博士論文

Clinical effectiveness of tumor marker des-gamma-carboxyprothrombin in Chinese patients with hepatocellular carcinoma

(中国人肝細胞癌患者における腫瘍マーカー異常プロトロンビンの有効性に関する研究)

宋　培培

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ABSTRACT

Background and Aim: Although des-γ-carboxy prothrombin (DCP) is known to be an useful serum biomarker for the diagnosis of hepatocellular carcinoma (HCC) and could serve as a preoperative indicator in assessing HCC progression, the clinical usefulness of DCP in Chinese patients with HCC predominantly caused by hepatitis B virus (HBV) infection has yet been fully confirmed. This large-scale, multi-center case-controlled study aimed to ascertain the clinical utility of DCP in HCC diagnosis as well as the relation between DCP expression and the prognosis for Chinese HCC patients.

Methods: Subjects were 1,153 individuals from three major hospitals in China, including 550 cases in HCC group, 164 cases in Malignant disease group, 181 cases in Benign disease group, 85 cases in Chronic liver disease group, and 173 cases in Normal group. Serum levels of DCP and AFP were measured and clinicopathological features were determined for all subjects. The variables of tumor number, tumor differentiation, microvascular invasion, satellite node, and TNM stage from 112 HCC cases were also collected.

Results: The levels of DCP and AFP were significantly higher in HCC group (550 patients, 74.18% with HBV infection) than that in other four groups ($P < 0.001$). Receiver operating curves (ROC) indicated the optimal cut-off value was 86 mAU/mL for DCP with a sensitivity of 71.50% and specificity of 86.30%, and 21 ng/mL for AFP with a sensitivity of 68.00% and specificity of 93.20%. The area under ROC curve was 0.846 for DCP, 0.832

for AFP, and 0.890 for the combination of DCP and AFP. The combination of DCP and AFP resulted in a higher Youden index and a sensitivity of approximately 90%, even for small tumors. Furthermore, the high level of serum DCP was significantly associated with larger tumor size, poorly differentiated tumor, presence of microvascular invasion, more advanced TNM stage, or presence of tumor recurrence. The 3-year survival for HCC patients with high serum DCP level was significantly poorer than those with low serum DCP levels (3-year survival rate: 54.53% *vs.* 81.82%, $P = 0.007$).

Conclusions*:* The simultaneous measurement of DCP and AFP could achieve a better sensitivity in diagnosing Chinese HCC patients, even for small tumors. Serum DCP could serve as a preoperative indicator in assessing progression for Chinese patients with HCC. To improve the ability of serum biomarkers in HCC diagnosis and prognosis, the combined testing of DCP and AFP is suggested to be widely used in China.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most prevalent malignancy and the second leading cause of cancer deaths worldwide, with an estimated global incidence of 782,000 new cases and nearly 746,000 deaths in 2012 (1, 2). HCC is prevalent in Eastern and South-Eastern Asia, with an incidence of 31.9/100,000 and 22.2/100,000, where the major risk factor is hepatitis B virus (HBV) (1, 2). Of particular note is the fact that China alone accounts for 50% of HCC cases worldwide, with a total prevalence of 26-32/10,000 and a prevalence as high as 70-80/10,000 in some areas (3, 4).

HCC is one of the most difficult to treat malignances. Surgical resection and liver transplantation are thought to be curative therapies for HCC. However, fewer than 30% of all patients are eligible candidates due to poor liver function, advanced disease at the time of diagnosis, limited donor livers, or a combination of those factors (5, 6). For intermediate-to-late stage HCC, locoregional therapies including transcatheter arterial chemoembolization (TACE) and radiofrequency ablation (RFA) are available but suited to a limited portion of patients with liver tumors qualifying for palliative care (7). To achieve the most from available therapies, the most promising strategy is to diagnose and treat high-risk populations early on since HCC might be detected in an early curable stage and result in long-term survival - evidence showed that the normal overall 5-year survival rate for patients with HCC was 40%, but with a liver resection to treat early HCC, the 5-year survival rate could rise to 60-70% (8, 9).

In order to determine how HCC is detected in its early stages worldwide, we made a systematically literature reviewed in 2012 consisting of 3,008 papers included in the

PubMed database from 2001 to 2011 (10, 11). The characteristic 17 guidelines for HCC management worldwide have also been analyzed, including 5 guidelines from the America, 7 from Asia, and 5 from Europe (Table 1) (6, 12-27). Results of literature analysis showed that surveillance and early diagnosis for high-risk patients with HBV- or HCV-related chronic liver diseases could improve the rate of early HCC detection and the rate of curative treatment, which had been confirmed by several cohort studies and recommended by the guidelines for HCC (28-30). For the diagnostic tools, worldwide, while imaging diagnostic tools are widely used in Western countries, the serum biomarkers are still regarded as useful tools for HCC early diagnosis in Asian countries (Figure 1) (10) (31, 32).

In China, the most prevalent early diagnostic tools have been serum alpha-fetoprotein (AFP) and ultrasonography until now (Table 2) (10, 31, 33-36). AFP is the serum biomarker most widely used in China since 1970s, and its clinical usefulness was confirmed by a randomized controlled trial of 18,816 Chinese patients in 2004 (9). However, AFP levels are normal in up to 40% of patients with HCC, particularly during the early stage of the disease (low sensitivity) (37, 38), and elevated AFP levels are seen in patients with cirrhosis or exacerbation of chronic hepatitis (low specificity) (39, 40). Furthermore, some studies have indicated that AFP has substantially limited diagnostic accuracy in detecting small HCC (41). Thus, other reliable serum biomarkers need to be identified to complement AFP in order to improve clinical outcomes for patients.

Worldwide, a number of studies have looked at des-gamma-carboxy prothrombin (DCP), also known as prothrombin induced by vitamin K absence-II (PIVKA-II). Numerous studies have found that the combined testing of DCP and AFP has a sensitivity of 47.5-94.0% and a specificity of 53.3-98.5% in HCC early diagnosis, and these figures

Table 1. Characteristics of current 17 guidelines for HCC around the world

Areas	Guidelines/Approach	Content	Evaluation measures	Draft by
America				
2005	AASLD Guideline/LA (6)	D&T + S	evidence categories and recommendation grades; dissemination evaluation	American Association for the Study of Liver Disease
2007	ACS Guideline/EC (12)	D&T	—	American College of Surgeons
2009	NCCN Guideline/EC (13)	D&T + E + S	consensus categories	National Comprehensive Cancer Network
2010	WGO Guideline/EC (14)	D&T + E + P + S	resource-based recommendations	World Gastroenterology Organization
2010	NCI Guideline/EC (15)	D&T + E	—	United States National Cancer Institute
Asia				
2004	Korean Guideline/LA (16)	D&T	evidence categories and recommendation grades	Korean Liver Cancer Study Group and National Cancer Center
2005	J-HCC Guideline/LA†(17)	D&T + P + S	evidence categories and recommendation grades; dissemination evaluation draft; evaluation prior to publication	Japanese Ministry of Health, Labor, and Welfare
2006	SGA Guideline/LA (18)	D&T + E + P	evidence categories and recommendation grades	Saudi Gastroenterology Association
2007	JSH Guideline/EC (19)	D&T + S	question and answer analyser system	Japan Society of Hepatology
2009	AOS Guideline/EC (20)	D&T + P + S	evidence categories and recommendation grades; resource-based recommendations	Asian Oncology Summit 2009
2009	Chinese Guideline/EC (21)	D&T	—	Chinese Society of Liver Cancer, Chinese Society of Clinical Oncology, Chinese Society of Hepatology Liver Cancer Study Group
2010	APASL Guideline/EC† (22)	D&T + E + P + S	evidence categories and consensus grade	Asian-Pacific Association for the Study of the Liver
Europe				
2001	EASL Guideline/EC (23)	D&T + E + P + S	—	European Association for the Study of the Liver
2003	BSG Guideline/LA (24)	D&T + E + S	evidence categories and recommendation grades	British Society of Gastroenterology
2004	BASL Guideline/EC (25)	D&T + E + P + S	—	Belgian Association for the Study of the Liver
2008	ESMO Guideline/EC (26)	D&T + E + P + S + F	dissemination evaluation	European Society for Medical Oncology
2009	GOIM Guideline/EC (27)	D&T + E	—	Italian Southern Oncological Group

LA, literature analysis; EC, expert consensus; † Experts consist of radiologists, statisticians, and other experts besides hepatologists; the others were drafted by hepatologists; E, epidemiology; P, prevention; S, surveillance; D&T, diagnosis and treatment; E, epidemiology; P, prevention; S, surveillance; F, follow-up.

