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## Clinical and Translational Research

# Clinical relevance of increased serum preneoplastic antigen in hepatitis C-related hepatocellular carcinoma

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## Abstract

### BACKGROUND

The prognosis of hepatocellular carcinoma (HCC) patients remains poor despite advances in treatment modalities and diagnosis. It is important to identify useful markers for the early detection of HCC in patients. Preneoplastic antigen (PNA), originally reported in a rat carcinogenesis model, is increased in the tissues and serum of HCC patients.

### AIM

To determine the diagnostic value of PNA for discriminating HCC and to characterize PNA-positive HCC.

### METHODS

Patients with hepatitis C virus (HCV)-related hepatic disorders were prospectively enrolled in this study, which included patients with hepatitis, with cirrhosis, and with HCC. A novel enzyme-linked immunosorbent assay was developed to measure serum PNA concentrations in patients.

### RESULTS

A, Akatsuka T, and Okita K have no conflicts of interest to declare.

**Data sharing statement:** The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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Serum PNA concentrations were measured in 89 controls and 141 patients with HCV infections (50 hepatitis, 44 cirrhosis, and 47 HCC). Compared with control and non-HCC patients, PNA was increased in HCC. On receiver operating characteristic curve analysis, the sensitivity of PNA was similar to the HCC markers des- $\gamma$ -carboxy-prothrombin (DCP) and  $\alpha$ -fetoprotein (AFP), but the specificity of PNA was lower. There was no correlation between PNA and AFP and a significant but weak correlation between PNA and DCP in HCC patients. Importantly, the correlations with biochemical markers were completely different for PNA, AFP, and DCP; glutamyl transpeptidase was highly correlated with PNA, but not with AFP or DCP, and was significantly higher in PNA-high patients than in PNA-low patients with HCV-related HCC.

## CONCLUSION

PNA may have the potential to diagnose a novel type of HCC in which glutamyl transpeptidase is positively expressed but AFP or DCP is weakly or negatively expressed.

**Key words:** Serum preneoplastic antigen; Hepatitis C virus; Hepatocellular carcinoma; Des- $\gamma$ -carboxy-prothrombin;  $\alpha$ -Fetoprotein; Sensitivity; Specificity

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**Core tip:** Despite advances in treatment modalities and diagnosis, the prognosis of hepatocellular carcinoma (HCC) patients remains poor. It is important to identify useful markers for the early detection of HCC. Preneoplastic antigen (PNA) is increased in the tissues and serum of HCC patients. Therefore, we investigated the diagnostic value of PNA to discriminate HCC. We found that PNA had a comparable diagnostic value to  $\alpha$ -fetoprotein and des- $\gamma$ -carboxy-prothrombin. PNA may have the potential to diagnose a novel type of HCC in which glutamyl transpeptidase is positively expressed, but  $\alpha$ -fetoprotein or des- $\gamma$ -carboxy-prothrombin is weakly or negatively expressed.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, and the primary risk factors for HCC include chronic infection by hepatitis B virus (HBV) or hepatitis C virus (HCV) and nonalcoholic fatty liver disease<sup>[1,2]</sup>. The overall survival rate of HCC patients has improved with advances in treatment modalities and diagnosis<sup>[3,4]</sup>. The surveillance rate for HCC patients has increased in Japan; thus, patients are more likely to be diagnosed in the earlier stage of the disease, and the survival rate has increased<sup>[5]</sup>. The HCC markers  $\alpha$ -fetoprotein (AFP) and des- $\gamma$ -carboxy-prothrombin (DCP), also known as PIVKA-II, have been used for the early-stage screening and diagnosis of HCC<sup>[6]</sup>.

Although HCC treatment modalities and diagnosis have developed and improved, the prognosis of HCC patients at advanced stages remains poor<sup>[7,8]</sup>. Therefore, it is important to develop useful markers for the early detection of HCC.

