

PIVKA-II is a useful tool for diagnostic characterization of ultrasound-detected liver nodules in cirrhotic patients

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Abstract

Protein induced by vitamin K absence-II (PIVKA-II) is a potential screening marker for hepatocellular carcinoma (HCC). Limited data are available about its utility in discriminating neoplastic from regenerative nodules at ultrasound (US) evaluation in cirrhotic patients. Aim of this study was to investigate the diagnostic utility of PIVKA-II in cases showing liver nodules of uncertain diagnosis at US.

Ninety cirrhotics with US evidence of liver nodule(s) were enrolled. All patients underwent blood sampling within 1 week of US and were thereafter followed up. HCC was confirmed in 40/90 cases, and in all cases it was in a very early/early stage. All sera were tested for PIVKA-II and alpha-fetoprotein (AFP) at the end of follow-up. PIVKA-II at a cut off of 60 mAU/mL was significantly associated with HCC at both univariate and multivariate analysis ($P=.016$ and $P=.032$, respectively). AFP at a cut off of 6.5 ng/mL was not associated with HCC at univariate analysis ($P=.246$). ROC curves showed that PIVKA-II had 60% sensitivity, 88% specificity, 80% positive predictive value (PPV), and 73% negative predictive value (NPV), whereas AFP had 67% sensitivity, 68% specificity, 63% PPV, and 72% NPV. AUROC curves showed that the combination of both biomarkers increased the diagnostic accuracy for HCC (AUC 0.76; sensitivity 70%, specificity 94%, PPV 91%, and NPV 79%).

In conclusion, PIVKA-II is a useful tool for the diagnostic definition of US-detected liver nodules in cirrhotic patients, and it provides high diagnostic accuracy for HCC when combined with AFP.

Abbreviations: AFP = alpha-fetoprotein, CI = confidence interval, C-P = Child–Pugh, HCC = hepatocellular carcinoma, PIVKA-II = protein induced by vitamin K absence-II, US = ultrasound.

Keywords: AFP, cirrhosis, HCC, liver nodules, PIVKA-II

1. Introduction

Hepatocellular carcinoma (HCC) is the 2nd most common cause of death from cancer in males and the 6th in females worldwide.^[1] In fact, despite a quite large number of treatment options, the 5-year survival of patients with HCC is lower than 15%.^[2] This dramatically poor outcome is also related to the fact that most HCCs are diagnosed at advanced stages, when patients are not eligible for curative therapies such as surgical resection or liver transplantation, and often they are not treatable at all with any of the possible approaches even with palliative therapies.^[3]

Actually, it is a general belief that the chances of surviving of HCC patients may be significantly increased by in-time diagnosis that might make it possible to choose the best treatment for each individual patient.^[4] On this basis, international guidelines recommend screening for HCC in high-risk patients (namely, patients with cirrhosis of any etiology), and in particular abdominal ultrasonography (US) twice per year for surveillance.^[5,6] However, in a meta-analysis of prospective cohort studies, US appeared to be unsatisfactory in HCC detection at early stages, showing a pooled sensitivity of only 63%.^[7] Therefore, alternative diagnostic approaches are needed, and – in any case – US controls would need to be complemented by additional tools to become efficacious in early HCC detection. In particular, the availability of a biomarker helpful for differential diagnosis between regenerative/dysplastic and neoplastic nodules in patients with cirrhosis and US evidence of nodular lesions would be extremely useful in clinical practice. The alpha-fetoprotein (AFP) serological test has been widely used in this context for many years. However, even if a low-level cutoff is used (ie, 10–20 ng/mL), AFP diagnostic sensitivity is around 60% while specificity becomes inadequate.^[8] Furthermore, AFP levels may be elevated in a number of nonspecific conditions such as chronic liver disease and malignancies other than HCC.^[9–12] Therefore, the latest international guidelines no longer recommend AFP testing for HCC surveillance even in combination with US.^[5,6] Hence, more sensitive and accurate diagnostic biomarkers for early diagnosis of HCC in high-risk patients are urgently needed. Protein induced by vitamin K absence-II (PIVKA-II) – also known as des- γ -carboxy-prothrombin – has been identified

