Combined detection of liver stiffness and C-reactive protein in patients with hepatitis B virus-related liver cirrhosis, with and without hepatocellular carcinoma

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Abstract. The aim of the present study was to investigate the usefulness of combined detection of liver stiffness (LS) and serum C-reactive protein (CRP) level in patients with hepatitis B virus (HBV)-related liver cirrhosis (LC). A total of 156 cases of previously untreated patients with HBV-related LC were classified into the LC group [LC without hepatocellular carcinoma (HCC)] and the HCC group (LC with HCC). Comparative analyses of LS and serum CRP level were conducted between these two groups. LS values and serum CRP levels were found to be significantly higher in the HCC group compared with those in the LC group (P<0.01). The LS values and serum CRP levels were not significantly different between α-fetoprotein (AFP)-positive and -negative patients. A high LS value was a high-risk factor for HCC in patients with chronic hepatitis B. The CRP-positive rate was significantly higher in the HCC group compared with that in LC group in a subset of patients with high LS values (P<0.01). In conclusion, the combined detection of LS and serum CRP may complement the measurement of AFP in the diagnosis of HBV-related HCC, improve the identification of patients with AFP-negative HCC and help distinguish HCC from LC.

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Introduction

Hepatocellular carcinoma (HCC) is the most frequent primary malignancy of the liver (1) and a prevalent malignancy with poor outcome. Approximately 600,000 new cases are diagnosed annually, among which 55% in China (2). The majority of HCC cases (~80%) are associated with chronic hepatitis B virus (HBV) infection-related liver cirrhosis (LC) (3,4). The main treatment used for primary liver cancer is surgery. Asian series have reported a 5-year survival rate of ~70% following resection of HCCs sized <2 cm, while the current 5-year survival rate of advanced liver cancer is ~10%, only marginally better compared with that of pancreatic cancer (5). Therefore, early diagnosis is crucial for improving the survival rate of patients with primary liver cancer. However, due to the lack of effective methods for early diagnosis, only 30-40% of patients with primary liver cancer are timely diagnosed and treated at an early stage. The measurement of α-fetoprotein (AFP) levels is widely used as a surveillance test for HCC (6). Several studies of AFP in HCC surveillance revealed that its sensitivity is estimated at 39-64%, its specificity at 76-91%, its positive predictive value at 9-32% and its negative value at ≤30% (6). The sensitivity, specificity and positive predictive value of ultrasound for HCC have been reported to be 71-78, 90-93 and 14-73%, respectively. Adding ultrasound to AFP measurement in the screening of individuals at higher risk of liver cancer may significantly increase the early diagnosis rate; approximately one-third of the patients may be diagnosed and treated at an early stage. Despite the large number of studies on the immunohistochemistry of HCC, such as AFP-L3, α1,6-fucosyltransferase, glypican-3, Dickkopf-1 and heat shock protein 70 (7), absolute positive and negative markers for HCC are still lacking, and even those characterized by very high sensitivity and specificity do not exhibit universal diagnostic usefulness. Therefore, the diagnosis of HCC at an early stage remains a prerequisite for favorable prognosis.

Liver stiffness measurement (LSM) using transient elastography (TE FibroScan®; Echosens, Paris, France) has been shown to be correlated with liver fibrosis in various chronic liver diseases (8-11). LSM correlates well with the stage of

liver fibrosis and may serve as a predictor of HCC development among patients with chronic hepatitis (12-16). It remains questionable whether LSM alone may be applied as a predictive marker of primary liver cancer at the clinical setting. Therefore, it is necessary to identify markers that may be combined with liver stiffness (LS) to improve the prediction of HCC.

C-reactive protein (CRP) is an acute-phase protein synthesized by the liver. Rapid, marked increases in CRP levels occur with inflammation, infection, trauma and tissue necrosis (17-21). Several studies have been performed on the association of CRP with the severity of inflammation in liver disease, such as fatty liver and chronic hepatitis C (22-25). A persistently elevated CRP level following initial treatment is an independent marker of a poor prognosis, and normalization of the CRP level after initial treatment is associated with improved outcome in HCC patients (26-28). As regards the ability of CRP to predict HBV-related HCC, it remains unknown. A total of 156 LC cases with and without HCC were compared in terms of LS and high-sensitivity CPR (hs-CRP), to investigate the potential of combined detection of LS and serum CRP level in the diagnosis of HCC in patients with chronic hepatitis B.