The expression of preneoplastic antigen (PNA) was originally reported in a study of hyperplastic nodules in a rat experimental carcinogenesis model<sup>[9]</sup>. Immunostaining approaches showed that PNA was present in the cytoplasm of hepatocytes in hyperplastic nodules and in primary hepatomas<sup>[9,10]</sup>. Purification and biochemical characterization indicated that PNA was composed of microsomal epoxide hydrolase (mEH) and other binding proteins<sup>[11,12]</sup>. The mEH gene was hypomethylated in nodules and hepatomas induced by chemical carcinogens<sup>[13]</sup>.

Evidence that PNA expression in human liver tissues is increased in pathological states has been accumulating. Immunohistochemical analysis of human tissues showed that mEH was positive in normal hepatocytes and HCC tissues, but less or



negatively expressed in other tumors, even if they metastasized to liver tissues<sup>[14,15]</sup>. Localization of mEH in the membrane changed during liver pathogenesis, such as neoplasia<sup>[16]</sup> or hepatitis infection<sup>[17]</sup>. An autoantibody response to mEH was detected in the serum of patients infected with HCV<sup>[18]</sup>. PNA was also detected in the culture medium of human HCC cell lines<sup>[19]</sup>. These data suggest that immunological detection of PNA may be a promising diagnostic tool for HCC.

To gain insight into PNA expression in liver diseases, we developed a highly sensitive enzyme-linked immunosorbent assay (ELISA) to measure PNA and determined serum PNA concentrations in patients with HCV-related hepatitis, cirrhosis, or HCC. The characteristics of PNA-positive HCC are also discussed in relation to biochemical markers.

## MATERIALS AND METHODS

### **Patients**

In Japan Community Health Care Organization (JCHO) Shimonoseki Medical Center, patients with HCV-related hepatic disorders were prospectively enrolled in this study, which included patients with hepatitis, with cirrhosis, and with HCC. All subjects fulfilled the criteria for a diagnosis of hepatitis, cirrhosis, or HCC with HCV infection, regardless of treatment history. The study protocol was approved by the Human Ethics Committee of JCHO Shimonoseki Medical Center (Approval date: May 29, 2015). Informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

### **Clinical and laboratory assessments**

Blood samples were collected from patients after written, informed consent was confirmed. Biochemical markers were assessed routinely in JCHO Shimonoseki Medical Center including albumin, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GTP), and total bilirubin levels, as well as HCC markers including DCP and AFP. A fibrosis marker of type IV collagen was measured with a latex immunoassay by Sekisui Medical Co., Ltd. (Tokyo, Japan).

### **Preparation of monoclonal antibodies and measurement of serum PNA**

Human mEH, a component of PNA, was produced in a recombinant baculovirus system<sup>[20]</sup>, and the solubilized form of mEH was purified with sequential steps by column chromatography<sup>[16]</sup>. The development of anti-mEH monoclonal antibody 2G2 has been previously described<sup>[19]</sup>. For preparation of the PNA-specific antibody, PNA fractions were purified from the culture medium of Huh.1 (human HCC) and LN-71 (human glioblastoma) cells by ammonium sulfate precipitation followed by the same methods for mEH purification. Female BALB/c mice aged 6 wk, purchased from Tokyo Laboratory Animal Science Co., Ltd. (Tokyo, Japan), were injected s.c. with 2  $\mu$ g of PNA four times, along with Freund's complete adjuvant for the first injection, with incomplete adjuvant for the second injection, and no adjuvant for the third and fourth injections. Their spleen cells were harvested and hybridized with NS-1 myeloma cells, as described previously<sup>[21]</sup>. Hybridoma clone 6G2 producing PNA-specific antibody was selected by screening each culture supernatant by ELISA against mEH and PNA. The mEH-specific 2G2 antibody (IgG1) was purified using an IgG Purification Kit-A (Chemical Dojin Co., Ltd., Tokyo, Japan), and PNA-specific antibody 6G2 (IgM) was purified using a HiTrap IgY Purification HP column (GE Healthcare Japan, Tokyo, Japan).

The ELISA for PNA was developed using a combination of anti-PNA antibody fixed on the plate and horseradish peroxidase-conjugated anti-mEH antibody for detection. Sera from control individuals were collected from healthy volunteers in Japan or purchased from BioreclamationIVT (Westbury, NY, United States).