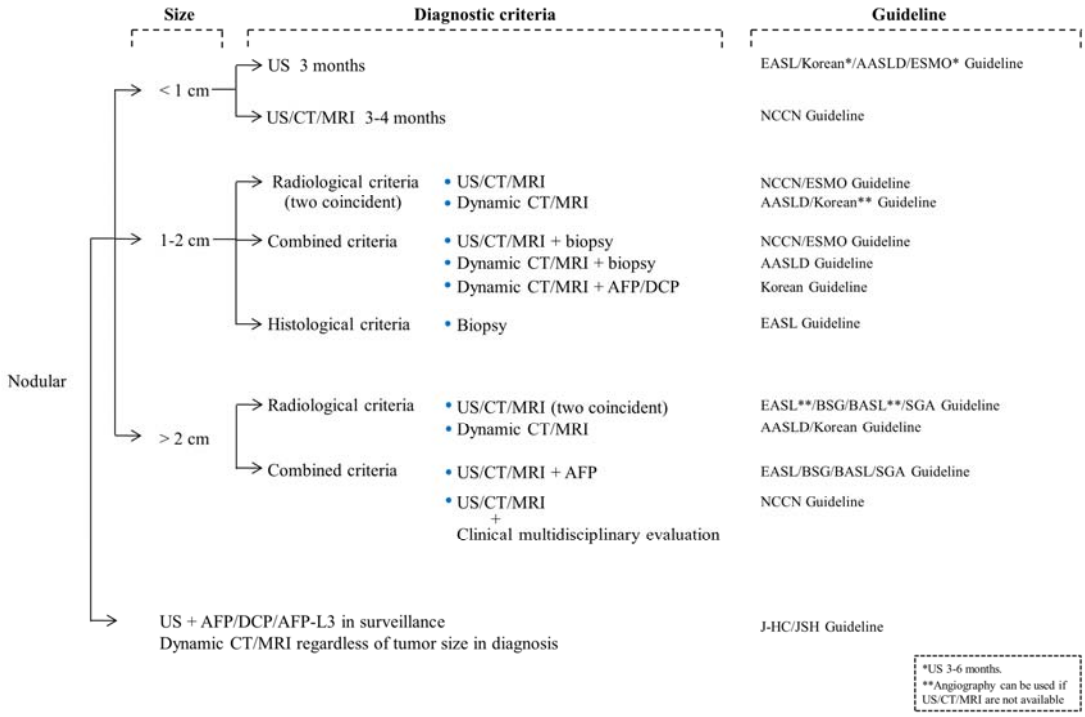

Figure 1. The diagnostic algorithm in current 17 guidelines for HCC management worldwide.

Worldwide, while imaging diagnostic tools are widely used in Western countries, the serum biomarkers are still regarded as useful tools for HCC early diagnosis in Asian countries. In Japan, US and AFP/DCP/AFP-L3 are recommended to be performed every 3-4 months for the highest-risk group (HBV- or HCV-related liver cirrhosis patients) and every 6-month for the high-risk group (patients with HBV- or HCV-related chronic liver disease or liver cirrhosis due to other causes). US, ultrasonography; CT, computed tomography; MRI, magnetic resonance imaging.

Table 2. The current status to screen for and diagnose HCC in China

Items	Current status in China	Ref.
Prevalence	The second most common cancer in urban areas and most common in rural areas; with an overall prevalence rate of 26-32/10,000, even up to 70-80/10,000 in some areas	(3, 4)
Mortality	The second leading cause of cancer-related deaths in males and the third leading cause of cancer-related deaths in females; with a total mortality rate of 26.26/100,000	(33)
Etiological factors	85% of patients with HBV infection, 10% of patients with HCV infection, and a small minority involve HBV and HCV	(3, 4)
Major at-risk population	People with HBV infection; 93 million HBV carriers, 20 million of these with chronic HBV infection	(33, 34)
Screening and Diagnostic algorithm	The test of ultrasonography and AFP every 6 months for the population ages 35-40 at risk for developing HCC	(10, 31)
Treatment algorithm	Comprehensive therapy predominantly in the form of surgery	(35)
Early detection	Most patients with HCC present with advanced-stage disease	(10, 36)

are higher than those for either marker alone (32, 42-46). Furthermore, several studies have showed the potential clinical usefulness of DCP as a preoperative indicator in assessing HCC progression and could also benefit post-treatment monitoring (47-51).

In Japan, DCP and AFP are widely and routinely used as serum biomarkers in HCC early diagnosis, which benefit more than 60% of patients that could be detected in the early stage (52), besides, using DCP as an indicator for HCC recurrence and post-treatment monitoring have also been widely used in clinical practice. However, DCP was just widely used in clinical practice in Japan and South Korea (53), yet has not been widely used clinically in China until now. Furthermore, unlike in Japan and Western countries, the main etiological factor for HCC in China is chronic infection with HBV, which accounts for 85% of all cases (3, 4). In order to assess the diagnostic value of DCP in Chinese patients with HCC, two studies published in 2002 (involving 60 patients with HCC and 30 patients with cirrhosis) (54) and 2003 (involving 120 patients with HCC and 90 patients with cirrhosis) (55) have indicated that the combined testing of DCP and AFP had a sensitivity of 78.3%, which is higher than that for DCP or AFP alone. However, the published studies were small in scale, and the prognostic value of DCP in Chinese patients predominantly caused by HBV infection has not been identified. Thus, the multiple-center studies of larger pools of serum samples from patients with HCC need to be conducted to provide further validation.

Given the rising incidence of HCC in China and the lack of substantial data on DCP's role as a serum biomarker in HCC diagnosis and prognosis for Chinese patients predominantly caused by HBV infection, we conducted this large-scale, multi-center case-controlled study to further investigate the clinical effectiveness of serum biomarker DCP for Chinese patients with HCC.

2. Patients and methods

2.1. Study population

The subject pool consisted of 1,153 cases from the Hepato-Biliary-Pancreatic Surgery Division at the Southwest Hospital of the Third Military Medical University, the Tianjin Medical University Cancer Hospital, and the 302 Military Hospital of China between 2001 and 2012 (Figure 2). This study was approved by institutional review boards, and clinicopathological information on each subject was collected.

Five groups of subjects were enrolled: *1*) HCC group, which involved HCC patients proved by pathology after hepatic resection; *2*) Malignant disease group, which involved patients with non-HCC malignant disease of the liver, bile ducts, or pancreas, including carcinoma of the gallbladder, cholangiocarcinoma, and pancreatic carcinoma underwent surgery; *3*) Benign disease group, which involved patients with benign disease of the liver, bile ducts, or pancreas, including cholangiolithiasis, cholecystitis, and hepatic cysts underwent surgery; *4*) Chronic liver disease group, which involved patients with progressivity of hepatitis or liver cirrhosis; and *5*) Normal group, which involved normal healthy subjects without finding of abnormity index by laboratory examination and imaging examination. None of cases in 5 groups received warfarin or other vitamin K inhibitor during the week prior to blood samples collection.

For patients in HCC group, HCC was clinically diagnosed based on the Chinese clinical guideline of HCC (10) and pathologic findings from resected specimens were confirmed for each patient. Information on demographic data and tumor characteristics

Figure 2. The source of subject pool in the present study.

The subject pool of 1,153 cases were from the Hepato-Biliary-Pancreatic Surgery Division at the Southwest Hospital of the Third Military Medical University in Chongqing, the Tianjin Medical University Cancer Hospital in Tianjin, and the 302 Military Hospital of China in Beijing between 2001 and 2012. All testing was conducted at the Southwest Hospital of the Third Military Medical University in Chongqing by the same group of laboratory technicians.

were recorded for each patient. No patients had received any previous therapy to treat HCC such as TACE, RFA, percutaneous ethanol injection (PEI), or resection and they underwent surgery for the first time at the Hospital. A detailed clinical evaluation was done before surgery for all patients.

For patients in Malignant disease group and Benign disease group, patients were diagnosed with either benign or malignant diseases of the liver, bile ducts, or pancreas other than HCC based on laboratory examination, imaging findings including those from ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI), as well as pathologic findings from resected specimens. Detailed diagnostic information was also recorded. All of the patients with HCC and patients without HCC were treated depending on their condition and stage of their disease.

For patients with progressivity of hepatitis or liver cirrhosis in Chronic liver disease group, HBV infection was diagnosed based on a positive result for hepatitis B surface antigen (HBsAg), and HCV infection was confirmed based on a positive result for anti-HCV and HCV RNA.

2.2. Blood samples

For patients in HCC group, Malignant disease group and Benign disease group, a 2-mL sample of peripheral blood was obtained within a week before surgery and immediately centrifuged into serum and plasma. Blood samples were also obtained from patients in Chronic liver disease group and healthy subjects in Normal group at the time of enrollment. The samples of serum and plasma were stored in aliquots in a refrigerator at -80°C until

testing.

2.3. Serological detection of DCP and AFP

Serum DCP levels were measured with an electrochemiluminescence immunoassay using a highly sensitive DCP determination kit (ED036, Eisai, Tokyo, Japan) in accordance with the manufacturer's instructions. The assay principle was showed in Figure 3. The range of detection was 10.00-200,000.00 mAU/mL. Serum AFP levels were tested using a commercial ELISA kit in accordance with instructions from the manufacturer (Biocell Biotech, Zhengzhou, China).