Patients and methods

Patients. A total of 156 patients with HBV-related LC in our hospital were included in this study. The patients were divided into the LC group (LC without HCC) and the HCC group (LC with HCC). These cases were confirmed by clinical presentation, diagnostic ultrasound imaging and laboratory diagnosis. LC was diagnosed according to the guideline of prevention and treatment for chronic hepatitis B, 2010 version (29); HCC was diagnosed according to the criteria formulated by the Chinese Anti-Cancer Association. All the included patients had not been treated with any hepatoprotective, antifibrotic or antiviral drugs. In addition, 50 healthy individuals, without autoimmune or metabolic diseases, without a history of drug or alcohol abuse or other factors that may cause liver damage, and without hematological diseases, were used as the control group. Patients with conditions likely to affect the serum CRP level, such as acute infections, rheumatoid arthritis, gout, asthma, chronic lung disease, myocardial infarction and stroke, were excluded.

Parameters

LSM. Scanning was performed using transient elastography FibroScan® (Echosens) according to the method described in the manufacturer's instructions. The LS grading criteria were as follows: >17.5 kPa cirrhosis; patients were subgrouped with a 5-kPa interval into 17.5-22.5, 22.6-27.5 and >27.6 kPa groups.

Serum CRP levels. Measurement was performed using the latex enhanced turbidimetric method with the reagent purchased from Roche Diagnostics GmbH (Mannheim, Germany) using the Roche Modular P800 analyzer. The serum CRP normal reference range was 0-2.87 mg/l, with serum hs-CRP >2.87 mg/l considered as positive.

AFP. The plasma levels of AFP were measured using a commercial ELISA kit (Abbott Laboratories Company, Chicago, IL, USA) in accordance with the manufacturer's

Table I. LSM values and serum hs-CRP levels (mean ± SD).

Groups	No.	hs-CRP (mg/l)	LSM values (kPa)
Healthy	50	0.78±1.07	4.25±0.74
LC	89	9.47±19.56	27.81±18.46
HCC	67	30.78±15.17	39.72±29.05
t-test		-0.964	-1.735
P-value		0.0337	0.0093

LSM, liver stiffness measurement; LC, liver cirrhosis; hs-CRP, high-sensitivity C-reactive protein; HCC, hepatocellular carcinoma.

Table II. LSM values and serum hs-CRP levels in AFP-positive and -negative patients (mean ± SD).

AFP status	No.	hs-CRP (mg/ml)	LSM values (kPa)
Positive	35	13.58±27.16	48.95±28.59
Negative	32	8.66±17.72	28.64±26.83
t-test		0.815	1.706
P-value		0.419	1.104

LSM, liver stiffness measurement; AFP, α-fetoprotein; hs-CRP, high-sensitivity C-reactive protein.

instructions. The HCC group was divided into AFP-positive (>20 ng/ml) and -negative (≤20 ng/ml).

Statistical analysis. The statistical analysis was performed using the SPSS v.17.0 software package (SPSS Inc., Chicago, IL, USA). Quantitative data were analyzed using the independent-samples t-test and expressed as mean ± standard deviation. The count data were analyzed using the χ^2 test. P<0.05 was considered to indicate a statistically significant difference. Correlation analyses were conducted using the Pearson's and Spearman's correlation tests.

Results

Clinical data. A total of 156 cases of previously untreated patients with LC were included, 125 of whom were men and 31 were women (mean age, 38±17.1 years). The patients were divided into the LC group (LC without HCC, n=89) and the HCC group (LC with HCC, n=67). Of the 50 healthy controls, 41 were men and 9 were women (mean age, 38±9.4 years). The LSM values and serum CRP levels were found to be significantly higher in the HCC group compared with those in the LC group (P<0.05 and P<0.01, respectively; Table I).

Comparisons of LSM values and serum CRP levels in AFP-positive and -negative patients. The HCC group was subdivided into the AFP-positive (AFP >20 ng/ml) and AFP-negative (AFP ≤20 ng/ml) groups. The LSM values and

Table III. Proportion analysis of HCC, LSM grade and serum hs-CRP positivity rate.