### **Receiver operating characteristic curves for assessing the diagnosis of HCC**

The receiver operating characteristic (ROC) curve was obtained by calculating the sensitivity and specificity of the assay at every possible cut-off point and plotting sensitivity against [1-specificity] in SPSS for Windows (SPSS Japan, Tokyo, Japan). The area under the ROC curve (AUROC) was calculated to determine the diagnostic accuracy of the assay. Appropriate cut-off points were examined for balancing the sensitivity and specificity of the ROC curve, and the optimal cut-off point was identified as that yielding the minimal value for  $[(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2]$  or the maximal value for  $[\text{sensitivity} + \text{specificity} - 1]$ <sup>[22]</sup>.

### **Statistical analysis**

Statistical analysis was performed using SPSS for Windows (SPSS Japan, Tokyo, Japan). Differences in mean values between groups were assessed by the Mann-Whitney *U* test.  $\chi^2$  tests were used to compare univariate associations of categorical variables. Correlations between two parameters were analyzed using Spearman's rank correlation coefficient. A statistical review of this study was performed by a biomedical statistician.

## RESULTS

Of the 141 patients diagnosed as HCV-positive, 50 had hepatitis, 44 had cirrhosis, and 47 had HCC (Table 1). Compared with patients with hepatitis and cirrhosis, HCC patients had higher percentages of male and older patients. The percentage of male patients was 40% (20/50) in the hepatitis group, 41% (18/44) in the cirrhosis group, and 53% (25/47) in the HCC group. The median age was 70.2 years, 71.7 years, and 75.3 years in the three groups, respectively. The results of biochemical markers differed among the groups (Table 1). With the progression of hepatic disorders from hepatitis to cirrhosis, serum albumin decreased, whereas ALP, total bilirubin, and type IV collagen increased significantly. With the progression to HCC, AST and GTP increased significantly. It is important to note that no biological markers differed between cirrhosis and HCC, suggesting no significant deterioration of liver functions in HCV-related HCC.

Next, serum PNA was measured in patients with hepatitis, cirrhosis, and HCC, and the results were compared with those of 89 control subjects (Figure 1). Serum PNA did not differ between control and hepatitis patients, but it was slightly higher in cirrhosis than in control ( $P = 0.004$ ) and hepatitis patients ( $P = 0.017$ ). In contrast, PNA increased significantly in HCC compared with control ( $P < 0.001$ ) and hepatitis patients ( $P < 0.001$ ). PNA concentrations were over 10 times higher in HCC patients than in cirrhosis patients, although the difference between the two groups was not significant ( $P = 0.077$ ) due to the wide variations in data, especially in HCC patients.

The diagnostic value of serum PNA in HCC was determined and compared with the two HCC markers AFP and DCP (Figure 2). The ROC curves were obtained by calculating the sensitivity and specificity of these markers to differentiate HCC from hepatitis. The AUROC was 0.745 for PNA, 0.824 for AFP, and 0.793 for DCP. The ROC curve was also obtained to differentiate HCC from hepatitis and cirrhosis, and the AUROC was 0.680 for PNA and 0.754 for AFP and DCP. These data indicate that the diagnostic value of PNA was comparable to AFP and DCP to differentiate HCC from hepatitis or from hepatitis and cirrhosis.

Balancing sensitivity and specificity of the ROC curve indicated that the optimal cut-off point of serum PNA for predicting HCC was 5 ng/mL. Using this cut-off point, the sensitivity and specificity of PNA were determined and compared with those of AFP and DCP (Table 2). In differentiating HCC from hepatitis, the sensitivity and specificity were 63.8% and 78.0% for PNA, 61.7% and 88.0% for AFP, and 61.7% and 94.0% for DCP, respectively. In differentiating HCC from hepatitis and cirrhosis, the sensitivity and specificity were 63.8% and 66.0% for PNA, 61.7% and 75.5% for AFP, and 61.7% and 83.0% for DCP, respectively. These data suggest that the sensitivity of PNA was similar to AFP and DCP, but the specificity of PNA was lower than of AFP and DCP.