All testing was conducted at the Southwest Hospital of the Third Military Medical University by the same group of laboratory technicians, and none of technicians was informed of the subject’s status prior to testing.

2.4. Data collection and analysis

For 1,153 cases, the clinicopathological variables of age, gender, HBsAg, anti-HCV, levels of DCP and AFP, tumor size, and histological pathology were examined. To investigate the relationship between DCP expression and the prognosis for Chinese patients with HCC, the variables of tumor number, tumor differentiation, microvascular invasion, satellite node, and TNM stage from112 HBV-related HCC cases were also collected.

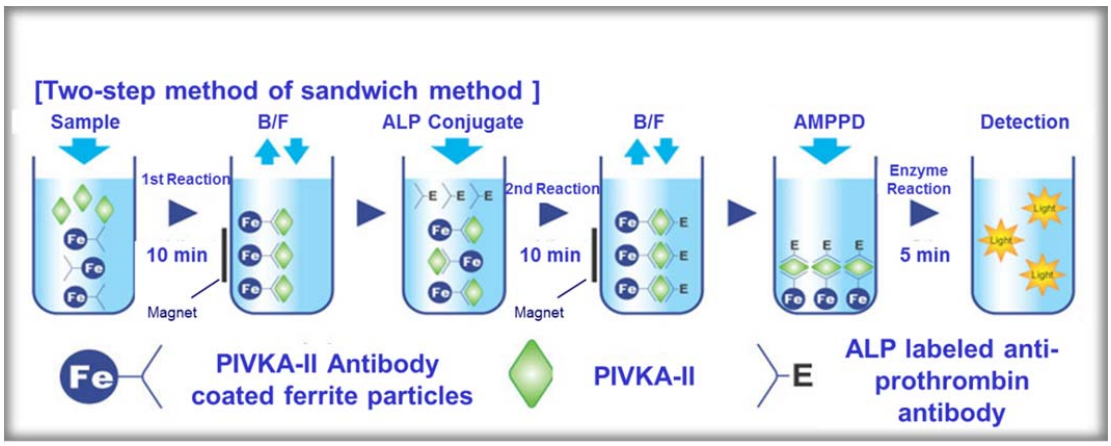

Figure 3. The assay principle of DCP (PIVKA-II).

Serum DCP levels were measured with an electrochemiluminescence immunoassay using a highly sensitive DCP determination kit (ED036, Eisai, Tokyo, Japan) in accordance with the manufacturer's instructions. The range of detection was 10.00-200,000.00 mAU/mL, using the two-step method of sandwich method. B/F, Bound/Free Separation; ALP, Alkaline phosphatase; ALP Conjugate, ALP labeled anti-prothrombin antibody; AMPPD, 3-(2‘-spiroadamantane)-4-methoxy-4-(3”-phosphoryloxy) phenyl-1,2-dioxetane salt.

Continuous variables were expressed as median (range) and compared between groups using the Wilcoxon rank-sum test. Categorical data were compared using the χ^2 test. Descriptive statistics for the transformed marker were compared using box plots and then using analysis of variance. Youden's index was calculated as an index of sensitivity and specificity.

To determine the optimal cut-off values for DCP and AFP to diagnose HCC, receiver operating characteristic (ROC) curves were created using all possible cut-offs for each assay. The area under ROC curves (AUROC) were also been calculated. A bivariate normal distribution for the two markers was assumed. A 2-tailed *P* value of < 0.05 was used to determine statistical significance.

To ascertain the prognostic value of DCP in Chinese patients with HCC predominantly caused by HBV infection, survival curves were calculated using the Kaplan-Meier method and compared to the results of the log-rank test. All variables that had *P* value of < 0.05 in univariate analysis were selected for multivariate analysis, which was performed using Cox's proportional-hazards model.

All statistical analyses were performed using the statistical software package SPSS® version 22.0 for Windows® (SPSS, Chicago, Illinois, USA).

3. Results

3.1. Clinical utility of simultaneous measurement of DCP and AFP in diagnosing patients with HCC among a total of 1,153 subjects

3.1.1. Baseline characteristics

As shown in Table 3, there were 550 cases in HCC group, 164 in Malignant disease group, 181 in Benign disease group, 85 in Chronic liver disease group, and 173 in Normal group.

Among a total of 1,153 cases, 876 cases (75.98%) were male and 277 (24.02%) were female, with a median age of 46 years (range: 12-83 years).

For the 550 patients with HCC, 74.18% (408 patients) were infected with HBV, which was significantly higher than that in patients with malignant disease and patients with benign disease ($P < 0.001$). Chronic liver disease group included 79 patients who were positive for HBsAg. Of 1,153 subjects in total, only 17 patients were positive for anti-HCV antibodies (10 cases in HCC group, 1 in Benign disease group, and 6 in Chronic liver disease group).

3.1.2. Results of DCP and AFP measurement

The median levels of DCP and AFP in patients with HCC were 516.50 mAU/mL (range: 10.00-200,000.00 mAU/mL) and 237.40 ng/mL (range: 0.24-1,939,000.00 ng/mL), which

Table 3. Laboratory results for five groups of subjects

Items	HCC group (*n* = 550)	Malignant Disease group (*n* = 164)	Benign disease group (*n* = 181*)	Chronic liver disease group (*n* = 85)	Normal group (*n* = 173)	*P***
Age (year) median (range)	51 (15-82)	56 (31-83)	50 (12-83)	32 (22-46)	28 (21-46)	
Gender (male / female)	480 / 70	110 / 54	84 / 97	70 / 15	132 / 41	
HBsAg						< 0.001
positive (cases)	408	43†[a]	44†[b]	79	0	
Anti-HCV						
positive (cases)	10	0	1‡	6	0	
DCP level (mAU/mL)						< 0.001
median	516.50	27.93	20.00	48.78	29.91	
minimum	< 10.00	< 10.00	< 10.00	22.20	< 10.00	
maximum	> 200,000.00	48,193.50	129,297.83	178.78	104.97	
AFP level (ng/mL)						< 0.001
median	237.40	2.81	2.30	7.00	6.00	
minimum	0.24	0.20	0.20	1.00	0.00	
maximum	1,939,000.00	3,098.00	1,082.20	25.00	30.00	

* HBsAg results were missing for 11 patients; †[a] including 6 patients with HBV-related cirrhosis proven by pathology, †[b] including 20 patients with HBV-related cirrhosis proven by pathology; ‡ including 1 patients with HCV-related cirrhosis proven by pathology. ** patients with HCC *vs.* the other four groups of subjects, respectively.

were significantly higher than those in the other four groups of subjects ($P < 0.001$) (Table 3). There was no significant correlation between serum levels of DCP and AFP ($R^2 = 0.154$) (Figure 4).

When a cut-off value of 40 mAU/ml for DCP, reported to be the upper limit of normal for Japanese subjects (56), was used, 82.91% of patients in HCC group, 38.31% of patients in Malignant disease group, 27.75% of patients in Benign disease group, 61.18% of patients in Chronic liver disease group, and 34.68% of subjects in Normal group had elevated DCP levels.

When using a cut-off value of 10 ng/mL for AFP as the upper limit of normal reported in Chinese subjects, 74.80% of patients in HCC group, 20.83% of patients in Malignant disease group, 12.50% of patients in Benign disease group, 28.24% of patients in Chronic liver disease group, and 30.06% of subjects in Normal group had elevated AFP levels (Figure 5).

3.1.3. Optimal cut-off values for DCP and AFP in differentiating patients with HCC from the other four groups of subjects studied

In order to determine optimal cut-off values that could balance the false-positive rate and the false-negative rate with the best positive predictive value (PPV) and negative predictive value (NPV) that could best distinguish patients with HCC from the other four groups of subjects, ROC curves were plotted for DCP and for AFP.

As shown in Figure 6, the optimal cut-off value for DCP was 86 mAU/ml, which

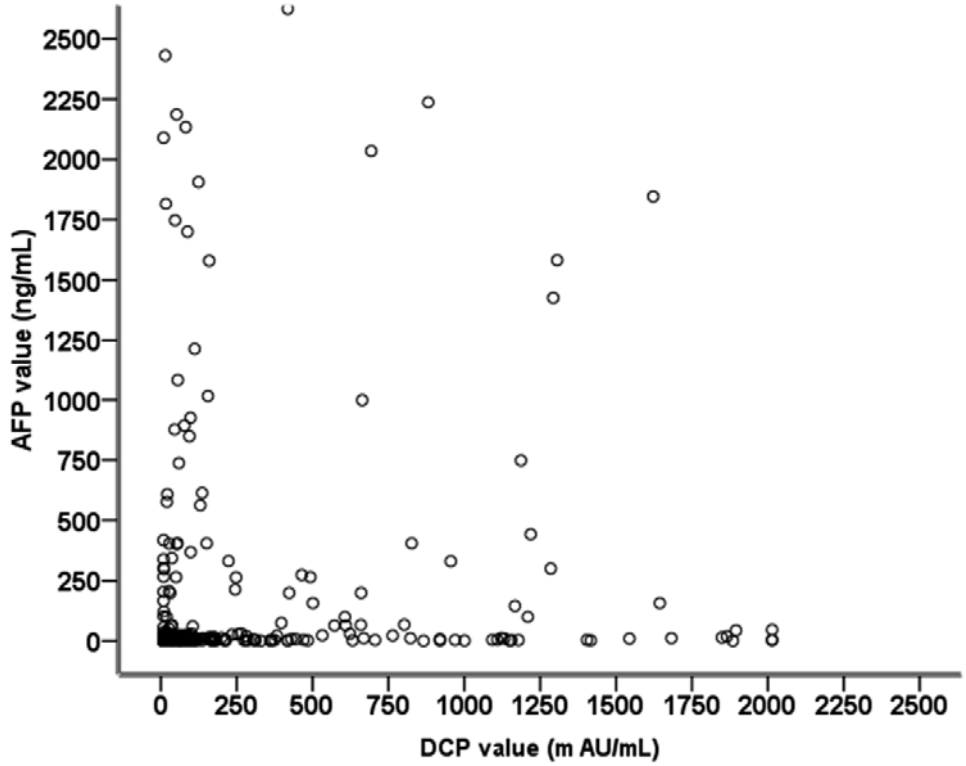

Figure 4. The correlation between serum levels of DCP and AFP.

