Am J Cancer Res 2023;13(6):2588-2597
www.ajcr.us /ISSN:2156-6976/ajcr0149892

Brief Communication

High PIVKA-II level and ASAP score predict 1-year risk of hepatocellular carcinoma in non-cirrhotic chronic hepatitis B patients

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Received February 28, 2023; Accepted April 21, 2023; Epub June 15, 2023; Published June 30, 2023

Abstract: Protein induced by Vitamin K absence or antagonists-II (PIVKA-II) is a diagnostic marker of hepatocellular carcinoma (HCC). We aimed to investigate the predictive role of PIVKA-II and ASAP score for development of HCC in 1 year among untreated patients of chronic hepatitis B (CHB). We conducted this case-control study to include untreated CHB patients followed at the National Taiwan University Hospital and grouped into HCC and matched non-HCC groups. Their archived serum samples were assayed for PIVKA-II levels 1 year before HCC, at HCC or their last serum sample. A total of 69 HCC cases and 102 non-HCC controls were recruited. Baseline PIVKA-II level was significantly higher in the HCC group than in the control group and it could predict HCC development in 1 year with an area under the receiver operating characteristic curve of 0.76. Multivariable analysis adjusting age, sex, liver function and alpha-fetoprotein level showed that baseline PIVKA-II ≥31 mAU/mL (vs. <31 mAU/mL) increased 12.5-fold risk (95% CI: 4.9-31.7) of HCC in 1 year, and even in patients with normal alpha-fetoprotein levels. The ASAP score, a combination of age, sex, alpha-fetoprotein and PIVKA-II, increases the predictability for HCC in 1 year. We concluded that both high PIVKA-II level and ASAP score may predict HCC development in 1 year in untreated CHB patients, especially in patients with normal alpha-fetoprotein level.

Keywords: Protein induced by vitamin K absence or antagonists-II, Des-gamma-carboxy prothrombin, DCP, liver cancer, HCC, biomarker, tumor marker

Introduction

According to the World Health Organization, HCC is the fifth most common malignancy worldwide and the second most cancer-related death [1]. Chronic Hepatitis B (CHB) is a major cause of HCC and contributes to approximately 50% of HCC [2]. Several potent antiviral therapies have been developed and effectively reduce the risk of HCC development [3]; however, there are still many patients who do not fulfill the criteria to start antiviral therapies or do not have the access to antiviral agents. As prognosis is highly related to the stage of HCC, early detection of HCC is vital to improve the overall survival [4]. HCC surveillance to detect early-stage HCC and to reduce mortality is recommended by current guidelines [5-8]. All current guidelines recommended regular ultrasonographic examination every 6 months in at risk patients [5-9]. Ultrasound alone yielded a sensitivity of 63%, which was not very effective for detecting early-HCC [10]. Alpha-fetoprotein (AFP) had been suggested as a surveillance option combined with ultrasonography by the Asian Pacific Association for the Study of the Liver (APASL), American Association for the Study of Liver Diseases (AASLD), Korean Liver Cancer Association (KLCA) and the Japan Society of Hepatology (JSH) guideline [5-8];

however, AFP was less specific for HCC in case of active hepatitis [11], and also showed poor sensitivity for small HCC [12, 13].

PIVKA-II, also known as Des-gamma-carboxy prothrombin, was first reported in 1984 that elevated in patients with HCC and might serve as a diagnostic tumor marker [14]. PIVKA-II shows greater diagnostic accuracy than AFP in early stage HCC in a systemic review [15]; however, the generalizability and accuracy remained to be validated [15]. PIVKA-II had been used as a complementary marker to AFP to increase the diagnostic rate (such as the GALAD score and ASAP model) [16, 17]. PIVKA-II, AFP and AFP-L3 had been recommended by the JSH consensus for HCC surveillance [6].

The role of PIVKA-II level to predict the development of HCC remains limited. Our previous study targeting on patients of CHB-related cirrhosis on antiviral therapy, we found PIVKA-II and AFP at time of virological suppression may predict the development of subsequent HCC [18]. In this study, we would like to investigate the predictive role of PIVKA-II for HCC in treatment-naive CHB patients.