The correlations of PNA with AFP and DCP in the serum of HCC patients were evaluated (Figure 3). Spearman's correlation analysis clearly indicated no correlation between PNA and AFP, with a correlation index of 0.229 ( $P = 0.121$ ). A significant but weak correlation was seen between PNA and DCP, with a correlation index of 0.313 ( $P = 0.032$ ). These data indicated that PNA was positive in many patients who were negative for AFP or DCP.

To determine the differences in disease characteristics, the correlations of PNA, AFP, and DCP with biochemical markers in the sera of HCC patients were compared (Table 3). Spearman's correlation analysis indicated significant correlations of PNA with AST, ALP, and GTP. The highest correlation was seen between PNA and GTP, with a correlation index of 0.666 ( $P < 0.001$ ). In contrast, AFP was significantly correlated with all biochemical markers with the exception of GTP. DCP was significantly correlated only with albumin and ALP. These data indicate that the correlations with biochemical markers were completely different for PNA, AFP, and DCP in HCC patients, and that GTP was highly correlated with PNA, but not with AFP or DCP. HCC patients were then divided into PNA-high and PNA-low patients by the cut-off of the median PNA value (8 ng/mL), and biochemical markers were compared between PNA-high and PNA-low patients (Table 4). Statistical analyses indicated that AST, ALP, and GTP were significantly higher in PNA-high patients



**Table 1** Characteristics of patients with hepatitis C virus-related hepatitis, cirrhosis, and hepatocellular carcinoma

	1: Hepatitis (n = 50)		2: Cirrhosis (n = 44)		3: HCC (n = 47)		1 vs 2	1 vs 3	2 vs 3
	Mean	SD	Mean	SD	Mean	SD	P value	P value	P value
Sex (male/female)	20/30		18/26		25/22		0.360	0.048	0.101
Age (yr)	70.2	(11.7)	71.8	(10.4)	75.3	(9.0)	0.558	0.018	0.102
Albumin (g/L)	4.2	(0.3)	3.7	(0.6)	3.7	(0.9)	< 0.001	< 0.001	0.962
AST (U/L)	44	(35)	46	(20)	60	(51)	0.056	0.033	0.861
ALT (U/L)	41	(42)	33	(20)	45	(48)	0.967	0.667	0.674
ALP (U/L)	259	(60)	345	(141)	362	(312)	0.001	0.084	0.212
Bilirubin total (mg/dL)	0.8	(0.4)	1.2	(0.7)	1.2	(1.1)	0.001	0.003	0.477
GTP (U/L)	46	(61)	47	(44)	105	(212)	0.186	0.001	0.078
Type IV collagen (ng/mL)	159	(69)	237	(88)	207	(94)	< 0.001	0.003	0.075

Results are shown as means with SD. Differences in mean values between each group were assessed with Mann-Whitney *U* tests, and differences in the sex ratio were assessed with  $\chi^2$  tests. HCC: Hepatocellular carcinoma; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase ; ALP: Alkaline phosphatase; GTP: Glutamyl transeptidase.

than in PNA-low patients with HCV-related HCC. The mean GTP value was 183.2 U/L in PNA-high patients, which was almost 6 times higher than the mean GTP value (31.0 U/L) in PNA-low patients ( $P < 0.0001$ ).