Serum levels of DCP and AFP were plotted and their relation was compared. The median levels of DCP and AFP in patients with HCC were 516.50 mAU/mL (range: 10.00-200,000.00 mAU/mL) and 237.40 ng/mL (range: 0.24-1,939,000.00 ng/mL). There was no significant correlation between serum levels of DCP and AFP ($R^2 = 0.154$).

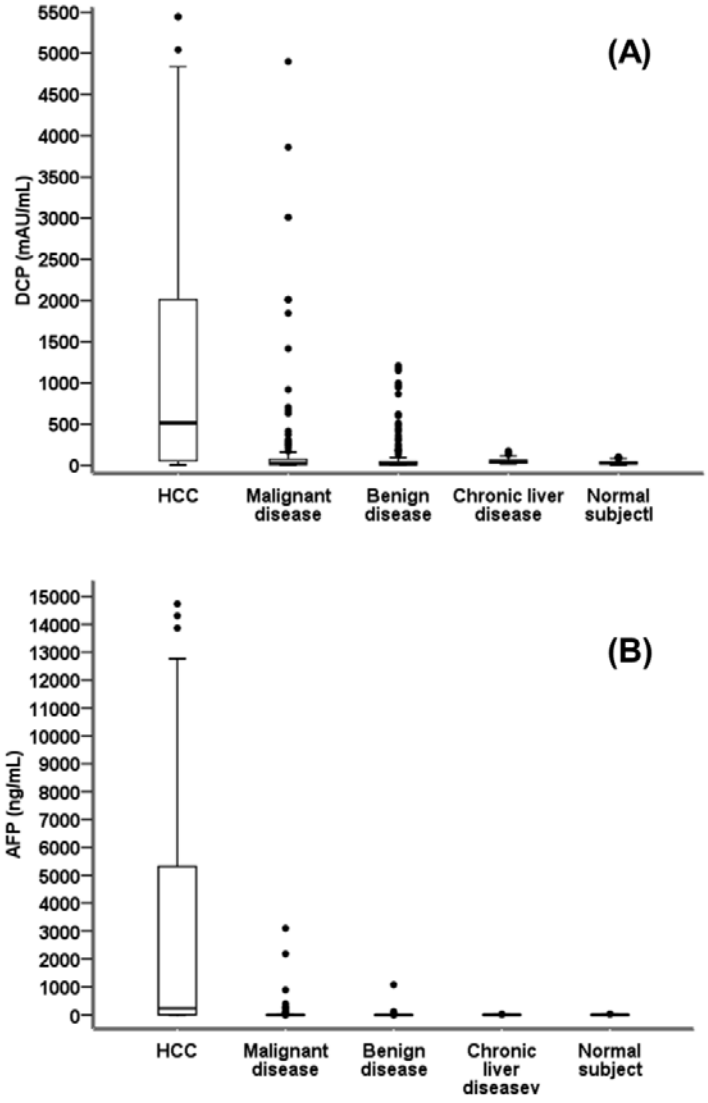

Figure 5. Scatter plot for DCP levels (A) and AFP levels (B) in 5 groups of subjects.

When a cut-off value of 40 mAU/ml for DCP, reported to be the upper limit of normal for Japanese subjects, was used, 82.91% of patients in HCC group had elevated DCP levels. When using a cut-off value of 10 ng/mL for AFP as the upper limit of normal reported in Chinese subjects, 74.80% of patients in HCC group had elevated AFP levels.

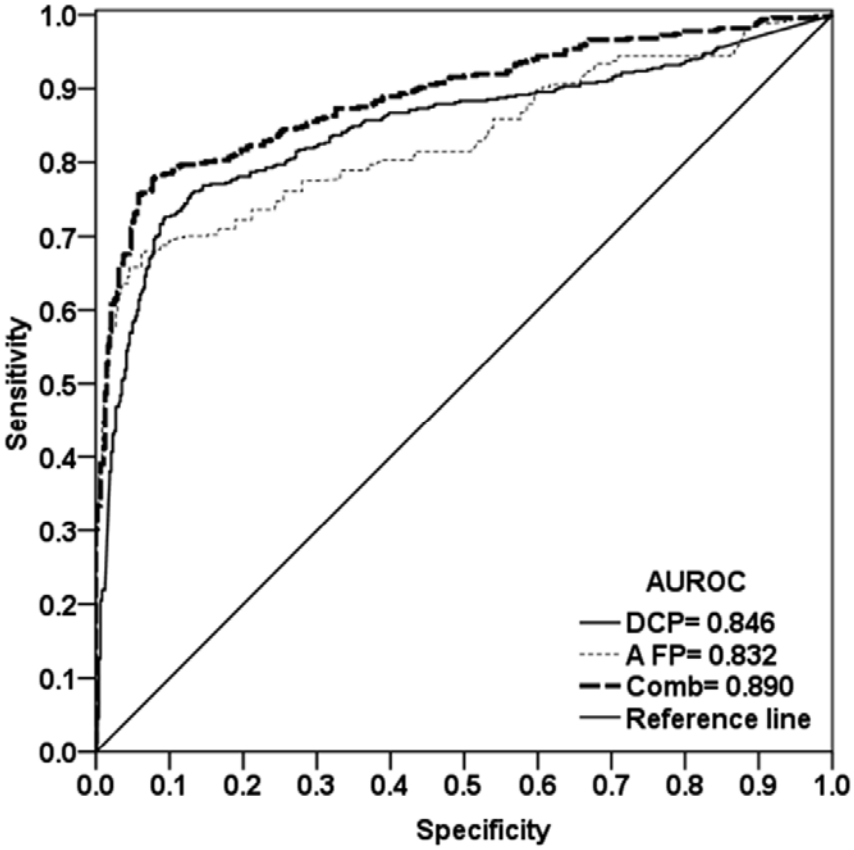

Figure 6. ROC curves comparing DCP and AFP levels in patients with HCC *vs.* patients without HCC.

The curves show an optimal cut-off value for DCP of 86 mAU/mL and for AFP of 21 ng/mL. The AUROC was 0.846 for DCP, 0.832 for AFP, and 0.890 for the combination of DCP and AFP. RCO, receiver-operating characteristic; AUROC, area under the ROC curve.

yielded a sensitivity of 71.50% and specificity of 86.30% in differentiating patients with HCC from the other four groups of subjects; the optimal cut-off value for AFP was 21 ng/mL, which yielded a sensitivity of 68.00% and specificity of 93.20% in differentiating patients with HCC from the other four groups of subjects. The AUROC was 0.846 (95% CI, 0.794-0.863, $P < 0.001$) for DCP, 0.832 (95% CI, 0.817-0.879, $P < 0.001$) for AFP, and 0.890 (95% CI, 0.869- 0.911, $P < 0.001$) for the combination of DCP and AFP.

When using DCP with the cut-off value of 86 mAU/ml, 71.45% of patients in HCC group, 22.08% of patients in Malignant disease group, 15.61% of patients in Benign disease group, 11.76% of patients in Chronic liver disease group, and 5.20% of subjects in Normal group had elevated DCP levels.

When using AFP with the cut-off value of 21 ng/mL, 68.01% of patients in HCC group, 9.03% of patients in Malignant disease group, 7.29% of patients in Benign disease group, 3.53% of patients in Chronic liver disease group, and 5.20% of subjects in Normal group had elevated AFP levels.