Materials and methods

Patient enrollment

This was a retrospective case-control study conducted in National Taiwan University Hospital, a tertiary medical center in Taipei, Taiwan. Patients with treatment-naïve chronic hepatitis B (CHB) without cirrhosis previously included in the Elucidation of Risk Factors for Disease Control or Advancement in Taiwanese Hepatitis B Carriers (ERADICATE-B) cohort were screened [19]. HCC surveillance was performed by abdominal ultrasonography and AFP level at least once every six months. HCC was diagnosed by either histology or two typical dynamic images by computed tomography or magnetic resonance imaging, with an AFP level of ≥200 ng/mL before 2010 and one typical dynamic imaging study after 2010 according to the AASLD guidelines [20]. These patients were categorized into HCC and non-HCC groups.

Because we planned to investigate PIVKA-II level as a predictive marker, we included patients with stored serum in the -20°C freezer at the hepatitis research center of the National Taiwan University Hospital. For the HCC group, we included patients with stored serum at 1 year before the diagnosis of HCC (as baseline) and at the time of HCC. We screened age and sex matched patients without HCC, and included those with stored serum at least 1 year before (as baseline) their last follow-up in our hospital to confirm their absence of HCC at the end of follow-up. Baseline PIVKA-II levels were measured to predict the development of HCC in 1 year. People with incomplete clinical data, unavailable stored sera, a history of HCC, and patients who received vitamin K or warfarin, which might interfere the levels of PIVKA-II were excluded. The clinical characteristics of patients were collected, including age, sex, platelet, total bilirubin, AST, ALT, HBeAg, HBV DNA, and AFP.

This study was approved by the Institutional Review Board of the National Taiwan University Hospital (200909047R) and conformed to the ethical principles for medical research involving human subjects of the Declaration of Helsinki updated in 2013. All patients provided written informed consents before enrollment.

Measurement of serum PIVKA-II

Serum PIVKA-II concentrations were analyzed by the ARCHITECT i2000SR immunoassay analyzer (Abbott Laboratories, North Chicago, IL) through the manufacturer's instructions. The ARCHITECT PIVKA-II assay is a two-step chemiluminescent microparticle immunoassay for the quantitative measurement of PIVKA-II in human serum or plasma. The PIVKA-II present in the sample binds to the anti-PIVKA-II coated microparticles in the capture step. After washing, the analyte particles were mixed with acridinium-labeled anti-prothrombin conjugate to create a reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs) and the final results are expressed as arbitrary units (mAU/mL) for the quantitative measurement. The precision of the assay was below 10% CV, with a measuring range from 20 to 30000 mAU/mL [21]. All clinical data, including the presence of HCC, were blinded to laboratory technicians to avoid bias.

Calculation of ASAP score

The ASAP score was calculated as ln (p/(1-p)) = ln -7.57711770 + 0.04666357 * age -

Table 1. Baseline (1-year before outcome date) characteristics of enrolled patients

Parameters	N	Non-HCC	HCC	*P*-value
N		102	69	
Age, year		56 (48-63)	56 (49-63)	0.819
sex				
Male		80 (78)	53 (77)	0.803
Female		22 (22)	16 (23)	
HBeAg	35			
Negative		16 (94)	13 (72)	0.086
Positive		1 (6)	5 (28)	
HBV DNA, log_{10} IU/mL	42	1.3 (1.3-2.9)	4.6 (2.8-5.3)	<.001
AST, U/L	159	23 (19-27)	38 (28-51)	<.001
ALT, U/L	167	23 (16-31)	38 (27-54)	<.001
Platelet, k/uL	76	197 (172-218)	120 (76-176)	0.003
Total bilirubin, mg/dL	89	0.9 (0.7-1.0)	0.8 (0.6-1.0)	0.594
AFP, ng/mL	148	2.7 (2.2-4.4)	11 (5.4-20)	<.001
<20		96 (98)	40 (80)	<.001
≥20		2 (2)	10 (20)	
PIVKA-II, mAU/mL	171	24 (20-30)	36 (26-71)	0.013
<31		84 (82)	24 (35)	<.001
≥31		18 (18)	45 (65)	

Note: Data are expressed as median (interquartile range) or number (percentage). Abbreviations: AST: aspartate transferase, ALT: alanine aminotransferase, AFP: alpha-fetoprotein, PIVKA-II: Prothrombin induced by Vitamin K Absence of Antagonist-II, HCC: hepatocellular carcinoma.