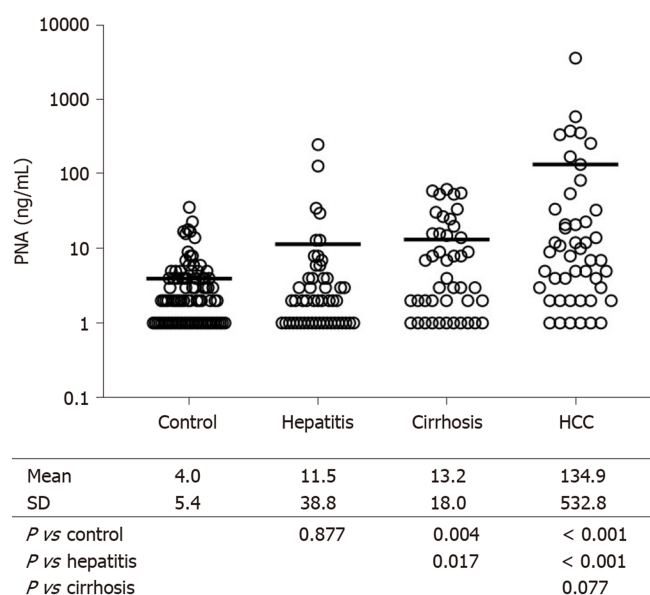
## DISCUSSION

HCC patients consist of heterogeneous populations, and the subtypes of HCC are characterized by clinical phenotypes such as cell differentiation and tumor size, and molecular phenotypes associated with gene mutations and transcriptional modification<sup>[23,24]</sup>. In addition, the immune microenvironment, which is affected by both clinical and molecular phenotypes, may have a large impact on patient prognosis<sup>[25]</sup> and be activated in a certain population of HCC patients<sup>[26]</sup>. Therefore, the development of a novel biomarker that can diagnose HCC and further characterize its phenotype in the clinical setting is warranted.

In this study, PNA was aberrantly increased in the serum of HCV-related HCC patients. The mechanism and function of the PNA increase in the serum of HCC patients are unclear. A component of PNA, mEH, is a drug metabolizing enzyme on the endoplasmic reticulum membrane that catalyzes the hydration of reactive epoxide. In addition, mEH plays a role in bile acid transport on the plasma membrane of hepatocytes<sup>[27]</sup>, and bile acid regulates hepatic tumor development<sup>[28]</sup>. Studies with a number of monoclonal antibodies recognizing different portions of mEH showed that mEH was located predominantly inside hepatocytes, while located abundantly on the surface of HCC cells<sup>[19,29]</sup>. Recent studies have shown that mEH plays a key role in the hydrolysis of fatty acids such as epoxyeicosatrienoic acid (EET)<sup>[30,31]</sup>. As EET regulates tumor cell growth, metastasis, angiogenesis, and inflammation in cancer<sup>[30,32,33]</sup>, the induction of mEH in HCC may reflect the dynamic change of localization and functions of mEH during carcinogenesis. The present data showing that serum PNA was increased in a limited number of non-HCC cirrhotic patients may suggest the change of mEH in preneoplastic stages.

The early detection of HCC allows patients to receive curative treatment and achieve long-term survival. The routine practice of screening high-risk patients for HCC contributes to the detection of HCC nodules in the early stages in more than 60% of patients in Japan<sup>[34]</sup>. The HCC markers AFP and DCP have been used to screen and diagnose HCC at an earlier stage. An immunohistochemical study of small HCC tissues showed that AFP-positive HCCs were more malignant than AFP-negative and DCP-positive HCCs<sup>[35]</sup>. DCP was more efficient than AFP for the diagnosis of early HCC and for the prediction of microvascular invasion<sup>[36]</sup>. The combined use of AFP and DCP was useful for predicting the aggressiveness of early-stage HCC in patients given local treatment<sup>[37,38]</sup>. However, their sensitivity and specificity are lower for early-stage HCC than for advanced stage HCC<sup>[6]</sup>. It appears that PNA has the potential to diagnose a novel population of HCC patients in which AFP or DCP is weakly or negatively expressed. Further analysis of PNA may clarify its potential for the detection of HCC nodules in the early stage.

In the present study, serum GTP was highly correlated with PNA, but not with



**Figure 1** Serum preneoplastic antigen concentrations in controls and in patients with hepatitis C virus-related hepatitis, cirrhosis, or hepatocellular carcinoma. Serum preneoplastic antigen concentrations were measured by ELISA. The results from each patient are plotted, and the means are shown by horizontal bars. The differences between groups were evaluated by Mann-Whitney *U* tests. The mean with SD and *P* value for comparison are summarized in the table. *n* = 89 in control, 50 in hepatitis, 44 in cirrhosis, and 47 in hepatocellular carcinoma.

