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Original

Positioning of novel tumor marker NX-PVKA-R in the diagnosis of hepatocellular carcinoma in comparison with PIVKA-II

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SUMMARY

Background/Aims : To compare the use of protein induced by vitamin K absence or antagonist II (PIVKA-II) measured conventionally with the ratio between PIVKA-II measured using P-11 and P-16 antibodies (NX-PVKA) and PIVKA-II measured conventionally (NX-PVKA-R) in terms of false-positive results for hepatocellular carcinoma (HCC). **Methodology** : Subjects comprised 318 patients with chronic liver disease, including 8 patients receiving warfarin treatment, which can result in false-positive results for HCC. HCC was present in 65 patients (HCC group) and absent in 253 (non-HCC group). PIVKA-II was measured conventionally. NX-PVKA-R was calculated as PIVKA-II/NX-PVKA. **Results** : Both PIVKA-II and NX-PVKA-R were significantly higher in the HCC group than in the non-HCC group ($p<0.0001$ each). False-positive results were seen in 9.5% of non-HCC patients with PIVKA-II, and in 10.3% with NX-PVKA-R. False-positive results were seen for all 8 patients (100%) on warfarin with PIVKA-II, but for 0% with NX-PVKA-R. Sensitivity, specificity, and accuracy were all lower for NX-PVKA-R than PIVKA-II. **Conclusions** : NX-PVKA-R is not more useful than PIVKA-II for diagnosing HCC, but is very useful in subpopulations such as patients on warfarin and patients with jaundice. The characteristics of NX-PVKA-R can be best exploited by selecting patients in which these factors are present.

Key Words : protein induced by vitamin K absence or antagonist II (PIVKA-II) ; NX-PVKA-R ; hepatocellular carcinoma

INTRODUCTION

Protein induced by vitamin K absence or antagonist II (PIVKA-II) is an abnormal form of prothrombin produced in patients with vitamin K deficiency. In 1984, Liebman et al. reported PIVKA-II as a novel tumor marker in hepatocellular carcinoma (HCC)[1]. With PIVKA-II, the 10 γ-carboxyglutamic acid (Gla) residues near the N-terminal of factor II (prothrombin), a vitamin K-dependent protein involved in blood coagulation, are not synthesized normally, and all or some of the glutamic acid residues (Glu residues) appear in the blood without modification. Although highly specific to HCC, PIVKA-II shows false-positive results in the presence of vitamin K deficiency due to cholestasis or warfarin treatment.

PIVKA-II exists with 1-10 Glu residues, but con-

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Table 1 Ckinical characteristics of 318 patients

	HCC (n=65)		non HCC (n=253)	
Sex (M : F)	42 : 23		143 : 110	
Age (mean±SD)	72.5±10.0		65.2±12.0	
Cause of liver disease	HBV	9	HBV	30
	HCV	42	HCV	184
	HBV+HCV	1	HBV+HCV	1
	Alcohol	5	Alcohol	17
	NASH	2	AIH	2
	Other	6	PBC	5
			Willson	1
			Other	13
Tumor factors				
Size (mean±SD cm)	4.2±3.5			
Number (single : multiple)	25 : 40			
Venous invasion (+ : −)	8 : 57			
Distant metastasis (+ : −)	1 : 64			

HBV, hepatitis B virus ; HCV, hepatitis C virus ; NASH, non alcoholic steatohepatitis
AIH, autoimmun hepatitis ; PBC, primary biliary cirrhosis ; Wilson, Wilson's desease

ventional HCC diagnosis uses a monoclonal antibody (MU-3 antibody) that is highly reactive to types of PIVKA-II containing larger numbers of Glu residues[2]. When the MU-3 antibody is used, discriminating between PIVKA-II induced by HCC and PIVKA-II caused by vitamin K deficiency is difficult. On the other hand, monoclonal antibodies that are highly reactive to PIVKA-II containing a small number of Glu residues have been developed (P-11 and P-16 antibodies), so using the ratio between PIVKA-II measured using these antibodies (NX-PVKA) and the conventional measurement of PIVKA-II (NX-PVKA-R) allows the elimination of false-positive results due to vitamin K deficiency resulting from factors such as warfarin treatment[3].