0.57611693 * sex + 0.42243533 * ln (AFP) + 1.10518910 * ln (PIVKA-II), which had been used to detect HCC among patients with hepatitis B [17].

Statistical analysis

Continuous data are reported as median (interquartile range), and differences between groups were compared by Student's t-test, or paired t-test as appropriate. Categorical data are reported as numbers (percentages), and differences between groups were evaluated by chi-square test as appropriate.

The receiver operating characteristic (ROC) curve was used to explore the optimal cut-off value (by maximizing the Youden Index) for the PIVKA-II and ASAP score value for predicting occurrence of HCC in 1 year. The univariate and multivariable logistic regression analysis were used to identify predictors for HCC. The statistical analyses were performed by using STATA (version 16; Stata Corp, College Station, TX, USA) and all tests were 2-sided and a *p* value <0.05 was considered statistically significant.

Result

Baseline characteristics

Totally, 171 non-cirrhotic, untreated HBV carriers were collected into analysis, including 69 HCC cases and 102 non-HCC controls. The median age was 55 years and 78% were men (**Table 1**). The baseline median PIVKA-II (36 vs. 24 mAU/mL, P=0.013), and AFP (11 vs. 2.7 ng/mL, P<0.001) were significantly higher in the HCC group than in the control group. The AST (38 vs. 23 U/L, P<0.001), ALT (38 vs. 23 U/L, P<0.001), and HBVDNA (4.6 vs. 1.3 log_{10} IU/mL, P<0.001) were also significantly higher in the HCC group compared to the control group. Of these HCC patients, 80% had AFP level <20 ng/mL, while 65% had PIVKA-II ≥31 mAU/mL, which indicates PIVKA-II might serve as a supplementary predictor of HCC for those with normal AFP level.

PIVKA-II predicts HCC development in 1 year

In the HCC group, the median PIVKA-II level increased significantly until the development of HCC (from 36 to 44 mAU/mL, P=0.029), which confirmed that PIVKA-II as the tumor marker of HCC and might be used for HCC surveillance. We further explored whether we can use the baseline PIVKA-II to predict HCC development in 1 year. The receiver operating characteristic (ROC) curve was plotted for PIVKA-II in predicting HCC and the area under the ROC curve (AUROC) was 0.76 (95% confidence interval [CI]: 0.68-0.94) (**Figure 1**). The optimal cut-off value for PIVKA-II was 31 mAU/mL with a sensitivity of 65% and specificity of 83% to predict HCC development in 1 year (Supplementary Tables 1, 2).

AFP ≥20 ng/mL or PIVKA-II ≥31 mAU/mL predict HCC development in 1 year

We further evaluated the predictors of HCC including the PIVKA-II level. Univariate analysis showed that baseline PIVKA-II ≥31 mAU/mL,

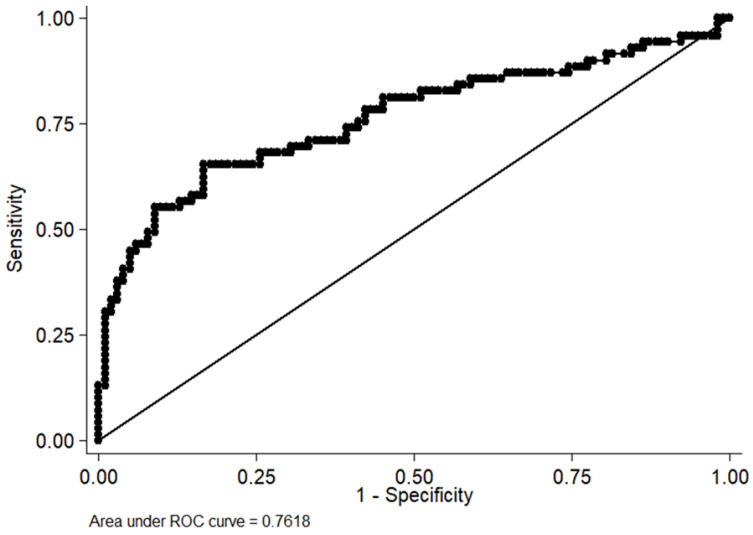

Figure 1. The receiver operating characteristic (ROC) curve of PIVKA-II for predicting HCC in 1 year.