AFP or DCP, and it was significantly higher in PNA-high patients than in PNA-low patients with HCV-related HCC. Therefore, PNA could potentially be used to diagnose a novel type of HCC in which GTP is positively expressed, but AFP or DCP is weakly or negatively expressed.

HCC patients with elevated serum GTP had a lower survival rate after resection<sup>[39]</sup> and transarterial chemoembolization<sup>[40]</sup>. The increase of GTP in early-stage HCC and the aggressive phenotype of HCC highly expressing GTP have been examined. Immunohistochemical and enzyme histochemical analysis showed the localization of GTP in preneoplastic foci at the early stage in a rat carcinogenesis model<sup>[41]</sup> and in hepatocellular foci of HCC patients with HCV infection<sup>[42]</sup>. The meta-analysis indicated that pretreatment serum GTP was a predictor of poor overall survival, recurrence-free survival, and disease-free survival in HCC patients<sup>[43,44]</sup>. The ectopic expression of GTP accelerated tumor cell growth, metastasis, and resistance to chemotherapy<sup>[45,46]</sup>. Therefore, PNA-positive HCC may have an aggressive phenotype with a higher risk for disease progression and a poor prognosis. The prognostic value of PNA for survival will be determined in the follow-up evaluation of the patients enrolled in this study.

A limitation of this study is the etiologies of the hepatic disorders. The risk factors for hepatitis, cirrhosis, and HCC include chronic infection by HBV or HCV and hepatic steatosis and inflammation in nonalcoholic fatty liver disease. In the present study, however, only patients with HCV-related hepatic disorders were enrolled. Further studies are warranted to identify the changes of PNA in hepatitis, cirrhosis, and HCC with other etiologies.

In conclusion, the diagnostic value of PNA to discriminate HCC was comparable to AFP and DCP. PNA may have the potential to diagnose a novel type of HCC in which GTP is positively expressed, but AFP or DCP is weakly or negatively expressed. Further studies are needed to determine the diagnostic value of PNA for HCC patients in comparison with other HCC markers.

**Table 2 Sensitivity and specificity of preneoplastic antigen,  $\alpha$ -fetoprotein, and des- $\gamma$ -carboxy-prothrombin in patients with hepatitis C virus-related hepatocellular carcinoma**

	(A) HCC vs hepatitis		(B) HCC vs hepatitis/cirrhosis	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
PNA	63.8	78.0	63.8	66.0
AFP	61.7	88.0	61.7	75.5
DCP	61.7	94.0	61.7	83.0

The sensitivity and specificity of markers were determined to discriminate hepatocellular carcinoma patients from (A) hepatitis patients and (B) hepatitis and cirrhosis patients. The cut-off value for positivity was 5 ng/mL for preneoplastic antigen, 10 ng/mL for  $\alpha$ -fetoprotein, and 40 mAU/mL for des- $\gamma$ -carboxy-prothrombin. HCC: Hepatocellular carcinoma; PNA: Preneoplastic antigen; AFP:  $\alpha$ -Fetoprotein; DCP: Des- $\gamma$ -carboxy-prothrombin.

**Table 3 Correlations of preneoplastic antigen,  $\alpha$ -fetoprotein, and des- $\gamma$ -carboxy-prothrombin with laboratory tests in patients with hepatitis C virus-related hepatocellular carcinoma**