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as a serum biomarker linked to HCC. It is an abnormal prothrombin molecule produced as a consequence of an acquired defect in the posttranslational carboxylation of the prothrombin precursor in malignant cells.^[13,14] A quite large body of evidence indicates that PIVKA-II levels in the blood are increased in patients with HCC and – importantly – an increasing amount of data indicates that it is more sensitive than AFP for differentiating HCC at all stages from patients with cirrhosis or chronic hepatitis.^[15–19] In addition, several studies from Asian countries have shown that serum PIVKA-II levels correlate with the HCC stage, as well as with survival of HCC patients, and a combination of PIVKA-II and AFP is currently used in Japan for HCC diagnosis and surveillance in accordance with the recommendations of the Clinical Practice Guidelines for HCC of that country.^[20–22] On the contrary, studies concerning the diagnostic use of PIVKA-II in Western countries are still limited, and indeed they have produced controversial results.^[16,17,23–25] The aim of this study was to investigate the possible utility of PIVKA-II – compared to or in combination with AFP – as a biomarker in cases with US evidence of liver nodules of uncertain diagnosis.

2. Materials and methods

2.1. Patients

Ninety cirrhotic patients who had evidence of liver nodule(s) at US examination for the first time and who consecutively attended the liver unit of the University Hospital of Messina from November 2011 to October 2013 were enrolled. All of them underwent blood sampling within 1 week before or after the US identification of liver nodules, and the corresponding serum samples were aliquoted and stored at -80°C until testing. Diagnosis of cirrhosis was based on a combination of clinical, biochemical, imaging and endoscopic findings in 86/90 patients, and on histological evaluation in the remaining 4 patients. All patients were followed up for at least 18 months after US nodule (s) detection through imaging techniques – contrast enhanced computed tomography and/or magnetic resonance imaging – and/or nodule needle biopsy performed according to the American Association for the Study of Liver Disease guidelines for HCC management.^[5] Contrast enhanced computed tomography and magnetic resonance imaging consisted of 4 phases (unenhanced, arterial, venous, and delayed phases) in order to assess the presence of the typical features of HCC nodules (arterial hypervascularity followed by venous and/or delayed phase “washout”). Briefly, the route map for the diagnosis of HCC was the following, in accordance to American Association for the Study of Liver Disease guidelines^[5]: nodules $<1\text{ cm}$ diameter were followed up with US until growing to $>1\text{ cm}$ diameter (no cases in our cohort); nodules $>1\text{ cm}$ diameter presenting the typical imaging pattern of arterial hypervascularity followed by “washout” were diagnosed as HCC; and nodules $>1\text{ cm}$ diameter without computed tomography and magnetic resonance imaging typical imaging features of HCC underwent needle biopsy. Nodules revealed at US were confirmed to be neoplastic in 40/90 cases whereas HCC was not confirmed in 50/90 cases. Nodule biopsy was performed in 7 cases, and HCC diagnosis was histologically confirmed in 5 cases and not confirmed in 2. All HCCs were in the very early/early stage according to the Barcelona Clinic Liver Cancer staging system.^[5] No patient was under treatment with vitamin K or warfarin. Written informed consent was obtained from each patient. The study protocol conformed to the principles of the Declaration of

Helsinki, and it was reviewed and approved by the local Ethics Committee.

2.2. Serum tests

PIVKA-II and AFP serum levels were measured on a Lumipulse G1200 (Fujirebio Inc.), using the LUMIPULSE G PIVKA-II kit and the LUMIPULSE G AFP-N kit (Fujirebio Tokyo, Japan), respectively, according to the manufacturer’s instructions. All tests were performed in duplicate.