This study examined the usefulness of NX-PVKA-R in the diagnosis of HCC in comparison to the conventional use of PIVKA-II alone.

METHODOLOGY

Patients

Subjects comprised 318 patients with chronic liver disease who consulted the Department of Gastroenterology at Dokkyo Medical University Koshigaya Hospital between March and September 2011. These patients included 21 patients with jaundice with a total bilirubin level ≥2.0 mg/dl, and 8 patients receiving warfarin treatment.

The cause of liver disease was hepatitis B virus (HBV) in 39 patients, hepatitis C virus (HCV) in 226 patients, HBV+HCV in 2 patients, excessive alcohol consumption in 22 patients, and other causes in 29 patients. HCC was diagnosed using dynamic computed tomography (CT) or digital-subtraction angiography (DSA). HCC was present in 65 patients and absent in 253 (Table 1). HCC tumor factors are shown in Table 1, but due to the small number of patients in this study, relationships between HCC tumor stage and PIVKA-II and NX-PVLA-R were not examined. All 8 patients on warfarin were without HCC.

Measurement of PIVKA-II and NX-PVKA

PIVKA-II was measured using the Picolumi PIVKA-II (cut-off, 40 mAU/ml ; Eidia, Tokyo, Japan) method[4]. This method employs mouse anti-human PIVKA-II antibody MU-3 and rabbit anti-human prothrombin polyclonal antibody.

NX-PVKA was measured using sandwich electrochemiluminescence immunoassay. This method employs novel mouse anti-human PIVKA-II monoclonal antibodies P11 and P16 (Sekisui Medical, Tokyo, Japan), generated by immunization with PIVKA-II from a warfarin user[3]. NX-PVKA-R was calculated as PIVKA-II/NX-PVKA.

Table 2 Clinical parameters of 318 patients

	HCC (n=65)	non HCC (n=253)	t-test
ALT (IU/L)	46.1±31.8	44.5±47.4	P=0.307
T-Bil (mg/dL)	1.2±1.1	1.2±2.6	P=0.283
Alb (g/dLl)	3.5±0.6	4.0±0.6	P=0.819
PT (%)	93.7±14.2	98.2±21.5	P=0.006
Platelet ($\times 10^4/\mu$L)	12.0±7.8	14.1±7.5	P=0.286

ALT, alanine aminotransferase ; T-Bil, total bilirubin ; Alb, albumin ; PT (%), prothrombinnactivity

Clinical parameters

Blood biochemical parameters as hepatitis activity, liver residual function, and liver fibrosis, serum alanine aminotransferase (ALT) levels, serum total bilirubin, albumin concentrations, platelet cell count, and prothrombin activity (PT%) were measured. After approval by the Institution Review Board of Dokkyo Medical University Koshigaya Hospital, the present study was conducted only after written informed consent was obtained from the patients.

Statistical analysis

Continuous data are expressed as mean±standard deviation (SD). The Mann-Whitney U test was used in comparisons between two groups. Values of $p<0.05$ were regarded as statistically significant.

RESULTS

Clinical parameters of HCC groups and non-HCC group

Blood biochemical parameters of patients in the group with HCC (HCC group) and the group without HCC (non-HCC group) were shown in Table 2. No significant differences between groups were seen with respect to ALT, total bilirubin, albumin concentration, or platelet count, but PT% was significantly higher in the non-HCC group.

Cutt-off valur of NX-PVKA-R

Figure 1 shows the receiver operating characteristic (ROC) curve for NX-PVKA-R. Using this curve, 1.28 was calculated as the cut-off value maximizing the Youden index. Significant figures for NX-PVKA-R were considered to be those figures up to one decimal place, so the cut-off value was rounded to 1.3, and this value was used in subsequent calculations.