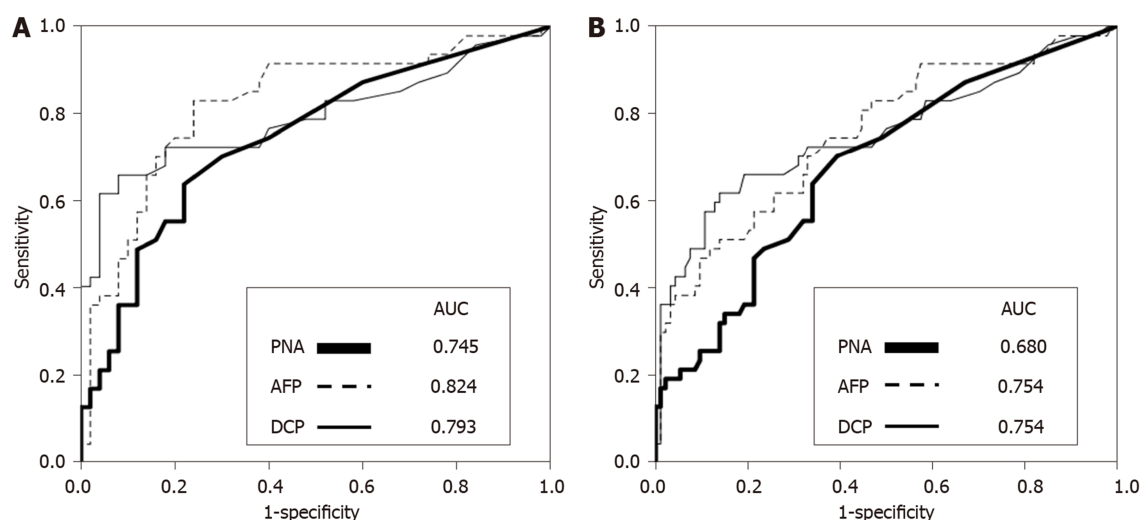
	PNA		AFP		DCP	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Albumin	-0.173	0.245	-0.416	0.004	-0.339	0.020
AST	0.325	0.026	0.626	< 0.001	0.232	0.117
ALT	0.227	0.125	0.438	0.002	-0.038	0.799
ALP	0.582	< 0.001	0.459	0.001	0.302	0.039
Bilirubin	0.226	0.130	0.366	0.012	0.030	0.841
GTP	0.666	< 0.001	0.274	0.062	0.082	0.585
Type IV collagen	0.107	0.476	0.498	< 0.001	0.246	0.095

The correlation index (*r*) and 2-sided *P* value were calculated by Spearman's correlation analysis. PNA: Preneoplastic antigen; AFP:  $\alpha$ -Fetoprotein; DCP: Des- $\gamma$ -carboxy-prothrombin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GTP: Glutamyl transeptidase.

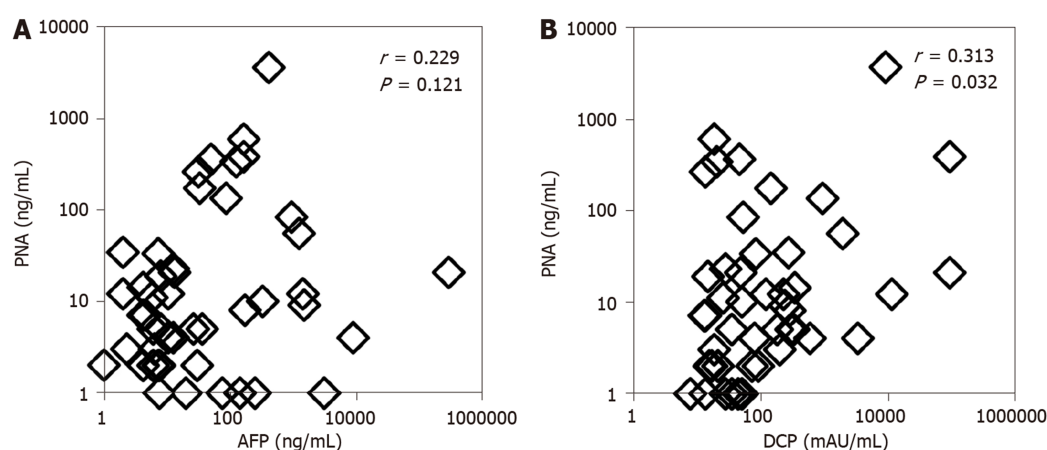
**Table 4 Comparison of biochemical markers between preneoplastic antigen-high and preneoplastic antigen-low patients with hepatitis C virus-related hepatocellular carcinoma**