AFP ≥20 ng/mL, and high ALT level were significantly associated with the development of HCC in 1 year (**Table 2**). Multivariable logistic regression analysis after controlled age, sex, ALT and AFP levels showed baseline PIVKA-II ≥31 mAU/mL increased 12.5-fold risk of HCC (vs. <31 mAU/mL, OR: 12.5, 95% CI: 4.9-31.7) in 1 year.

PIVKA-II level ≥31 mAU/mL predicts HCC in patients with normal AFP level <20 ng/mL

In our treatment-naïve CHB cohort, 80% of patients have normal AFP level 1 year before their HCC development. In order to investigate whether PIVKA-II helps to predict HCC in these patients, 136 patients (AFP <20 ng/mL) were used for subgroup analysis, including 40 HCC cases and 96 non-HCC controls. The baseline PIVKA-II level was predictive of 1-year HCC with an AUROC of 0.80 (95% CI: 0.72-0.89) (**Figure 2**). We further investigate the risk predictor for HCC in this subgroup of patients. Univariate analysis showed that baseline PIVKA-II ≥31 mAU/mL and higher ALT levels were significantly associated with the development of HCC in 1 year (**Table 3**). Multivariable logistic regression analysis after adjustment of confounding factors showed baseline PIVKA-II ≥31 mAU/mL increased 12.4-fold risk of HCC in 1 year (vs. <31 mAU/mL, OR: 12.4, 95% CI: 4.7-32.7).

High ASAP score predicts HCC in 1 year

Furthermore, we investigated the predictive performance of PIVKA-II in combination with AFP level and the ASAP score was evaluated. The ASAP scores were significantly higher in the HCC group than in the control group (0.48 vs. 0.25, P<0.001). The ROC curve was plotted for ASAP score in predicting HCC and the area under the ROC curve (AUROC) was 0.90 (95% CI: 0.85-0.95) (**Figure 3**) and 0.89 (95% CI: 0.83-0.95) in subgroup analysis of AFP level <20 ng/mL (Supplementary Figure 1). The optimal cutoff is 0.31 yielded by Youden Index to predict of 1-year HCC, with a sensitivity of 92.5% and a specificity of 71.7% (Supplementary Tables 3, 4). In our cohort, 60% patients with ASAP score ≥0.31 developed HCC in 1 year, compare with 4% in those with ASAP score <0.31 (**Figure 4**). Multivariable logistic regression analysis after adjustment of confounding factors showed baseline ASAP score >0.31 increased 111-fold risk of HCC (OR: 111.16, 95% CI: 21.4-577.4) (**Table 4**).

Discussion

In our study, we found that among untreated non-cirrhotic chronic hepatitis B patients, PIVKA-II ≥31 mAU/mL was associated with 12.5-fold risk of developing HCC in 1 year, which could be a potential predictor for future HCC development. Notably, patients with normal AFP level (<20 ng/mL), PIVKA-II ≥31 mAU/mL were still associated with 12.4-fold increased risk of HCC in 1 year. The combination of PIVKA-II and AFP into the ASAP score can predict subsequent HCC in 1 year with improved accuracy.

Surveillance in high-risk populations is an effective measure for HCC early detection, because we can identify early-stage HCC for curative therapy. Asian chronic non-cirrhotic HBV carriers for females >50 years have a 0.3-0.6% HCC risk per year and for males >40 years have a 0.4-0.6% HCC risk per year [6]. Therefore, a surveillance strategy for HCC is recommended. The efficacy of HCC surveillance every 6 months had been demonstrated in a large prospective randomized controlled trial in China [22]. Current guidelines suggest

Table 2. Univariate and multivariable analysis for the predictors of HCC in 1 year