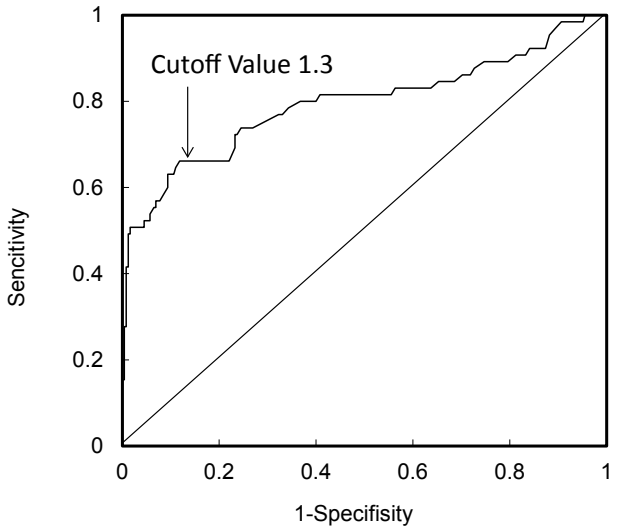

Figure 1 The receiver operating characteristic (ROC) curve for NX-PVKA-R. Using this curve, 1.28 was calculated as the cut-off value maximizing the Youden index. Significant figures for NX-PVKA-R were considered to be those figures up to one decimal place, so the cut-off value was rounded to 1.3, and this value was used in subsequent calculations.

Comparison of PIVKA-II and NX-PVKA-R

PIVKA-II and NX-PVKA-R values for the 65 patients in the HCC group and the 253 patients in the non-HCC group were shown in Figure 2. The HCC group presented significantly higher values ($p<0.0001$) for both PIVKA-II (Figure 2a) and NX-PVKA-R (Figure 2b). PIVKA-II yielded false-positive results in 24 of 253 patients in the non-HCC group (9.5%), and NX-PVKA-R yielded false-positive results in 26 of these patients (10.3%).

Figure 3 shows PIVKA-II and NX-PVKA-R values for the 8 patients without HCC who were on warfarin. PIVKA-II yielded false-positive results for all 8 patients, but NX-PVKA-R showed values below the cut-

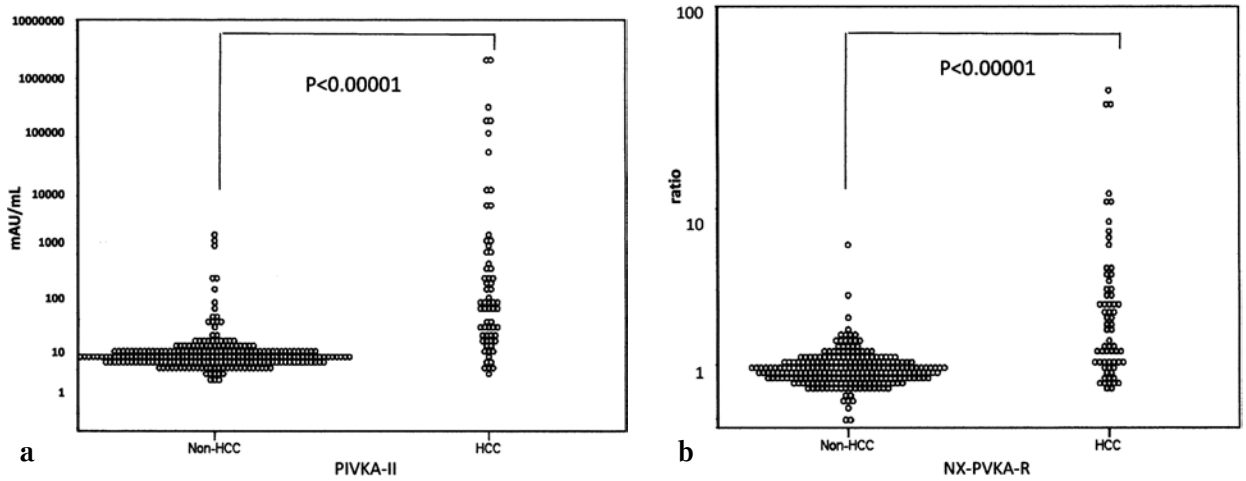

Figure 2 PIVKA-II and NX-PVKA-R values for the 65 patients in the HCC group and the 253 patients in the non-HCC group. The HCC group presented significantly higher values ($p<0.0001$) for both PIVKA-II (Fig. 2a) and NX-PVKA-R (Fig. 2b).