LSM grade (kPa)	Case (no.)	LC group		HCC group	
		CRP+, no. (%)	CRP-, no. (%)	CRP+, no. (%)	CRP-, no. (%)
17.5-22.5	0	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)
22.6-27.5	24	0/10 (0.0)	10/10 (100.0)	0/14 (0.0)	14/14 (100.0)
>27.6	132	30/79 (37.97)	49/79 (62.03)	34/53 (64.15)	19/53 (35.85)
χ^2 test		8.847		24.89	
P-value		0.003		0.000	

LSM, liver stiffness measurement; hs-CRP, high-sensitivity C-reactive protein; HCC, hepatocellular carcinoma.

serum CRP levels were not significantly different between AFP-positive and -negative patients (P>0.05, Table II).

Association of LSM values and serum CRP levels with HCC. The proportional analysis of HCC and CRP positivity rate among 3 stratifications of LSM revealed that a high LSM value was a high-risk factor for HCC. In a subset of patients with LSM values >27.6 kPa, the CRP positivity rate was significantly higher in the HCC group (64.15%) compared with that in the LC group (37.97%) (P<0.01, Table III).

Discussion

The diagnosis of HCC at an early stage is a prerequisite for improved prognosis. LSM using FibroScan transient elastography has been shown to be correlated with liver fibrosis in various chronic liver diseases. The LSM cut-off value of 17.5 kPa is considered optimal for diagnosing LC (8-11) and the LSM value is useful in predicting HCC (12-16). Among a total of 156 patients with HBV-related cirrhosis, the LSM values were significantly higher in the HCC group (39.72±29.05 kPa) compared with those in the LC group (27.81± 8.46 kPa) (P<0.05). The LSM values were not significantly different between AFP-positive and -negative patients (P>0.05). The cut-off value was set at 17.5 kPa, and patients were subgrouped with a 5-kPa interval into the 17.5-22.5, 22.6-27.5 and >27.6 kPa groups. This subgroup analysis revealed that a high LSM value (>27.6 kPa) was a high-risk factor for HCC, which was consistent with earlier findings, indicating the usefulness of LSM in predicting HCC. Furthermore, it was observed that 59.85% of patients with LSM values of >27.6 kPa did not have HCC. This positive predictive value was significantly lower compared with that of ultrasound combined with AFP. Therefore, it is necessary to identify markers to combine with LS in order to improve the prediction of HCC.

The majority of the cases of HCC are associated with cirrhosis related to chronic hepatitis (1,3,9). CRP is an acute-phase protein synthesized by the liver. Several studies have been performed to determine the association of CRP with the severity of inflammation in liver diseases, such as fatty liver and chronic hepatitis C. A persistently elevated CRP level following initial treatment is an independent marker of a poor prognosis, and normalization of the CRP level after initial treatment is associated with an improved outcome in HCC patients (17-29). We observed

that serum CRP levels were significantly higher in the HCC group (30.78±15.17 mg/l) compared with those in the LC group (9.47±19.56 mg/l) (P<0.01), whereas they were not significantly different between AFP-positive and -negative patients (P>0.05). These results may be explained by the fact that AFP-positive HCC patients may have larger tumors and exhibit more severe inflammation due to the invasion of the portal system. The cut-off value for LSM was set at 17.5 kPa, and patients were subgrouped with a 5-kPa interval into 17.5-22.5, 22.6-27.5 and >27.6 kPa groups. Patients with serum CRP levels >2.87 mg/l were defined as positive, whereas those with serum CRP levels <2.87 mg/l were defined as negative. A proportion analysis was performed of HCC and serum CRP positivity rate among the 3 LSM grades. It was demonstrated that a high LSM value was a high-risk factor for HCC. In a subset of patients with LSM values >27.6 kPa, the CRP positivity rate was significantly higher in the HCC group (64.15%) compared with that in the LC group (37.97%) (P<0.01). These results demonstrate the association of HCC with combined detection of LS and serum CRP.

In conclusion, the combined detection of LS and serum CRP may complement the measurement of AFP level in the diagnosis of HBV-related HCC, facilitate the identification of patients with AFP-negative HCC, and help distinguish HCC from LC.

Acknowledgements

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