2.3. Statistical analyses

Considering PIVKA-II as the primary parameter in distinguishing HCC from non-HCC cirrhotic patients with the confidence level $\alpha=0.050$, a sample size of 40 per group was estimated to be sufficient to gain 80% power. Continuous variables were expressed as mean \pm standard deviation or median and ranges as appropriate, and were compared by using the nonparametric Mann–Whitney test. Categorical variables were expressed in absolute values and percentage and were compared with the Chi-square test. Log transformation was used on the AFP and PIVKA-II values to account for the large range of values among the groups for both markers. The descriptive statistics for the transformed markers were compared by box plots and then by analysis of variance. The nonparametric approach was used when the continuous variables were not normally distributed, as verified by Kolmogorov–Smirnov test. To determine the optimal cut-off value for PIVKA-II and AFP in the diagnosis of HCC, receiver operating characteristic curves were constructed using all possible cut-offs for each assay. The areas under the receiver-operating characteristic curves were calculated and compared. Sensitivity, specificity, negative predictive value, and positive predictive value of the 2 examined markers (considered alone and combined with each other) were evaluated to discriminate between cirrhotic patients with and without HCC (HCC patients and non-HCC patients, respectively). Univariate logistic regression analysis was used to identify predictors of HCC. Variables with P value $<.05$ in univariate analysis were then subjected to multivariate analysis and included in the logistic model. A 2-tailed P value of $<.05$ was used to determine statistical significance. All statistical analyses were performed using SPSS software version 17.0. (SPSS Inc., an IBM Company, Chicago, IL).

3. Results

Demographic and clinical characteristics of the 90 consecutive patients with evidence of liver nodules at US examination (40 with and 50 without HCC as verified by 2nd level imaging) are shown in Table 1. HCC patients were older than non-HCC ones ($P=.009$). Ten HCC patients had Child–Pugh (C-P) class A cirrhosis and 30 had C-P class B or C cirrhosis whereas 29/50 were in C-P class A and 21 in class B/C in the non-HCC group ($P=.002$) (Table 1). Viral hepatitis etiology was significantly more common in HCC than non-HCC patients (31/40 vs 27/50, $P=.02$) whereas alcoholic etiology of the cirrhosis was significantly more frequent in non-HCC than HCC patients (10/50 vs 1/40, $P=.02$). No differences were found in the number of liver nodules detected at US examination between the 2 groups (Table 1), and all nodules were within 3 cm in diameter.

Median serum levels of both PIVKA-II and AFP were significantly higher in patients with HCC than in those without

Table 1
Clinical-demographic characteristics of 90 cirrhotic patients with neoplastic or nonneoplastic liver nodules.

	HCC 40 cases	Non-HCC 50 cases	P
Male sex, n, %	29 (72.5)	36 (72)	.95
Age (mean±SD, y)	68.8±10.4	62.9±10.3	.009
Etiology of liver disease, n, %			
Viral	31 (77.5)	27 (54)	
Cryptogenic/NAFLD	8 (20)	13 (26)	.02
Alcoholic	1 (2.5)	10 (20)	
Child–Pugh stage, n, %			
Child–Pugh A cirrhosis	10 (25)	29 (58)	.002
Child–Pugh B or C cirrhosis	30 (75)	21 (42)	
Number of liver nodules detected at US, n, %			
1	35 (87.5)	42 (84)	.63
2–3	5 (12.5)	8 (16)	

HCC=hepatocellular carcinoma, NAFLD=nonalcoholic fatty liver disease, SD=standard deviations, US=ultrasonography.

(PIVKA-II levels, 75 mAU/mL [AU=Anson unit], range: 10–8, 675 vs 28, range: 11–449, $P < .001$; AFP levels, 9.3 ng/mL, range: 2.4–2000 vs 4.5, range: 1.1–1551, $P < .001$) (Fig. 1A, B). However, at univariate analysis, PIVKA-II but not AFP values were significantly associated with presence of HCC ($P = .016$ and $P = .246$, respectively). In addition, older age, viral etiology of the liver disease, and the C-P class B/C cirrhosis were also significantly associated with HCC at univariate analysis ($P = .011$, $P = .023$, and $P = .002$, respectively) (Table 2). At multivariate analysis, PIVKA-II, viral etiology, and C-P class B/C maintained statistical significance and were independent predictors of HCC ($P = .032$, $P = .006$, and $P = .011$, respectively) (Table 2).