Parameters	Univariate		Multivariable	
	OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Age (1 year increase)	1.00 (0.96-1.03)	0.753	1.04 (1.00-1.09)	0.071
Male, sex	0.91 (0.44-1.09)	0.803	0.67 (0.24-1.86)	0.443
ALT (1 U/L increase)	1.02 (1.01-1.04)	0.003	1.01 (1.00-1.03)	0.056
AFP ≥20 vs. AFP <20	12.00 (2.52-57.24)	0.002	21.36 (3.58-127.50)	0.001
PIVKA-II ≥31 vs. PIVKA-II <31	8.75 (4.30-17.80)	<.001	12.53 (4.95-31.73)	<.001

Abbreviations: ALT: alanine aminotransferase, AFP: alpha-fetoprotein, PIVKA-II: Prothrombin induced by Vitamin K Absence of Antagonist-II, HCC: hepatocellular carcinoma, OR: odds ratio, CI: Confidence interval.

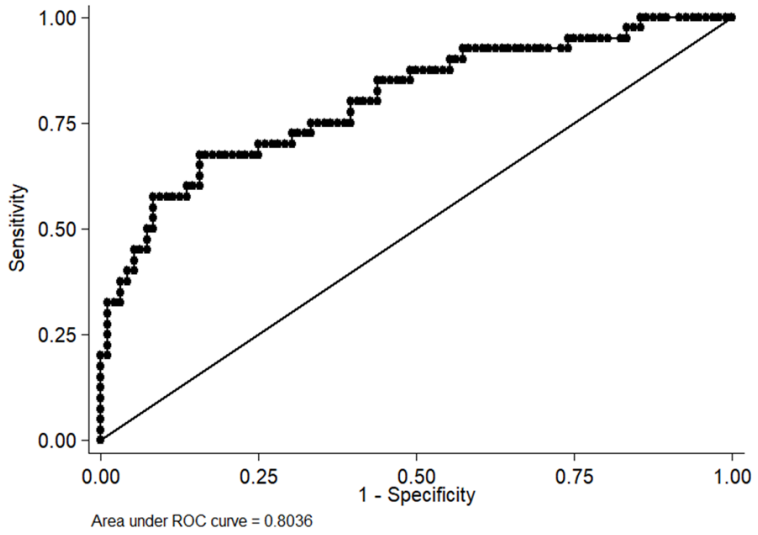

Figure 2. The receiver operating characteristic (ROC) curve of PIVKA-II for predicting HCC in 1 year in patients with AFP <20 ng/mL.

6-month abdominal ultrasonography as the standard protocol for HCC surveillance. However, for patients with obesity, liver atrophy, or marked cirrhosis, it is difficult to find small HCC by ultrasonography. Additional biomarker is therefore clinically needed.

AFP has already been widely used as a biomarker for HCC. Currently, ultrasonography with or without AFP has been recommended by APASL and AASLD for high-risk patients [5, 6]. However, for HCC size ≤3 cm, serum AFP level was frequently normal [13], which makes it insensitive for early detection of HCC.

PIVKA-II increases in malignant hepatocyte during vitamin K deficiency and supposed to reflect liver function under warfarin treatment first in 1963 [23]. In liver tissue of HCC, impaired vitamin-K dependent carboxylation pathway for prothrombin precursor leads to overproduction of prothrombin precursor with des-γ-carboxylation, and thus increased the serum PIVKA-II level [24]. The PIVKA-II levels significantly increased in HCC patients; therefore, it may serve as a diagnostic marker for HCC [25, 26]. PIVKA-II level is associated with portal vein invasion and advanced tumor stage [27]. PIVKA-II is not correlated with AFP [14], therefore, PIVKA-II might be able to complement the predictive role of AFP. PIVKA-II together with AFP reflect different mechanisms of hepatocarcinogenesis. The JSH guideline suggested AFP, AFP-L3, and PIVKA-II combine with ultrasound for high-risk group in their regular surveillance [28].