3.1.4. Sensitivity, specificity, and predictive values of DCP and AFP in differentiating patients with HCC from the other four groups of subjects studied

As shown in Table 4, DCP with a cut-off value of 86 mAU/mL had a high specificity and PPV but a lower sensitivity and NPV than a cut-off value of 40 mAU/mL. The Youden index for DCP with a cut-off value of 86 mAU/mL was 49.40% (HCC group *vs.* Malignant disease group), 55.90% (HCC group *vs.* Benign disease group), 58.70% (HCC group *vs.*

Table 4. The clinical utility of DCP and AFP with different cut-off values in differentiating HCC patients from other groups of subjects

DCP / AFP (cut-off value)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden Index (%)
DCP (86 mAU/mL)					
HCC group *vs.* Malignant disease group	71.50	77.90	92.04	43.32	49.40
HCC group *vs.* Benign disease group	71.50	84.40	93.57	48.18	55.90
HCC group *vs*. Chronic liver disease group	71.50	88.20	97.52	32.33	58.70
HCC group *vs*. Normal group	71.50	94.80	97.76	51.09	66.30
DCP (40 mAU/mL)					
HCC group *vs.* Malignant disease group	82.90	61.70	88.54	50.26	44.60
HCC group *vs.* Benign disease group	82.90	72.30	90.48	57.08	45.20
HCC group *vs*. Chronic liver disease group	82.90	38.80	89.76	25.98	21.70
HCC group *vs*. Normal group	82.90	65.30	88.37	54.59	48.20
AFP (10 ng/mL)					
HCC group *vs.* Malignant disease group	74.80	79.00	92.52	47.48	53.80
HCC group *vs.* Benign disease group	74.80	87.50	96.87	40.19	62.30
HCC group *vs*. Chronic liver disease group	74.80	71.80	93.92	32.80	46.60
HCC group *vs*. Normal group	74.80	69.90	87.71	49.19	44.70
AFP (21 ng/mL)					
HCC group *vs.* Malignant disease group	68.00	91.00	96.30	45.17	59.00
HCC group *vs.* Benign disease group	68.00	92.70	97.97	35.89	60.70
HCC group *vs*. Chronic liver disease group	68.00	96.50	99.12	34.02	64.50
HCC group *vs*. Normal group	68.00	94.80	97.40	50.77	62.80
AFP (400 ng/mL)					
HCC group *vs.* Malignant disease group	45.10	97.20	98.25	33.90	42.30
HCC group *vs.* Benign disease group	45.10	99.00	99.56	25.82	44.10
HCC group *vs*. Chronic liver disease group	45.10	100.00	100.00	23.74	54.90
HCC group *vs*. Normal group	45.10	100.00	100.00	38.79	54.90
AFP (21 ng/mL) + DCP (86 mAU/mL)					
HCC group *vs.* Malignant disease group	82.90	75.20	91.93	56.28	58.10
HCC group *vs.* Benign disease group	82.90	82.30	93.44	61.32	65.20
HCC group *vs*. Chronic liver disease group	82.90	84.70	97.23	43.37	67.60
HCC group *vs*. Normal group	82.90	90.80	96.61	62.55	73.70

PPV, positive predictive value; NPV, negative predictive value.

Chronic liver disease group), and 66.30% (HCC group *vs.* Normal group), which were higher than that of DCP with cut-off value of 40 mAU/mL.

As the cut-off AFP value increased from 10 ng/mL to 400 ng/mL, its specificity and PPV increased but its sensitivity and NPV decreased. The Youden index for AFP with a cut-off value of 21 ng/mL was 59.00% (HCC group *vs.* Malignant disease group), 60.70% (HCC group *vs.* Benign disease group), 64.50% (HCC group *vs.* Chronic liver disease group), and 62.80% (HCC group *vs.* Normal group), which were higher than those for AFP with a cut-off value of 10 ng/mL or 400 ng/mL. The combination of DCP with a cut-off value of 86 mAU/ml and AFP with a cut-off value of 21 ng/mL had a greater sensitivity and a higher Youden index than DCP or AFP alone in differentiating patients with HCC from the other four groups of subjects, regardless of other cut-off value chosen.

3.1.5. DCP and AFP levels for patients with HCC according to tumor size

As shown in Table 5, the median DCP level increased from 93.91 mAU/mL to 2,014.00 mAU/mL along with the enlargement of tumor size. For patients with a tumor > 10.0 cm, the median AFP level was 2,265.00 ng/mL, which was significantly higher than that in patients with a smaller tumor.

Among the 550 patients with HCC, 41 cases were with the tumor size of ≤ 2.0 cm, with the median values for DCP as 93.01 mAU/mL (range: 10-7,369.15 mAU/mL), and for AFP as 216.10 ng/mL (range: 0.24-59,615.00 ng/mL); 99 cases were with the tumor size of ≤ 3.0 cm, the median values for DCP as 134.59 mAU/mL (range: 10-46,825.61 mAU/mL), and for AFP as 297.63 ng/mL (range: 0.24-1,647,080.00 ng/mL); 205 cases were with the

tumor size of ≤ 5.0 cm, the median values for DCP as 280.87 mAU/mL (range: 10-200,000 mAU/mL), and for AFP as 206.40 ng/mL (range: 0.24-1,647,080.00 ng/mL).

3.1.6. The sensitivity of DCP and AFP in diagnosing patients with HCC according to tumor size

As shown in Table 6, the sensitivity of DCP with a cut-off value of 86 mAU/ml increased from 53.66% to 86.00% along the enlargement of tumor size. The combination of DCP with a cut-off value of 86 mAU/ml and AFP with a cut-off value of 21 ng/mL resulted in a sensitivity of approximately 90%, which was significantly higher than that of DCP or AFP alone.

For 41 cases with the tumor size of ≤ 2.0 cm, the combination of DCP with a cut-off value of 86 mAU/ml and AFP with a cut-off value of 21 ng/mL resulted in a sensitivity of 92.68%, which was higher than that of DCP (53.66%) or AFP (80.49%) alone ($P < 0.001$). For 99 cases with the tumor size of ≤ 3.0 cm, the combination of DCP and AFP with those cut-off values resulted in a sensitivity of 89.90%, which was higher than that of than that of DCP (62.63%) or AFP (77.78%) alone ($P < 0.001$). For 205 cases with the tumor size of ≤ 5.0 cm, the combination of DCP and AFP with those cut-off values resulted in a sensitivity of 88.78%, which was also higher than that of than that of DCP (68.78%) or AFP (67.80%) alone ($P < 0.001$).

Table 5. DCP and AFP levels in 550 patients with HCC according to tumor size

Size of tumor	Median		Minimum		Maximum	
	DCP (mAU/mL)	AFP (ng/mL)	DCP (mAU/mL)	AFP (ng/mL)	DCP (mAU/mL)	AFP (ng/mL)
≤ 2.0 cm	93.91	216.10	< 10.00	0.24	7,369.15	59,615.00
> 2.0 cm, ≤ 3.0 cm	191.82	391.93	< 10.00	1.83	46,825.61	1,647,080.00
> 3.0 cm, ≤ 4.0 cm	462.78	126.90	< 10.00	1.38	> 200,000.00	366,417.00
> 4.0 cm, ≤ 5.0 cm	556.88	174.00	< 10.00	0.82	111,170.15	1,193,000.00
> 5.0 cm, ≤ 10.0 cm	1,278.91	200.00	< 10.00	0.24	> 200,000.00	794,800.00
> 10.0 cm	2,014.00	2,265.00	< 10.00	1.00	> 200,000.00	1,939,000.00

Table 6. The sensitivity of DCP and AFP in the diagnosis of 550 patients with HCC according to tumor size

Size of tumor	DCP (86 mAU/mL) (%)	AFP (21 ng/mL) (%)	DCP (86 mAU/mL) + AFP (21 ng/mL) (%)
≤ 2.0 cm	53.66	80.49	92.68
> 2.0 cm, ≤ 3.0 cm	69.00	75.90	87.90
> 3.0 cm, ≤ 4.0 cm	76.50	58.80	88.20
> 4.0 cm, ≤ 5.0 cm	72.70	58.20	87.30
> 5.0 cm, ≤ 10.0 cm	80.50	65.80	88.40
> 10.0 cm	86.00	79.10	94.20

3.2. Relation between DCP expression and the prognosis for Chinese patients with HCC

3.2.1. General information

In order to ascertain the prognostic value of DCP in Chinese patients with HCC, we made a further analysis on 112 patients with HBV-related HCC who underwent surgical resection at the Hepato-Biliary-Pancreatic Surgery Division of Tianjin Medical University Cancer Hospital from February 2008 to October 2011. The date of prognosis for other patients with HCC that enrolled in the present study is now in collection.

Among 112 HCC cases, 95 were male and 17 were female with a median age of 54 years (range: 21-81 years),79 cases (70.54%) with the tumor size of > 3.0 cm, 90 cases (80.36%) with a single tumor, 67 cases (59.82%) with moderately differentiated tumor, 73 cases (65.18%) present microvascular invasion, 28 cases (25.00%) present satellite nodes, 33 cases (29.46%) with a more advanced TNM stage, and 52 cases (46.43%) present tumor recurrence (Table 7).