Receiver-operating characteristic curves were plotted to identify PIVKA-II and AFP cut-off values that would best distinguish cirrhotic patients with HCC nodules from patients with regenerative/dysplastic nodules. The optimal cut-off was 60 mAU/mL for PIVKA-II and 6.5 ng/mL for AFP. These values yielded a sensitivity and specificity of 60% and 88% (95% confidence interval [CI] 45%–74% and 76%–94%, respectively) for PIVKA-II and 67% and 68% (95% CI 52%–80% and 54%–79%, respectively) for AFP. The positive predictive value and negative predictive value were 80% and 73% for PIVKA-II and 63% and 72% for AFP, respectively (Table 3). Areas under the receiver-operating characteristic curves were 0.71 (95% CI 0.596–0.823) for PIVKA-II and 0.72 (95% CI 0.613–0.823) for

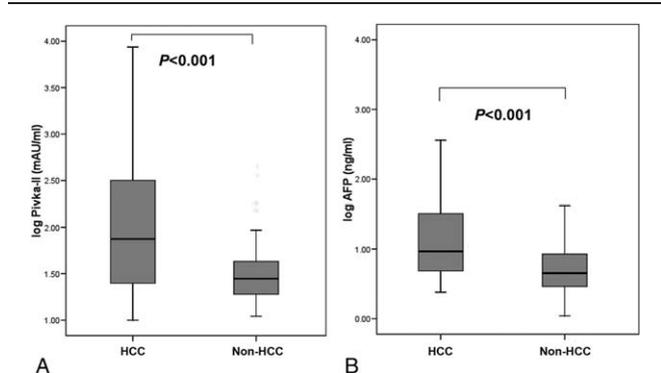


Figure 1. (A, B) Serum levels of PIVKA-II (A) and AFP (B) in HCC and non-HCC cases. Log transformation was used on the AFP and PIVKA-II values because of the large range of values for both markers. Boxes refer to 25th and 75th percentile values, lines indicate median levels, whereas the 95% confidence interval extends beyond the box. AFP=alpha-fetoprotein, HCC=hepatocellular carcinoma, PIVKA-II=protein induced by vitamin K absence-II.

AFP. Interestingly, by combining PIVKA-II and AFP values the diagnostic accuracy for HCC increased compared with either test alone (sensitivity 70%, specificity 94%, positive predictive value 91%, and negative predictive value 79%; area under the receiver-operating characteristic curve 0.76; 95% CI 0.665–0.862) (Fig. 2 and Table 3).

4. Discussion

This observational cohort study assessed the performance of AFP and PIVKA-II in the diagnosis of early HCC in cirrhotic patients with initial US evidence of suspicious liver nodules. The results showed that PIVKA-II is an independent predictor of HCC presence and a better diagnostic biomarker than AFP in discriminating between neoplastic and nonneoplastic lesions in cirrhotic livers. These findings are in accordance with data from other studies (Marrero et al, Durazo et al, and Poté et al)^[16,17,24] also reporting a better performance of PIVKA-II than AFP in differentiating cirrhotics with and without HCC. The relatively lower sensitivity of PIVKA-II found in this study – which, however, was counterbalanced by good specificity (88%) and positive predictive value (80%) – in comparison with previous ones is likely related to the inclusion of patients with very early or early stage HCC that are the most difficult to diagnose at a preclinical stage. Indeed, although PIVKA-II serum levels are routinely used in HCC screening in Japan and other Eastern

Table 2
Univariate and multivariate analyses of possible predictive factors of hepatocellular carcinoma in cirrhotic patients with liver nodules at ultrasound.

	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
PIVKA-II	1.006	1.001–1.011	.016	1.006	1.000–1.011	.032
AFP	1.001	0.999–1.002	.246	–	–	–
Age	1.057	1.013–1.104	.011	1.048	0.996–1.103	.073
Sex	0.975	0.385–2.469	.958	–	–	–
Viral etiology	2.934	1.161–7.417	.023	5.246	1.599–17.211	.006
Child–Pugh B/C	4.143	1.668–10.289	.002	4.508	1.422–14.291	.011
Number of liver nodules at US	0.750	0.225–2.500	.640	–	–	–

AFP=alpha-fetoprotein, CI=confidence interval, OR=odds ratio, PIVKA-II=protein induced by vitamin K absence-II, US=ultrasound.

Table 3

Performance characteristics of PIVKA-II, AFP, and combination of both biomarkers in the diagnosis of early hepatocellular carcinoma in patients with cirrhosis and ultrasound evidence of liver nodules.

