Clinical utility of simultaneous measurement of alpha-fetoprotein and des- γ -carboxy prothrombin for diagnosis of patients with hepatocellular carcinoma in China: A multi-center case-controlled study of 1,153 subjects

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Summary

This study aimed to investigate the clinical utility of simultaneous measurement of alpha-fetoprotein (AFP) and des- γ -carboxy prothrombin (DCP) for hepatocellular carcinoma (HCC) diagnosis in Chinese patients predominantly caused by hepatitis B virus infection by a multi-center case-controlled study. Subjects were 1,153 individuals from three major hospitals in China, including 550 cases in HCC group, 164 in Malignant disease group, 182 in Benign disease group, 85 in Chronic liver disease group, and 173 in Normal group. Serum levels of AFP and DCP were measured and clinicopathological features were determined for all subjects. Results showed that 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. The simultaneous measurement of AFP and DCP could achieve a better sensitivity in diagnosing Chinese HCC patients, even for small tumors.

Keywords: Serum biomarker, des- γ -carboxy prothrombin (DCP), alpha-fetoprotein (AFP), sensitivity, specificity

1. Introduction

Hepatocellular carcinoma (HCC) is 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). 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) (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).

While imaging diagnostic tools are widely used in Western countries, the serum biomarkers are still

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regarded as useful tools for HCC early diagnosis in Asian countries. In China, alpha-fetoprotein (AFP) is the serum biomarker most widely used in HCC early diagnosis (5), and its clinical usefulness was confirmed by a randomized controlled trial of 18,816 Chinese patients in 2004 (6). However, AFP levels are normal in up to 40% of patients with HCC, particularly during the early stage of the disease (low sensitivity) (7,8), and elevated AFP levels are seen in patients with cirrhosis or exacerbation of chronic hepatitis (low specificity) (9,10). Furthermore, some studies have indicated that AFP has substantially limited diagnostic accuracy in detecting small HCC (11). 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- γ -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 are higher than those for either marker alone (12-16). In Japan, DCP and AFP are widely and routinely used as serum biomarkers in HCC surveillance and diagnosis, which benefit the early diagnosis in more than 60% of patients (17). However, DCP is currently approved for use in Japan, South Korea, and Indonesia (18), yet has not been widely used in China. 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. 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) (19) and 2003 (involving 120 patients with HCC and 90 patients with cirrhosis) (20) 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 two studies were small in scale, 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 in Chinese patients, we conducted this large-scale, multi-center case-controlled study to further investigate the clinical utility of simultaneous measurement of AFP and DCP for HCC diagnosis in Chinese patients.

2. Materials 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. This study was approved by institutional review boards, and clinicopathological information on each subject was collected. Five groups of consecutive 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; 3) Benign disease group, which involved patients with benign disease of the liver, bile ducts, or pancreas, including cholangiolithiasis, cholecystitis, hepatic cysts; 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 risk factors for viral hepatitis. 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). None of cases received vitamin K during the week prior to inclusion in this study.

2.2. 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 range of detection was 10-200,000 mAU/mL. Serum AFP levels were tested using a commercial ELISA kit in accordance with instructions from the manufacturer (Biocell Biotech, Zhengzhou, China). For patients undergoing surgery, blood samples for measurement of DCP and AFP were obtained a week before surgery. Blood samples were spun, serum aliquoted, and stored at -80°C until testing. 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.

2.3. Data collection and analysis

Clinicopathological variables of age, gender, HBsAg, anti-HCV, levels of DCP and AFP, tumor size, and histological pathology were examined.

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

Table 1. 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**
Median age (range) (year)	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.

(ROC) curves were created using all possible cut-offs for each assay. A bivariate normal distribution for the two markers was assumed. A 2-tailed *p* value of < 0.05 was used to determine statistical significance. All statistical analyses were performed using the statistical software package SPSS[®] version 22.0 for Windows[®] (SPSS, Chicago, Illinois, USA).

3. Results

3.1. Baseline characteristics

As shown in Table 1, 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.

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).

The median levels of DCP and AFP in patients with HCC were 516.50 mAU/mL (range: 10-200,000 mAU/mL) and 237.40 ng/mL (range: 0.24-1,939,000 ng/mL), which were significantly higher than those in the other four groups of subjects ($P < 0.001$) (Table 1). There was no significant correlation between serum levels of DCP and AFP ($R^2 = 0.154$) (Figure 1). When a cut-off value of 40 mAU/ml, reported to be the upper limit of normal for Japanese subjects (21), 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

Figure 1. The correlation between serum levels of DCP and AFP ($R^2 = 0.154$).

group, and 34.68% of subjects in Normal group had elevated DCP levels. When using a cut-off value of 10 ng/mL 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 2).

