論文の内容の要旨

論文題目：

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)

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ABSTRACT

Background and Aim:

Hepatocellular carcinoma (HCC) is one of the most difficult to treat malignances. To achieve the most from available therapies for patients with HCC, 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. Serum biomarkers are attractive potential tools for HCC early diagnosis because they would enable non-invasive, objective, and reproducible assessments. In China, alpha-fetoprotein (AFP) has been recommended by HCC guidelines as a serum biomarker for diagnosis and it 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 soon be identified to complement AFP in order to improve clinical outcomes for patients with HCC in China.

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 are higher than those for either marker alone. 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. However, most of studies mentioned above were focusing on HCV-related HCC, there was few report about the clinical usefulness of DCP in Chinese patients with HCC predominantly caused by hepatitis B virus (HBV) infection, the relation

between DCP expression and the prognosis for patients with HBV-related HCC has yet been fully confirmed.

Aiming at ascertaining the clinical utility of DCP in diagnosing Chinese patients with HCC predominantly caused by HBV infection, and to investigate the relation between DCP expression and the prognosis for Chinese HCC patients, this large-scale, multi-center case-controlled study was conducted by our Japan-China joint research group.

Patients and Methods:

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 550 HCC patients proved by pathology after hepatic resection; *2*) Malignant disease group, which involved 164 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 181 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 85 patients with progressivity of hepatitis or liver cirrhosis; and *5*) Normal group, which involved 173 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, 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.

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. 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.

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 from 112 HBV-related HCC cases were also collected. All statistical analyses were performed using the statistical software package SPSS® version 22.0 for Windows® (SPSS, Chicago, Illinois, USA).

Results:

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.

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

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% in differentiating patients with HCC from the other four groups of subjects. The area under ROC curve (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. 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 higher Youden index and a greater sensitivity, regardless of other cut-off value chosen. The combination of the two serum markers resulted in a sensitivity of approximately 90%, which was significantly higher than that of DCP or AFP alone, the same was true even for a tumor smaller than 2.0 cm. Above results indicated that DCP could be a good candidate as a compliment to AFP in HCC diagnosis for Chinese patients predominantly caused by HBV infection.

Furthermore, a further analysis on 112 patients with HBV-related HCC was also conducted to ascertain the relation between DCP expression and the prognosis for Chinese patients with HCC. Among 112 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.

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

Results showed that high serum DCP levels were also 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%, $P = 0.035$). 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.

Conclusion:

The simultaneous measurement of DCP and AFP could achieve a better sensitivity in diagnosing Chinese patients with HCC, even for small tumors. 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 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.