	Sensitivity	Specificity	PPV	NPV
PIVKA-II	60%	88%	80%	73%
AFP	67%	68%	63%	72%
PIVKA-II+AFP	70%	94%	91%	79%

AFP=alpha-fetoprotein, NPV=negative predictive value, PIVKA-II=protein induced by vitamin K absence-II, PPV=positive predictive value.

world countries,^[22,26] results of their accuracy in Western areas come from few studies, which included patients at different HCC stages and showed conflicting results.^[16,17,23–25] Of interest, PIVKA-II combined with AFP provided even better results in terms of diagnostic accuracy and had a better sensitivity and specificity compared to either biomarker alone. These data confirm evidence from other studies indicating that PIVKA-II and AFP are complementary biomarkers,^[23,27–29] and this is consistent with the fact that their production occurs through different pathways.

Thus, the combination of PIVKA-II and AFP seems to be the most promising tool available at the moment to predict the presence of early HCC in cirrhotic patients with a positive US screening. This observation is of utmost clinical importance since US examination is the primary surveillance tool for cirrhotic patients, but it is not sensitive enough for an undisputed HCC identification in many patients with cirrhosis. Although AFP is no longer considered a valid biomarker in HCC surveillance in American and European practice guidelines, our study suggests that it might be of some use in enhancing the diagnostic accuracy of PIVKA-II if the 2 markers are tested together, and this is in accordance with Japanese guidelines.^[22] These results may encourage to perform further studies evaluating the role of PIVKA-II – alone or in combination with AFP – in large cohorts of patients with liver diseases. In particular, it may be of relevance to prospectively investigate patients with different stages of chronic liver diseases, as well as patients with HCC but without cirrhosis, as not rarely observed in patients with chronic hepatitis B virus infection or with nonalcoholic steatohepatitis.

The identification of reliable serum biomarkers is of utmost importance in the diagnosis of liver cancer, especially for detection and screening of HCC at early stages.^[30,31] In recent years, many studies have provided relevant evidence concerning the potential diagnostic significance of nonprotein serum markers (eg, methylated DNAs in circulating tumor cells, microRNAs, and long-noncoding RNAs in microvesicles and exosomes).^[31,32] However, protein biomarkers – such as PIVKA-II – quantifiable in serum are the most suitable for clinical routine evaluation and population screening, since these tests require very small amounts of serum, are low-cost, have high reproducibility, and samples do not need any particular pretreatment (ie, extraction or purification).

In conclusion, evaluation of serum PIVKA-II appears to be a powerful additional tool for HCC diagnosis. The inclusion of serum PIVKA measurement in the diagnostic work-up for HCC (of course in addition to imaging examinations and possibly with AFP evaluation) should be considered in daily clinical practice, though further large-scale prospective studies are needed to

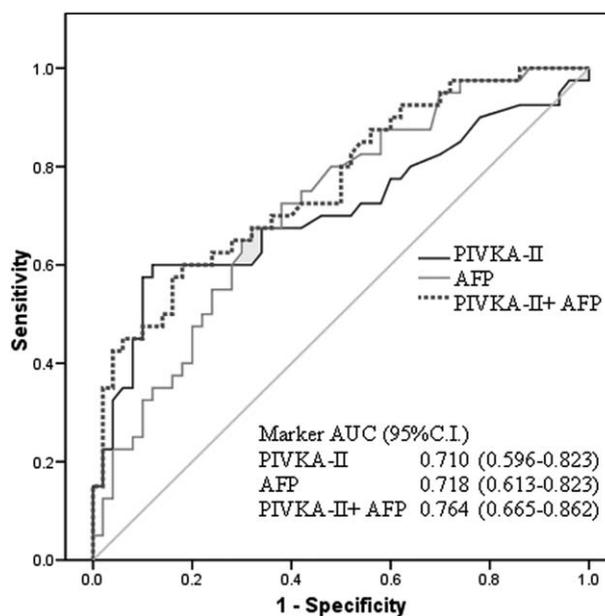


Figure 2. ROCs curve comparing serum levels of PIVKA-II, AFP, and a combination of PIVKA-II and AFP as screening markers for HCC. The AUROC curve is shown with its 95% confidence interval. AFP=alpha-fetoprotein, AUROC=area under the ROC, HCC=hepatocellular carcinoma, PIVKA-II=protein induced by vitamin K absence-II, ROC=receiver-operating characteristic.

finally confirm the utility of PIVKA-II for the detection of early HCC.

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