Currently, PIVKA-II has been considered as promising predictive biomarker for HCC in different clinical scenarios, including predicting prognosis of HCC patients [29], at time of anti-HBV therapy induced virological remission in patients of CHB-related cirrhosis [18], and to predict the complete responses of trans-arterial chemoembolization in patients of HCC [30]. Several studies supported that the combination of PIVKA-II and AFP had better diagnostic efficacy in HCC [31]. Most recent studies on PIVKA-II have concentrated on its diagnostic accuracy of HCC or its predictive role in HCC progression [32-34]. Few have focused on the capability of PIVKA-II in predict HCC before clinical diagnosis on a high-risk population. A case-control study with 189 CHB patients in 2016 indicated the benefit of combination of AFP and PIVKA-II for diagnosis and early detection of CHB-related HCC [35]. However, the predictive role of PIVKA-II 1 year before HCC had an

Table 3. Univariate and multivariable analysis for predictors of HCC in 1 year in patients with AFP <20 ng/mL

Parameters	Univariate		Multivariable	
	OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Age (1 year increase)	1.01 (0.97-1.05)	0.745	1.04 (0.99-1.09)	0.131
Male, sex	0.89 (0.38-2.11)	0.794	0.61 (0.21-1.79)	0.373
ALT (1 U/L increase)	1.03 (1.01-1.05)	0.006	1.03 (1.01-1.05)	0.005
PIVKA-II ≥31 vs. PIVKA-II <31	10.39 (4.43-24.35)	<.001	12.45 (4.74-32.66)	<.001

Abbreviations: ALT: alanine aminotransferase, PIVKA-II: Prothrombin induced by Vitamin K Absence of Antagonist-II, HCC: hepatocellular carcinoma, OR: odds ratio, CI: Confidence interval.

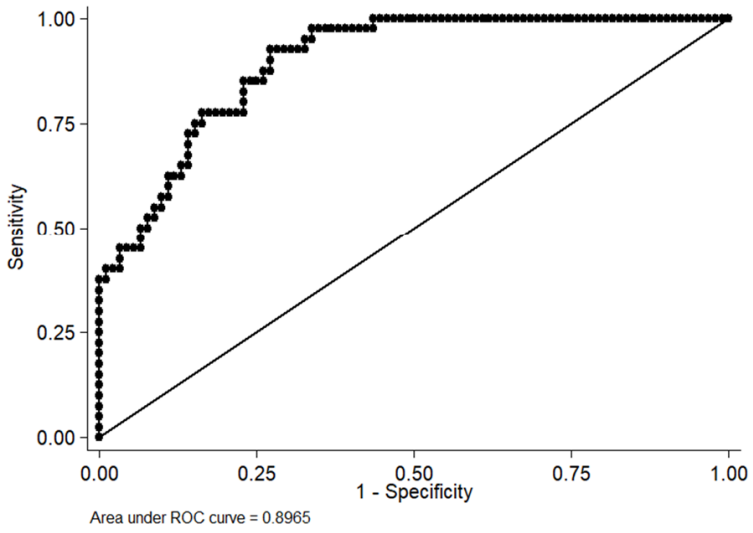

Figure 3. The receiver operating characteristic (ROC) curve of ASAP score for predicting HCC in 1 year.

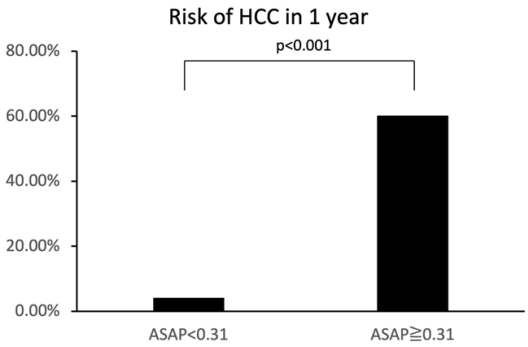

Figure 4. Using ASAP score 0.31 for HCC prediction in 1 year.

AUROC of 0.54 only and there were no suggested cut-off PIVKA-II values for HCC prediction. Serial AFP and PIVKA-II levels measured before HCC have the potential for HCC prediction, while PIVKA-II has a better AUROC than AFP before diagnosis [15]. Our study indicated the predictive role of PIVKA-II 1 year before HCC had an AUROC of 0.76 (95% confidence interval [CI]: 0.68-0.94) with an optimal cutoff value for PIVKA-II was 31 mAU/mL.