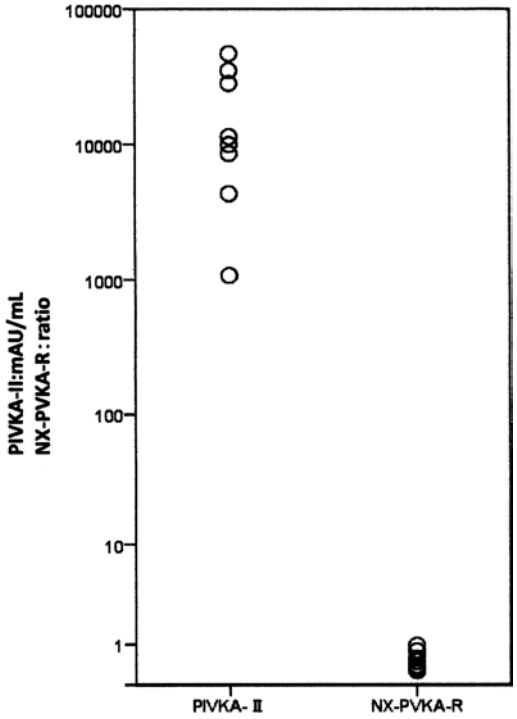

Figure 3 PIVKA-II and NX-PVKA-R values for the 8 patients without HCC who were on warfarin. PIVKA-II yielded false-positive results for all 8 patients, but NX-PVKA-R showed values below the cut-off point for all patients.

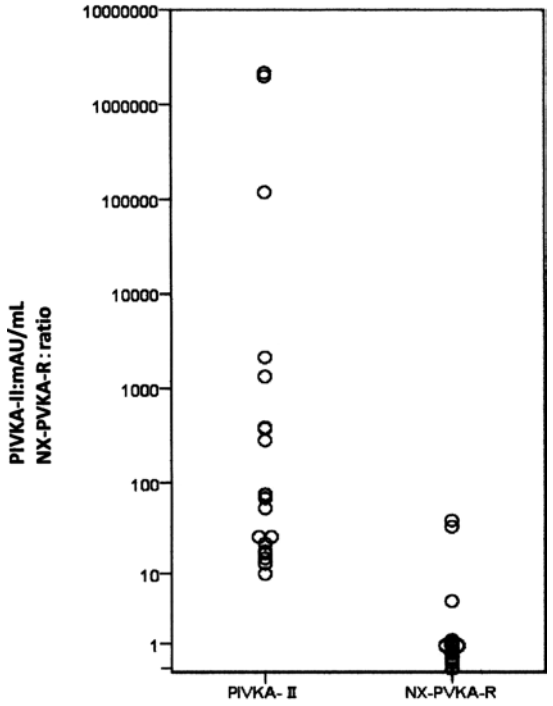

Figure 4 PIVKA-II and NX-PVKA-R values for the 21 jaundice patients with total bilirubin levels ≥2.0 mg/dL. PIVKA-II yielded false-positive results for 9 of the 17 patients without HCC (52.9%), whereas NX-PVKA-R showed false positives for 3 patients (17.7%).

off point for all patients. Figure 4 shows PIVKA-II and NX-PVKA-R values for the 21 jaundice patients with total bilirubin levels ≥2.0 mg/dL. PIVKA-II yielded false-positive results for 9 of the 17 patients without HCC (52.9%), whereas NX-PVKA-R showed false positives for 3 patients (17.6%).

The sensitivity, specificity and accuracy of PIVKA-II and NX-PVKA-R were calculated, and shown in Table 3. Sensitivity, specificity, and accuracy were all lower for NX-PVKA-R than PIVKA-II.