	PNA-high patients ( <i>n</i> = 23)		PNA-low patients ( <i>n</i> = 24)		<i>P</i> value
	Mean	SD	Mean	SD	
Albumin (g/L)	3.5	0.7	3.9	1.1	0.1348
AST (U/L)	73.2	58.6	47.0	36.1	0.0247
ALT (U/L)	58.3	61.8	31.7	18.9	0.3025
ALP (U/L)	463.4	393.2	264.2	135.9	0.0004
Bilirubin total (mg/dL)	1.4	1.4	1.0	0.7	0.6345
GTP (U/L)	183.2	278.7	31.0	12.5	< 0.0001
Type IV collagen (ng/mL)	225.2	104.3	189.4	76.3	0.2133

Hepatocellular carcinoma patients were divided into preneoplastic antigen-high and preneoplastic antigen-low groups using the median preneoplastic antigen value (8 ng/mL) as the cut-off. Results are shown as means with SD. Differences in mean values between each group were assessed with Mann-Whitney *U* tests. PNA: Preneoplastic antigen; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GTP: Glutamyl transeptidase.



**Figure 2** Receiver-operating characteristic analyses of preneoplastic antigen,  $\alpha$ -fetoprotein and des- $\gamma$ -carboxy-prothrombin in hepatitis C virus-related hepatocellular carcinoma. The sensitivity and specificity of markers were determined to discriminate hepatocellular carcinoma patients from hepatitis patients and cirrhosis patients. A: Hepatitis patients; B: Hepatitis and cirrhosis patients. The receiver-operating characteristic curve was obtained by plotting the sensitivity on the Y-axis against 1-specificity on the X-axis, and the area under the receiver-operating characteristic curve was calculated. PNA: Preneoplastic antigen; AFP:  $\alpha$ -Fetoprotein; DCP: Des- $\gamma$ -carboxy-prothrombin.



**Figure 3** Correlation analysis of preneoplastic antigen with  $\alpha$ -fetoprotein and des- $\gamma$ -carboxy-prothrombin in sera of patients with hepatitis C virus-related hepatocellular carcinoma. The correlation index ( $r$ ) and 2-sided  $P$  value were calculated by Spearman correlation analysis. AFP:  $\alpha$ -Fetoprotein; DCP: Des- $\gamma$ -carboxy-prothrombin; PNA: Preneoplastic antigen.

## ARTICLE HIGHLIGHTS

### Research background

The prognosis of hepatocellular carcinoma (HCC) patients remains poor despite advances in treatment modalities and diagnosis. The early detection of HCC allows patients to receive curative treatment and achieve long-term survival. It is important to identify useful markers for the early detection of HCC in patients.

### Research motivation

Preneoplastic antigen (PNA), originally reported in a rat carcinogenesis model, is increased in the tissues and serum of HCC patients. However, the diagnostic value of PNA for discriminating HCC remains to be determined.

### Research objectives

The objectives of this study are to determine the diagnostic value of PNA for discriminating HCC and to characterize PNA-positive HCC.

### Research methods

Patients with hepatitis C virus-related hepatic disorders were prospectively enrolled in this study, which included patients with hepatitis, with cirrhosis, and with HCC. A novel enzyme-

linked immunosorbent assay was developed to measure serum PNA concentrations in patients.

### Research results

Compared with control and non-HCC patients, PNA was increased in HCC. The sensitivity of PNA was similar to the HCC markers des-γ-carboxy-prothrombin (DCP) and α-fetoprotein (AFP), but the specificity of PNA was lower. There was no correlation between PNA and AFP and a significant but weak correlation between PNA and DCP in HCC patients. Importantly, the correlations with biochemical markers indicated that GTP was highly correlated with PNA, but not with AFP or DCP, and that it was significantly higher in PNA-high patients than in PNA-low patients with hepatitis C virus-related HCC.

### Research conclusions

PNA may have the potential to diagnose a novel type of HCC in which GTP is positively expressed but AFP or DCP is weakly or negatively expressed.

### Research perspectives

Further studies are needed to determine the diagnostic value of PNA for HCC patients in comparison with other HCC markers and to determine its potential for the detection of HCC nodules in the early stage. PNA-positive HCC may have an aggressive phenotype with a higher risk for disease progression and a poor prognosis. The prognostic value of PNA for survival will be determined in a follow-up evaluation of the patients enrolled in this study.

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