The GALAD score (gender, age, AFP-L3, AFP, and DCP) had been introduced for detecting HCC in chronic liver disease patients with an AUROC greater than 0.90 irrespective of HCC stages [16], and had been validated in several case-control cohorts [36, 37], however, AFP-L3 is not available in many countries, which limits its application. The simplified ASAP score, has been proposed to discriminate HCC from chronic HBV, HBV related cirrhosis, benign hepatic tumors, and healthy control participants from 11 hospitals in China [17]. The addition of AFP-L3 did not increase the diagnostic accuracy for HCC in both 2198 patients in the training cohort and 727 patients in the validation cohort. A cut-off value of 0.5256 of the ASAP score had been recommended as a diagnostic marker [17]. In another case-control study included 168 HCV-HCC patients and a control group of 193 HCV-infected patients, the ASAP score had higher AUROC compared to the GALAD score (0.917 vs. 0.894, P=0.057) in detect any stage of HCC, both in the overall and cirrhotic groups [38]. According to the AUROC result, the ASAP score was significantly better than the GALAD score in detection of early-stage (BCLC stage 0-A) HCC (P<0.05), suggesting the potential of ASAP score in early detection of HCC in patients of chronic hepatitis C [38]. The ASAP score had been explored in

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Parameters	Univariate		Multivariable	
	OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Age (1 year increase)	1.00 (0.96-1.03)	0.753	0.91 (0.85-0.97)	0.003
Male, sex	0.91 (0.44-1.09)	0.803	0.36 (0.10-1.34)	0.129
ALT (1 U/L increase)	1.02 (1.01-1.04)	0.003	1.01 (0.99-1.03)	0.185
ASAP ≥0.31 vs. ASAP <0.31	33.05 (9.35-116.88)	<.001	111.16 (21.40-577.44)	<.001

Abbreviations: ALT: alanine aminotransferase, HCC: hepatocellular carcinoma, OR: odds ratio, CI: Confidence interval.

a cohort of 1012 patients for HCC surveillance. It should be calibrated according to different etiology of liver disease for individual risk stratification [39]. Therefore, we investigated the predictive role of ASAP score in CHB related HCC, and confirmed baseline ASAP score >0.31 increased 111-fold risk of HCC after adjustment of confounding factors. Our research provides the first evidence to apply the ASAP score as an HCC prediction tool in non-cirrhotic HBV carriers.

Our study identifies the predictive role of PIVKA-II in treatment-naïve non-cirrhotic HBV patients, which represents a large population of high-risk patients in Asia. We identify a cut-off 31.0 mAU/mL of PIVKA-II, which is not high, because it is derived from non-cirrhotic patients, and for prediction of HCC 1 year before, rather than for diagnostic purposes (cut-off value: 37.5-45.0 mAU/mL) [33, 34]. Our data validate that combination of AFP and PIVKA-II increase sensitivity without decreasing specificity, although the cost-effectiveness remains to be assessed [6]. Besides, using ASAP score increased the AUROC up to 0.90 for predicting HCC in 1 year, which highlights combining PIVKA-II and AFP increased the predictive power and potential to early detect HCC in non-cirrhotic HBV carriers. Therefore, we suggest when patients have normal AFP during HCC surveillance, PIVKA-II may be measured and abnormal PIVKA-II level warns the risk of HCC. The high PVIKA-II or ASAP level reminds the physician to closely monitor HCC in at-risk patients, and may detect HCC at an early stage.

There are a few limitations of this study. First, the small sample size from a single center and the lack of external validation reduced our statistical power; thus, further studies with larger case numbers are required for confirmation of our results. Second, missing data and selection bias were unavoidable in this retrospective study, which limited the accuracy and analytic aspects relatively. The applicability of our results needs to be verified in different ethnicity and populations.

In conclusion, both high PIVKA II level and ASAP score are promising to predict 1-year HCC risk in untreated non-cirrhotic CHB patients. Further prospective studies are needed to validate these findings.