Table 3 Sensitivity, specificity and accuracy of PIVKA-II and NX-PVKA-R

	Cut off Value	Sensitivity	Specificity	Accuracy
PIVKA-Ⅱ	40 mAU/mL	74.2%	92.8%	89.0%
NX-PVKA-R	1.3	63.4%	89.2%	83.9%

DISCUSSION

The process by which prothrombin is synthesized in the liver first produces a precursor with 10 Glu residues in the Gla domain. This precursor is then released into the blood as an active coagulation factor through conversion of Glu residues to Gla residues by γ-glutamylcarboxylase (GGCX) in the presence of the reduced form of vitamin K, O_2, and CO_2. However, if a vitamin K deficiency is present or the patient is taking a vitamin K antagonist during this process, γ-carboxylation will not proceed normally, and several of the 10 Glu residues will remain, producing a prothrombin lacking blood-coagulating activity. This is called des-γ-carboxyprothrombin or PIVKA-II.

PIVKA-II is not only used for diagnosis of HCC, but also correlates with portal venous invasion of HCC[5], and is reportedly a factor that reflects tumor stage and survival rate in HCC[6]. PIVKA-II is also produced by non-cancerous tissues surrounding HCC[7, 8], and works not only as a growth factor for HCC[9~11], but also as a vascular endothelial growth factor for HCC, and is believed to play an important role in angiogenesis[12,13].

As discussed above, PIVKA-II is a very useful tumor marker in the diagnosis and prognosis prediction of HCC. However, PIVKA-II levels are also elevated in patients with vitamin K deficiency, such as those on warfarin, and in end-stage liver disease patients without HCC[14]. False-positive results can therefore be problematic. On the other hand, various types of PIVKA-II exist, based on the number of Glu residues present, and the types of PIVKA-II in patients taking vitamin K antagonists reportedly differ from those in patients with HCC[1,15]. In addition, the reagent for measuring PIVKA-II that is currently widely used in Japan (Picolumi PIVKA-II ; Eidia) uses a monoclonal antibody obtained from the MU-3 cell line. This reagent is highly reactive to PIVKA-II that contains a large number of Glu residues, but does not react with forms of PIVKA-II with 5-10 Gla residues[16]. The monoclonal antibodies used to measure NX-PVKA in the present study (P-11 and P-16 antibodies) are highly reactive to types of PIVKA-II containing only a small number of Glu residues. Toyoda et al. measured and compared PIVKA-II and NX-PVKA values in 20 patients with HCC and 56 patients without HCC on warfarin. PIVKA-II showed no significant difference between groups ($p=0.7952$), but patients without HCC on warfarin presented significantly higher values for NX-PVKA ($p=0.0291$). NX-PVKA-R showed significantly higher values in patients with HCC than in patients without HCC on warfarin ($p<0.0001$). Based on those results, NX-PVKA-R has been reported to identify patients on warfarin with elevated PIVKA-II due to HCC and is useful as a tumor marker for HCC in this patient subpopulation[3]. Although Toyoda et al. proposed 1.5 as the optimal cut-off value for NX-PVK-R in those studies, we independently created an ROC curve for our study, and used a cut-off of 1.3. In the future, further research with greater numbers of patients will be needed to assess the validity of this cut-off value.

Our study compared the usefulness of PIVKA-II and NX-PVKA-R for diagnosing HCC in 318 patients with chronic liver disease. Although only 8 patients were on warfarin, representing a small sample size, false-positive results were able to be eliminated for HCC diagnosis in all 8 patients. In addition, false-positive results were also able to be eliminated in 18 of 21 patients (82.4%) with jaundice resulting from impaired vitamin K absorption due to cholestasis. Although separate data are not presented here, the present findings suggest that NX-PVKA-R might be useful in alcoholic liver disease. The above findings once again confirmed the usefulness of NX-PVKA-R in the diagnosis of HCC in vitamin K-deficient patients.

Conversely, false-positive HCC diagnoses were seen in about 10% of cases with both PIVKA-II and NX-PVKA-R. Patients presenting false positives for PIV-

KA-II were patients on warfarin, jaundice patients and heavy drinkers, but, as discussed above, false positives caused by these factors can be avoided using NX-PVKA-R. Furthermore, one of the reasons false positives for NX-PVKA-R were present for about 10 % of patients was presumably the tendency for NX-PVKA-R to show high levels when PIVKA-II values, as the numerator, are low. When the sensitivity, specificity and accuracy according to eventual HCC diagnosis were examined, better results were obtained in each case for PIVKA-II than for NX-PVKA-R.