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

ROC curves were plotted to identify a cut-off value that would best distinguish patients with HCC from the other four groups of subjects. As shown in Figure 3, the optimal cut-off value for DCP was 86 mAU/ml, which yielded a sensitivity of 71.50% and specificity

Figure 2. Scatter plot for DCP levels (A) and AFP levels (B) in 5 groups of subjects.

of 86.30%; the optimal cut-off value for AFP was 21 ng/mL, which yielded a sensitivity of 68.00% and specificity of 93.20%. The area under the ROC curve 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.

3.3. 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 2, DCP with a cut-off value of 86 mAU/mL had a high specificity and positive predictive

Figure 3. ROC curve comparing DCP and AFP levels in patients with HCC versus patients without HCC (including patients with malignant disease, patients with benign disease, patients with chronic liver disease, and normal subjects). The curves show an optimal cut-off value for DCP of 86 mAU/mL and for AFP of 21 ng/mL. The area under the ROC curve was 0.846 for DCP, 0.832 for AFP, and 0.890 for the combination of DCP and AFP.

value (PPV) but a lower sensitivity and negative predictive value (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. 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.

3.4. DCP and AFP levels according to tumor size

In the 550 patients with HCC, the median DCP level increased from 93.91 mAU/mL to 2014.00 mAU/mL along with the enlargement of tumor size. For patients with a tumor > 10.0 cm, the median AFP level was

Table 2. 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.

Table 3. 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, ≤ 3.0cm	191.82	391.93	< 10.00	1.83	46,825.61	1,647,080.00
> 3.0 cm, ≤ 4.0cm	462.78	126.90	< 10.00	1.38	> 200,000.00	366,417.00
> 4.0 cm, ≤ 5.0cm	556.88	174.00	< 10.00	0.82	111,170.15	1,193,000.00
> 5.0 cm, ≤ 10.0cm	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

2,265.00 ng/mL, which was significantly higher than that in patients with a smaller tumor (Table 3).

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 (range: 0.24-1,647,080.00 ng/mL).

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

As shown in Table 4, the sensitivity of DCP with a cut-off value of 86 mAU/ml increased from 53.70% to 86.00% along the enlargement of tumor size. The combination of DCP with a cut-off value of 86 mAU/

Table 4. 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, ≤ 3.0cm	69.00	75.90	87.90
> 3.0 cm, ≤ 4.0cm	76.50	58.80	88.20
> 4.0 cm, ≤ 5.0cm	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

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.

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 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 DCP (68.78%) or AFP (67.80%) alone ($P < 0.001$).

4. Discussion

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). 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 (5,22). Early diagnosis of HCC is essential when curative interventions can be implemented to improve patient prognosis and long-term survival (23,24). Thus, 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.

Serum biomarkers are attractive potential tools for HCC early diagnosis because they would enable non-invasive, objective, and reproducible assessments (25). In China, AFP has been recommended by HCC guidelines as a serum biomarker for diagnosis and it has been widely used in clinical practice (26), but its disadvantage of low sensitivity, low specificity, and limited accuracy in detecting small HCC diminish its clinical utility in HCC early diagnosis (7-11). Thus, other reliable serum biomarkers need to soon be identified to complement AFP in order to improve

clinical outcomes for patients with HCC in China.

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 (27), differences in the sensitivity and specificity of DCP and AFP have been extensively discussed. 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% (15,28). 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 (28-31). These differences may be due to the use of different marker cut-off values in each study (40, 60, and 100 mAU/mL for DCP and 20-200 ng/mL for AFP), differences in underlying liver disease, tumor stage, or other aspects.

The present study analyzed the clinical utility of simultaneous measurement of AFP and DCP in differentiating Chinese HCC patients (71.18% with HBV infection) from those patients with non-HCC and normal subjects. Results showed that 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 the simultaneous measurement of AFP and DCP is effective 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 (30,32,33), it was also shown in our study that for patients with HCC, the median DCP level increased with a larger tumor. In the current 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.

In conclusion, the simultaneous measurement of AFP and DCP could achieve a better sensitivity in diagnosing Chinese HCC patients, even for small tumors. To improve the diagnostic ability of serum biomarkers for HCC in China, the combined usage of AFP and DCP is suggested by this multi-center case-controlled study.

Acknowledgements

This work was supported by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan and the Japan-China Liver Cancer Research Project of the International Research Cooperation Association for Bio & Socio-Sciences Advancement.

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(Received October 22, 2014; Accepted October 28, 2014)