Acknowledgements

We thank Miss Wan-Ting Yang, Shih-Wan Chou, Pei-Ying Chiang, and Pei-Ying Yang, for their administrative and statistical assistance, and the staff of the Department of Medical Research, National Taiwan University Hospital for the Integrated Medical Database (NTUH-iMD). We thank the 7th Core Laboratory of the Department of Medical Research, National Taiwan University Hospital for technical assistance. This work was supported by grants from the Ministry of Science and Technology, Taiwan (grant numbers MOST 109-2326-B-002-012-MY3, MOST 110-2326-B-400-004, MOST 110-2628-B-002-041), Ministry of Health and Welfare (MOHW111-TDU-B-221-114003), National Taiwan University Hospital (grant numbers VN-112-13, 112-TMU151, 112-S0245), and the Liver Disease Prevention & Treatment Research Foundation, Taiwan. The PIVKA-II assays were supported by Abbott. The funders have no roles in study design; data collection, analysis, interpretation of data, manuscript preparation or the decision to submit the paper for publication.

Disclosure of conflict of interest

None.

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Supplementary Table 1. The cut-off values for PIVKA-II for predicting HCC in 1 year

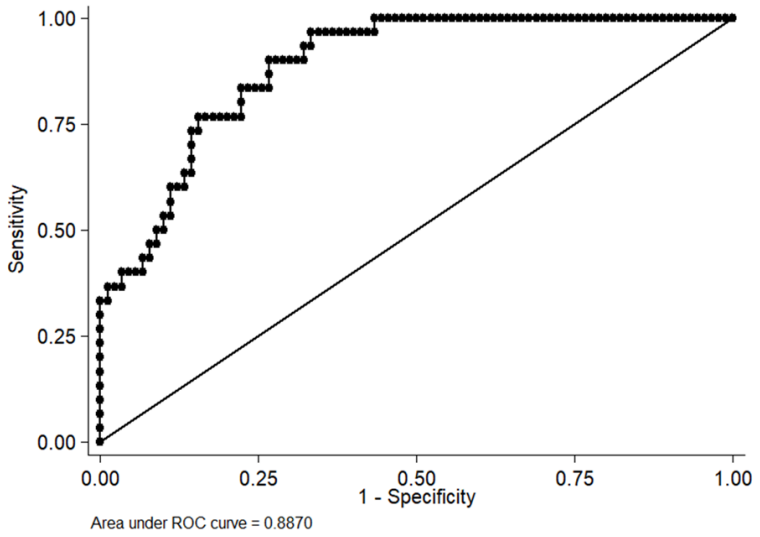
Cut-off value	Sensitivity (%)	Specificity (%)	Youden Index
20	88.41	22.55	0.1096
25	79.71	54.90	0.3461
31	65.22	81.37	0.4659
35	55.07	88.24	0.4331
40	46.38	92.16	0.3854
50	33.33	98.04	0.3137

N=171, AUROC=0.76 (95% CI: 0.68-0.84).

Supplementary Table 2. The cut-off values for PIVKA-II for predicting HCC in 1 year in patients with AFP <20 ng/mL

Cut-off value	Sensitivity (%)	Specificity (%)	Youden Index
15	100.00	3.13	0.0313
20	95.00	22.92	0.1792
25	82.5	56.25	0.3875
31	67.50	82.29	0.4979
35	57.50	88.54	0.4604
40	45.00	92.71	0.3771
50	32.50	96.88	0.2938

N=136, AUROC=0.80 (95% CI: 0.72-0.89).



Supplementary Figure 1. The receiver operating characteristic curve of ASAP score for predicting HCC in 1 year in patients with AFP <20 ng/mL.

Supplementary Table 3. The cut-off values for ASAP score for predicting HCC in 1 year

Cut-off value	Sensitivity (%)	Specificity (%)	Youden Index
0.25	100.00	48.91	0.4891
0.28	97.50	58.70	0.5620
0.31	92.50	71.74	0.6424
0.40	77.50	81.52	0.5902
0.50	45.00	94.57	0.3957
0.60	30.00	100.00	0.3000

N=132, AUROC=0.90 (95% CI: 0.85-0.95).

Supplementary Table 4. The cut-off values for ASAP score for predicting HCC in 1 year in patients with AFP <20 ng/mL

Cut-off point	Sensitivity (%)	Specificity (%)	Youden Index
0.25	100.00	50.00	0.5000
0.28	96.67	58.89	0.5556
0.31	90.00	72.22	0.6222
0.40	76.67	82.22	0.5889
0.50	40.00	94.44	0.3444
0.60	26.67	100.00	0.2667

N=120, AUROC=0.89 (95% CI: 0.83, 0.95).