CONCLUSIONS

NX-PVKA-R is not more useful than PIVKA-II for diagnosing HCC, but is very useful in subpopulations such as patients on warfarin, patients with jaundice, and the characteristics of NX-PVKA-R can be fully exploited by selecting those patients in which these factors are present.

REFERENCES

1) 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 **310** : 1427-1431, 1984.
2) Motohara K, Kuroki Y, Kan H, et al : Detection of vitamin K deficiency by use of an enzyme-linked immunosorbent assay for circulating abnormal prothrombin. Pediatr Res **19** : 354-357, 1985.
3) Toyoda H, Kumada T, Osaki Y, et al : Novel method to measure serum levels of des-gamma-carboxy prothrombin for hepatocellular carcinoma in patients taking warfarin : A preliminary report. Cancer Sci **103** : 921-925, 2012.
4) Okuda H, Nakanishi T, Takatsu K, et al : Measurement of serum levels of des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma by a revised enzyme immunoassay kit with increased sensitivity. Cancer **85** : 812-818, 1999.
5) Koike Y, Shiratori Y, Sato S, et al : Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma : a prospective analysis of 227 patients. Cancer **91** : 561-569, 2001.
6) Toyoda H, Kumada T, Osaki Y, et al : Role of tumor markers in assessment of tumor progression and prediction of outcomes in patients with hepatocellular carcinoma. Hepatol Res **37**(Suppl 2) : S166-S171, 2007.
7) Tang W, Kokudo N, Sugawara Y, et al : Des-gamma-carboxyprothrombin expression in cancer and/or non-cancer liver tissues : association with survival of patients with resectable hepatocellular carcinoma. Oncol Rep **13** : 25-30, 2005.
8) Yuan LW, Tang W, Kokudo N, et al : Measurement of des-gamma-carboxy prothrombin levels in cancer and non-cancer tissue in patients with hepatocellular carcinoma. Oncol Rep **12** : 269-273, 2004.
9) Fujikawa T, Shiraha H, Yamamoto K, Significance of des-gamma-carboxy prothrombin production in hepatocellular carcinoma. Acta Med Okayama **63** : 299-304, 2009.
10) Gao FJ, Cui SX, Chen MH, et al : Des-gamma-carboxy prothrombin increases the expression of angiogenic factors in human hepatocellular carcinoma cells. Life Sci **83** : 815-820, 2008.
11) Suzuki M, Shiraha H, Fujikawa T, et al : Des-gamma-carboxy prothrombin is a potential autologous growth factor for hepatocellular carcinoma. J Biol Chem **280** : 6409-6415, 2005.
12) Inagaki Y, Tang W, Xu H, et al : Des-gamma-carboxyprothrombin : clinical effectiveness and biochemical importance. Biosci Trends **2** : 53-60, 2008.
13) Fujikawa T, Shiraha H, Ueda N, et al : Des-gamma-carboxyl prothrombin-promoted vascular endothelial cell proliferation and migration. J Biol Chem **282** : 8741-8748, 2007.
14) Yamashiki N, Sugawara Y, Tamura S, et al : Diagnostic accuracy of alpha-fetoprotein and des-gamma-carboxy prothrombin in screening for hepatocellular carcinoma in liver transplant candidates. Hepatol Res **41** : 1199-1207, 2011.
15) Fujiyama S, Morishita T, Hashiguchi O, et al : Plasma abnormal prothrombin (des-gamma-carboxy prothrombin) as a marker of hepatocellular carcinoma. Cancer **61** : 1621-1628, 1988.
16) Naraki T, Kohno N, Saito H, et al : gamma-Carboxyglutamic acid content of hepatocellular carcinoma-associated des-gamma-carboxy prothrombin. Biochim Biophys Acta **1586** : 287-298, 2002.