3.2.2. Serum DCP level and its relationship to clinicopathological characteristics

Serum DCP level was determined in each of the 112 HCC patients, with a median value of 468.56 mAU/mL (range: 10.00-200,000.00 mAU/mL). Seventy-five of 112 patients (66.96%) showed serum DCP level of > 86 mAU/mL, which was identified as the optimal cut-off value in differentiating patients with HCC from the other four groups of subjects in

Table 7. Serum DCP level and clinicopathological characteristics in 112 patients with HBV-related HCC

Clinicopathological variables	DCP > 86 mAU/mL cases / total cases (%)	*P* value
Tumor size		
≤ 3.0 cm	15 / 33 (45.45%)	0.002
> 3.0 cm	60 / 79 (75.95%)	
Tumor number		
single	57 / 90 (63.33%)	0.085
multiple	18 / 22 (81.82%)	
Tumor differentiation		
well	10 / 19 (52.63%)	
moderate	46 / 67 (68.66%)	0.039*
poor	19 / 26 (73.08%)	
Microvascular invasion		
absent	9 / 39 (23.08%)	0.013
present	34 / 73 (46.58%)	
Satellite nodes		
absent	53 / 84 (63.09%)	0.132
present	22 / 28 (78.57%)	
TNM stage		
I+II	48 / 79 (60.76%)	0.031
III+IV	27 / 33 (81.82%)	
Recurrence		
absent	35 / 60 (58.33%)	0.035
present	40 / 52 (76.92%)	

*Showed statistically significant difference between the patients in poorly differentiated tumor group and the patients in well differentiated tumor group.

this study, and 37 of 112 patients (33.04%) showed DCP level of ≤ 86 mAU/mL.

As shown in Table 7, high serum DCP levels were significantly frequent in patients who were with larger tumor size (> 3.0 cm *vs.* ≤ 3.0 cm: 75.95% *vs.* 45.45%, P = 0.002), poorly differentiated tumor (poor *vs*. well: 73.08% *vs.* 52.63%, P = 0.039), presence of microvascular invasion (presence *vs.* absence: 46.58% vs 23.08%, P = 0.013), with a more advanced TNM stage (III+IV *vs*. I+II: 81.82% *vs.* 60.76%, P = 0.031), or presence of tumor recurrence (presence *vs.* absence: 76.92% *vs.* 58.33%, P = 0.035).

Besides, although the rate of patients with DCP > 86 mAU/mL was higher in multiple tumor group (81.82%) than that in single tumor group (63.33%), and higher in presence of satellite node group (78.57%) than that in absence of satellite node group (63.09%), there was no significantly statistic difference between the two groups, respectively.

3.2.3. The relationship between serum DCP level and postoperative survival

The relationship between high or low serum DCP levels (cut-off levels at 86 mAU/mL) and patient survival was analyzed. As shown in Figure 7, HCC patients with high serum DCP level showed significantly poorer prognosis than those with low serum DCP levels: 3-year survival rates were 54.53% and 81.82%, respectively, as determined by the Kaplan-Meier method (P = 0.007 by the log-rank test).

Results of univariate analysis indicated that the 3-year survival was also significantly worse in patients with larger tumor size (> 3.0 cm *vs.* ≤ 3.0 cm: 64.02% *vs.*

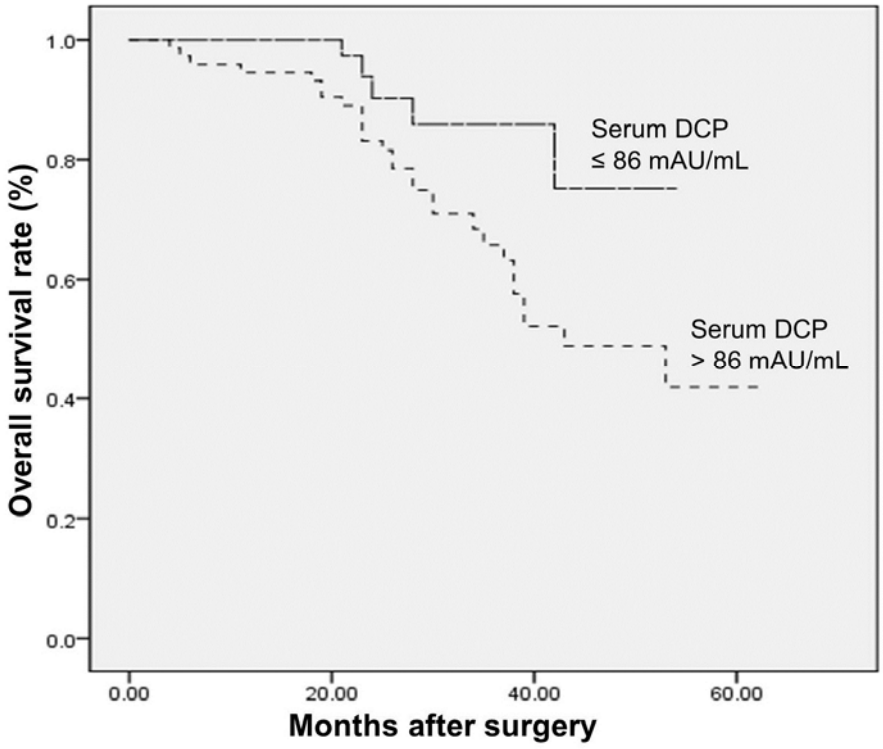

Figure 7. The curves of survival for 112 patients with HBV-related HCC according to serum DCP with cut-off value of 86 mAU/mL.

HCC patients with serum DCP level of > 86 mAU/mL showed significantly poorer prognosis than patients with serum DCP level of ≤ 86 mAU/mL (3-year survival rates: 54.53% *vs.* 81.82%, $P = 0.007$).

93.11%, $P = 0.020$), the presence of microvascular invasion (presence *vs.* absence: 61.02% *vs.* 85.91%, $P = 0.022$), a more advanced TNM stage (III+IV *vs.* I+II: 51.92% *vs.* 80.71%, $P < 0.001$), and the presence of tumor recurrence (present *vs.* absent: 50.23% *vs.* 78.45%, $P = 0.008$) (Table 8).

The 3-year survival rate of patients was not significantly different in the classification of serum AFP level (≤ 21 ng/mL *vs.* > 21 ng/mL: 72.56% *vs.* 67.23%), tumor number (single *vs.* multiple: 74.64% *vs.* 62.23%), satellite node (absence *vs.* presence: 73.43% *vs.* 70.52%) and tumor differentiation (well *vs.* moderate *vs.* poor: 82.80% *vs.* 71.91% *vs.* 68.43%).

All of above items with $P < 0.05$ in the univariate analysis were selected as variables for inclusion in the multivariate regression analysis by using Cox proportional hazard model. As shown in Table 9, after multivariate analysis, DCP > 86 mAU/mL (HR: 2.165, 95% CI: 1.048-4.521, $P = 0.047$), the presence of microvascular invasion (HR: 1.742, 95% CI: 1.016-4.326, $P = 0.048$) and a more advanced TNM stage (HR: 2.316, 95% CI: 1.125-4.770, $P = 0.023$) were identified as statistically significant risk factors for 3-year survival.

Table 8. Univariate analysis on risk factors of 3-year survival for112 patients with HBV-related HCC

Variables	cases	3-year survival rates (%)	*P* value
Serum DCP level			
≤ 86 mAU/mL	37	81.82	0.007
> 86 mAU/mL	75	54.53	
Serum AFP level			
≤ 21 ng/mL	41	72.56	0.112
> 21 ng/mL	71	67.23	
Tumor size			
≤ 3.0 cm	33	93.11	0.020
> 3.0 cm	79	64.02	
Tumor number			
single	90	74.64	0.132
multiple	22	62.23	
Tumor differentiation			
well	19	82.80	0.251
moderate	67	71.91	
poor	26	68.43	
Microvascular invasion			
absent	69	85.91	0.022
present	43	61.02	
Satellite nodes			
absent	84	73.43	0.139
present	28	70.52	
TNM stage			
I+II	79	80.71	< 0.001
III+IV	33	51.92	
Recurrence			
absent	60	78.45	0.008
present	52	50.23	

Table 9. Multivariate analysis on risk factors of 3-year survival for112 patients with HBV-related HCC

Variables	Hazard ratio (95% CI)	*P* value
Serum DCP level		0.047
≤ 86 mAU/mL *vs.* > 86 mAU/mL	2.165 (1.048-4.521)	
Microvascular invasion		0.048
absent *vs.* present	1.742 (1.016-4.326)	
TNM stage		0.023
I+II *vs.* III+IV	2.316 (1.125-4.770)	

4. Discussion

The present study is the first large-scale, multi-center case-controlled research conducted in China to investigate the clinical effectiveness of serum biomarker DCP for Chinese patients with HCC predominantly caused by HBV infection. Results indicated that DCP could be a good candidate as a compliment to AFP in diagnosing Chinese patients with HCC, with a sensitivity of approximately 90% for the combined testing of DCP and AFP, even for small tumor size. Besides, the relation between DCP expression and the prognosis for Chinese patients with HCC has also been confirmed by the present study, which suggested that serum DCP could serve as a preoperative indicator in assessing progression for Chinese patients with HCC.

Furthermore, this study not only provided evidence on the clinical effectiveness of serum DCP in HCC diagnosis and prognosis for Chinese patients with HCC, but also promoted DCP to be used in actual clinical practice. With the impetus of this Japan-China joint research project, currently, DCP has been used in actual clinical practice in some hospitals of China since DCP approved to be used in China in 2014. With the increased application in clinical practice, the test of DCP is expected to be routinely used to improve clinical outcomes for patients with HCC in China.

In China, HCC has currently become the second leading cause of cancer-related deaths in men and the third leading cause of such deaths in women, and its incidence has increased in the past few decades as a result of the high prevalence of its main etiological factor, chronic HBV infection (3, 4). People with HBV infection are the largest population at risk of developing HCC in China. In fact, 93 million HBV carriers are Chinese,

accounting for 2/3 of such patients worldwide, and about 20 million of these people have chronic HBV infection (33, 34). Evidence has shown that surgical resection and liver transplantation may offer the best opportunity for treating HCC yet are only available to early-detected patients (5, 6, 8, 9). During the past decades, as clinical techniques have developed in China, new techniques in treatment have also become available, such as laparoscopic surgery and minimally invasive robotic surgery. However, most HCC patient in China still suffered from advanced-stage disease, thus reducing the chance of curable treatment (57, 58). Therefore, strategies to diagnose HCC at an earlier stage are urgently needed in China when curable interventions can be offered to achieve long-term disease-free survival for Chinese patients with HCC.

The diagnosis tools should have an acceptable accuracy, accessibility, and affordability. In general, the tests used to diagnose HCC around the world include imaging diagnosis, serological diagnosis, and histological diagnosis. Among of them, a biopsy (also known as fine needle aspiration cytology, FNAC) has an overall sensitivity and specificity of 95.2% and 100%, but biopsy tests should be avoided if curative surgery is planned because the chance of needle track tumor seeding following a biopsy is 2.7% unless such a biopsy might change management of the patient or the major diagnostic doubt persists that cannot be resolved with imaging techniques or serological diagnosis (10).

Diagnostic imaging techniques include ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI), with a respective sensitivity of 60% (95% CI: 44-76%), 68% (95% CI: 55-80%) and 81% (95% CI: 70-91%) and a respective specificity of 97% (95% CI: 95-98%), 93% (95% CI: 89-96%) and 85% (95%CI: 77-93%) (10, 31, 33). Currently, ultrasound is the most common imaging tool used in HCC

diagnosis in China thanks to its features of simple, inexpensive, non-invasive, and allows real-time observation. However, the successful ultrasound detection relies on the available of ultrasound equipment, the expertise of the physician, and the echo texture of the liver. So the actual sensitivity and specificity of ultrasonography is difficult to evaluate due to the lack of standard in China.

Serum biomarkers are attractive potential tools for HCC early diagnosis because they would enable non-invasive, objective, and reproducible assessments (59). According to the Chinese Guidelines on HCC, serum AFP measurement and ultrasonography are recommended to be performed every 6 months for the people ages between 35 and 40 at risk for developing HCC (21). However, it should be noted that the disadvantage of low sensitivity, low specificity, and limited accuracy for AFP in detecting small HCC diminish its clinical utility in HCC early diagnosis (37-41). Thus, other reliable serum biomarkers need to be identified to complement AFP in order to improve clinical outcomes for patients with HCC in China.

Recent years have also seen many studies on the clinical usefulness of other serum biomarkers in detecting HCC early, including Golgi protein-73 (GP73), glypican-3 (GPC3) and gamma-glutamyltransferase (GGTII). Most recently, research on Dickkopf-1 (DKK1) and Midkine (MDK) as diagnostic serum biomarkers has garnered interest, which suggested that the novel serum biomarkers DKK1 and MDK can augment the measurement of AFP when diagnosing HCC, and particularly when diagnosing patients who are negative for AFP and/or who have HCC in an early stage (60, 61). However, these studies were small in scale and involved few patient, more such studies are needed before they can be included as valid biomarkers in strategies to diagnose patients who present with liver

masses. By contrast, the clinical usefulness of serum DCP in HCC diagnosis has been confirmed by a series of studies in many countries, especially in Japan and some Western countries, where HCC is predominantly caused by HCV infection.

DCP is an abnormal prothrombin that lacks carboxylation of specific amino-terminal glutamic acid residues. Since Liebman *et al.* found DCP to be a useful serum marker in diagnosing HCC in 1984 (62), differences in the sensitivity and specificity of DCP and AFP have been extensively discussed (Table 10) (42, 43, 54, 55, 63-74). In 8 large case-controlled studies, serum DCP was found to have a sensitivity of 48-62%, a specificity of 81-98%, and a diagnostic accuracy of 59-84% in differentiating patients with HCC from those with cirrhosis; in comparison, serum AFP was found to have a sensitivity of 40-54%, a specificity of 88-97%, and a diagnostic accuracy of 64-76% (45, 75). Although several studies of the two tumor markers have been reported, results of those studies conflicted with regard to the relative performance of those markers. Some studies showed that DCP has greater sensitivity than AFP, while other studies found no significant difference in the sensitivity of the two serum markers, but the combination of DCP and AFP, however, appeared to have greater sensitivity than either marker alone (70, 75-77). These differences may be due to the use of different marker cut-off values in each study (40, 80, 100 mAU/mL or higher value for DCP and 10-400 ng/mL for AFP), differences in underlying liver disease, tumor stage, or other aspects.

The clinical utility of DCP in diagnosing HCC at a reference level of 40 mAU/mL has been established by a number of retrospective and prospective studies (78). However, most of these studies were completed in Japan or Western counties, where HCC is predominantly caused by HCV infection (78-80). In Japan, more than 70% of patients with

Table 10. The exploration of clinical usefulness of using serum biomarker DCP to complement AFP in HCC early detection[*]

Marker	Cut-off value	Sensitivity	Specificity	Ref.
DCP + AFP	8 mAU/mL, 20 ng/mL	90.0% (90/100)	*N*	(63)
DCP + AFP	16 mAU/mL, 20 ng/mL	87.3% (55/63)	84.0% (158/188)	(64)
DCP + AFP	40 mAU/mL, 20 ng/mL	83.5% (76/91)	*N*	(65)
DCP + AFP	40 mAU/mL, 20 ng/mL	86.7% (52/60)	*N*	(66)
DCP + AFP	40 mAU/mL, 20 ng/mL	78.3% (94/120)	58.9% (53/90)	(55)
DCP + AFP	40 mAU/mL, 20 ng/mL	83.6% (51/61)	68.2% (45/66)	(67)
DCP + AFP	40 mAU/mL, 20 ng/mL	83.3% (204/245)	77.2% (206/267)	(68)
DCP + AFP	40 mAU/mL, 200 ng/mL	78.3% (83/106)	*N*	(69)
DCP + AFP	80 mAU/mL, 40 ng/mL	65.5% (19/29)	84.5% (596/705)	(70)
DCP + AFP	90 mAU/mL, 45 ng/mL	84.4% (130/154)	*N*	(71)
DCP + AFP	100 mAU/mL, 100 ng/mL	72.4% (55/76)	*N*	(72)
DCP + AFP	100 mAU/mL, 300 ng/mL	63.2% (48/76)	*N*	(72)
DCP + AFP	150 mAU/mL, 20 ng/mL	86% (-/-) [†]	63% (-/-) [†]	(42)
DCP + AFP	619 mAU/mL, 27 ng/mL	74% (-/-) [†]	87% (-/-) [†]	(42)
DCP + AFP	0.8 ng/mL, 45 ng/mL	88.3% (136/154)	*N*	(71)
DCP + AFP	20.24 ng/mL, 15 ng/mL	94.0% (47/50)	80.5% (33/41)	(73)
DCP + AFP	0.1μg/mL, 20 ng/mL	92.9% (65/70)	53.3% (24/45)	(74)
DCP + AFP	0.1 mg/mL, 400 ng/mL	85.7% (60/70)	82.2% (37/45)	(74)
DCP + AFP	40 mAU/ml, 20 ng/ml	78.3% (47/60)	56.7% (17/30)	(54)
DCP + AFP	8 mAU/mL, 20 ng/mL	66.7% (18/27)	*N*	(63)
DCP + AFP	16 mAU/mL, 20 ng/mL	82.9% (29/35)	84.0% (158/188)	(64)
DCP + AFP	40 mAU/mL, 20 ng/mL	59.4% (-/-) [†]	58.9% (53/90)	(55)
DCP + AFP	150 mAU/mL, 20 ng/mL	78% (-/-) [†]	62% (-/-) [†]	(42)
DCP + AFP	598 mAU/mL, 11 ng/mL	70% (-/-) [†]	80% (-/-) [†]	(42)
DCP + AFP	16 mAU/mL, 20 ng/mL	61.5% (8/13)	84.0% (158/188)	(64)
DCP + AFP	40 mAU/mL, 20 ng/mL	83.7% (36/43)	*N*	(65)
DCP + AFP	40 mAU/mL, 200 ng/mL	47.5% (29/61)	98.5% (132/134)	(43)

[*] In all studies indicated, patients with chronic hepatitis and/or liver cirrhosis were designated as the comparative non-HCC patient group. Sensitivity = True positive (TP) / (TP + Falsenegative (FN)), Specificity = True negative (TN) / (TN + False positive (FP)). [†] The patient distribution was not noted. *N*, Not noted or not investigated.

HCC are infected with hepatitis C virus (HCV), and approximately 15-20% of patients are infected with HBV (81), these figures are similar to those reported from the United States and Europe (82, 83). According to HCC Guidelines in Japan, ultrasonography and the simultaneous measurement of DCP and AFP are recommended to be performed every 3-4 months for the highest-risk group (HBV- or HCV-related liver cirrhosis patients) and every 6-month for the high-risk group (patients with HBV- or HCV-related chronic liver disease or liver cirrhosis due to other causes) (17, 19). Currently, in Japan, DCP and AFP are widely and routinely used as serum biomarkers for HCC early diagnosis, which benefit more than 60% of patients that could be detected in the early stage (52), and these tests have been covered by Japan' national health insurance.

The diagnostic effectiveness of DCP in HBV-related HCC is required for the diagnostic marker used in China. The present study analyzed the clinical utility of simultaneous measurement of DCP and AFP in differentiating Chinese HCC patients (74.18% with HBV infection) from those patients with non-HCC and normal subjects. Among 1,153 cases in this study, 550 cases were patients with HCC; for the control groups, patients with non-HCC disease of the liver, bile ducts, or pancreas underwent surgery – 164 cases in Malignant disease group and 181 cases in Benign disease group – were enrolled; besides, we also collected the blood samples and information from 85 patients in Chronic liver disease group, as well as 173 volunteers of healthy subject in Normal group as controls. Results showed that DCP was an useful serum biomarker in diagnosis for Chinese patients with HCC predominantly caused by HBV infection; for the cases enrolled in our study, DCP with a cut-off value of 86 mAU/mL had a high specificity, PPV, and Youden index than a cut-off value of 40 mAU/mL; the combined testing of DCP with a cut-off

value of 86 mAU/ml and AFP with a cut-off value of 21 ng/mL resulted in a greater sensitivity and higher Youden index than DCP or AFP alone in differentiating patients with HCC from the other four groups of subjects, which suggest that DCP could be a good candidate as a compliment to AFP in HCC diagnosis for Chinese patients predominantly caused by HBV infection.

Some studies have reported that the serum levels of DCP increase in relation to the size of HCC (76, 84, 85), it was also shown in our study that for patients with HCC, the median DCP level increased with a larger tumor, and the sensitivity of DCP with a cut-off value of 86 mAU/ml increased from 53.66% to 86.00% along the enlargement of tumor size. For the relation between AFP levels and tumor size, although the cases with tumor size of ≤ 2.0 cm and cases of 2-3 cm showed a relative high AFP level due to the classification difference, but in general, for cases ≤ 10.0 cm, the median AFP level was within 400 ng/mL (216.10 ng/mL for 41 cases ≤ 2.0 cm, 297.63 ng/mL for 99 cases ≤ 3.0 cm, 206.40 ng/mL for 205 cases ≤ 5.0 cm). In our study, the higher level of AFP was mainly in larger tumor size, showed a median AFP level of 2,265.00 ng/mL for patients with a tumor > 10.0 cm, which was significantly higher than that in patients with a smaller tumor, it was consistent with previous research findings. Likewise, although the sensitivity of AFP showed a relative high value for cases of ≤ 2.0 cm and cases of 2-3 cm due to the classification difference, in general, the sensitivity of AFP with a cut-off value of 21 ng/mL was 67.80% for 205 cases ≤ 5.0 cm. In the present study, the combination of DCP with a cut-off value of 86 mAU/mL and AFP with a cut-off value of 21 ng/mL resulted in a sensitivity of approximately 90%, which was significantly higher than that for DCP or AFP alone. The same was true even for a tumor smaller than 2.0 cm. These results suggest that the

simultaneous measurement of AFP and DCP may facilitate the diagnosis of patients with a broad range of HCC.

Besides the investigation on using DCP as a useful serum biomarker for HCC diagnosis, several studies have also showed the association between elevated level of serum DCP and a poor clinical outcome in HCC patients (69, 86-88). Results indicated that DCP was clinically effective as a serum biomarker for the prediction of patient prognosis (75, 89-91). Shirabe *et al.* examined 218 HCC patients who underwent surgical resection for HCC and concluded that serum DCP level is a predictor of microvascular invasion (92); Shimada *et al.* examined reported that serum DCP level is associated with vascular invasion and HCC recurrence (93); Inagaki *et al.* reported that patients with a high serum DCP level (5-year survival rate of 25.95%) had a significantly worse prognosis than those with a low serum DCP level (5-year survival rate: 25.95% *vs.* 55.56%; $P = 0.002$) (94). Some other studies have also showed serum DCP levels correlated significantly with clinicopathological factors of TNM stage, tumor size, and tumor differentiation (47, 89, 95).

However, all the studies mentioned above were focusing on HCV-related HCC, there was few report about the relationship between serum DCP level and HBV-related HCC. Focusing on the prognostic value of DCP in Chinese patients with HBV-related HCC, the present study indicated that high serum DCP levels were significantly frequent in patients who were with larger tumor size, poorly differentiated tumor, presence of microvascular invasion, more advanced TNM stage, or presence of tumor recurrence. In our study, the 3-year survival for HCC patients with high serum DCP levels was significantly poorer than that those with low serum DCP levels (3-year survival rate: 54.53%

vs. 81.82%, $P = 0.007$). These results suggest that DCP can be used for evaluation of HBV-related HCC prognosis and support the decision of treatment strategy.

There are some limitations in the present study. First, this study did involve a relatively large number of patients with HCC, but all of those patients underwent curative surgery. Hence, patients with HCC in a more advanced stage were not included in this study. Besides, very few cases in Malignant disease group and Benign disease group showed a high DCP level, although they have been confirmed as non-HCC cases based on laboratory examination, imaging findings, pathologic findings from resected specimen, and none of cases received warfarin or other vitamin K inhibitor during the week prior to blood samples collection, but the mechanism research on the relation between high level of DCP and disease status for these cases need to be further investigated. Second, although the relation between DCP expression and the prognosis for Chinese patients with HCC has also been confirmed by the present study, the date were from a small subjects group that involving 112 HBV-related HCC cases, the date of prognosis for other patients with HCC that enrolled in the present study is now in collection. Third, besides the clinical effectiveness of serum DCP in HCC diagnosis and prognosis, DCP has also been routinely used for the screening and post-treatment monitoring for HCC patients in some countries, such as Japan. For the clinical effectiveness of serum biomarker DCP as a screening tool and post-treatment monitoring tool for Chinese patients with HCC, more large-scale national prospective studies should be conducted to complement to this study and provide sufficient evidence.

5. Conclusion

Evidences have showed that early diagnosis of HCC is essential when curative interventions can be implemented to improve patients' prognosis and long-term survival. Due to the fact that there is greatly large number of HBV carrier in China and those people are expected to be affected chronic viral hepatitis, liver cirrhosis and HCC, therefore, the early diagnosis for Chinese HCC patients predominantly caused by HBV infection has important implications not only for China, but also for the worldwide to reduce the burden of disease.

In China, AFP as a serum biomarker has been widely used in clinical practice, but its disadvantage of low sensitivity, low specificity, and limited accuracy in detecting small HCC diminish its clinical utility in HCC early diagnosis. Thus, other reliable serum biomarkers need to be identified to complement AFP in order to improve clinical outcomes for patients with HCC in China.

Worldwide, although DCP is known to be useful serum biomarker for the diagnosis of HCC and could also serve as a preoperative indicator in assessing HCC progression, the clinical usefulness of DCP in Chinese patients with HCC predominantly caused by HBV infection has yet been fully confirmed.

This large-scale, multi-center case-controlled study involving 1,153 Chinese cases indicated that the simultaneous measurement of DCP and AFP could achieve a better sensitivity in diagnosing Chinese patients with HCC predominantly caused by HBV infection. The sensitivity of combined testing of two markers was significantly higher than that of either marker alone, the same was true even for a tumor smaller than 2.0 cm. To

improve the diagnostic ability of serum biomarkers for HCC in China, the combined usage of DCP and AFP is suggested by this study.

Furthermore, the relation between DCP expression and the prognosis for Chinese patients with HCC has also been confirmed by the present study, which suggested that serum DCP could serve as a preoperative indicator in assessing progression for Chinese patients with HCC.

With the impetus of this Japan-China joint research project, currently, DCP has been used in actual clinical practice in some hospitals of China since DCP approved to be used in China in 2014. With the increased application in clinical practice, the test of DCP is expected to be routinely used in China, with the goal of not only improving clinical outcomes for Chinese patients with HCC, but also reducing the disease burden globally due to the fact that China alone accounts for 50% of HCC cases worldwide.

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