

# Serum hyaluronic acid, procollagen type III and IV in histological diagnosis of liver fibrosis

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**OBJECTIVE:** To assess the significance of serum hyaluronic acid (HA), procollagen type III (PCIII), collagen type IV (CIV) in the histological diagnosis of liver fibrosis.

**METHODS:** The concentrations of serum HA, PCIII, CIV in 253 patients with chronic liver diseases were measured by radioimmunoassay. Liver biopsies were performed in all patients at the same time. The liver was pathologically evaluated by a pathologist according to a scoring system. Combined with the results of liver pathological diagnosis, the accuracy of serum HA, PCIII, CIV in diagnosing patients with hepatic fibrosis (staging  $\geq S_2$ ) or cirrhosis ( $S_4$ ) was assessed using the receiver operating curve (ROC).

**RESULTS:** The cutoff values of serum HA, PCIII and CIV for identifying patients with hepatic fibrosis ( $\geq S_2$ ) or cirrhosis ( $S_4$ ) were determined. The cutoff values of serum HA, PCIII and CIV for detecting patients with fibrosis (stage  $\geq S_2$ ) were 90  $\mu\text{g/L}$ , 90  $\mu\text{g/L}$ , 75  $\mu\text{g/L}$ , respectively; their sensitivity (Se) was 80.4%, 82%, 63.1%; their specificity (Spe) was 70.2%, 60.8%, 83.8%; their positive predictive values (PPV) were 86.7%, 83.5%, 90.4%; their negative predictive values (NPV) were 59.8%, 58.4%, 48.4%, respectively. The cutoff values for detecting patients with liver cirrhosis were 210  $\mu\text{g/L}$  for HA, 96.2% for Se, 85.3% for Spe, 65.4% for PPV, 98.8% for NPV; 150  $\mu\text{g/L}$  for PCIII, 76.4% for Se, 68.7% for Spe, 40.4% for PPV, 91.3% for NPV; 90  $\mu\text{g/L}$  for CIV, 80% for Se, 75.8% for Spe, 47.8% for PPV, 93.2% for NPV, respectively.

**CONCLUSIONS:** Serum HA, PCIII and CIV can be determined for an accurate diagnosis of hepatic fibrosis in various stages. HA is the best for screening liver cirrhosis.

(*HBPD Int* 2003; 2: 69–72)

**Key words:** hyaluronic acid; procollagen type III; collagen type IV; hepatic fibrosis; diagnosis; liver histology

## Introduction

Prolonged or recurrent hepatocellular damage leads to enhanced and persistent inflammation and repair with deposition of excessive extracellular matrix (ECM), a causative factor for hepatic fibro-

sis. As an important sequela of chronic liver diseases, hepatic fibrosis will ultimately progress to liver cirrhosis. How to prevent hepatic fibrosis from being cirrhotic is essential to the treatment of chronic hepatitis. It is particularly important to assess the degree of hepatic fibrosis in patients with chronic liver diseases.<sup>[1]</sup> But a simple and reproducible tool is necessary to monitor the progression of fibrogenesis. Liver biopsy as the gold standard for the diagnosis of hepatic fibrosis has its limitations in clinical practice such as sample error, complications, discomfort, invasiveness, etc. These prevent the use of liver biopsy as a procedure for the evaluation of hepatic fibrosis. B type ultrasound and CT are generally used for the diagnosis of he-

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*This study was supported by the grant from the Guangdong Provincial Science and Technology Foundation (No. A 1999–198).*

patic fibrosis or cirrhosis, but they fail to make early diagnosis. It has been suggested that serum markers of ECM can be detected for the assessment of hepatic fibrosis.<sup>[2]</sup> Studies<sup>[3,4]</sup> have shown that some serum markers of ECM are positively correlated with inflammatory activity and the degree of hepatic fibrosis, but how to detect accurately hepatic fibrosis by serum markers of ECM is problematic in the clinic. This study was to determine the accuracy (percentage of patients correctly classified) of serum HA, PCIII, CIV in assessing the stages of fibrosis and the most suitable variables for screening liver fibrosis.

## Methods

### Patients

253 consecutive patients with chronic viral hepatitis admitted to our hospital from July 1996 to February 1999 were enrolled in the study. Of these patients aged from 8 to 57 years, 219 were male and 34 female. 216 patients suffered from chronic hepatitis B, 23 chronic hepatitis C, 8 both chronic hepatitis B and C, 1 non-A to E hepatitis, and 5 liver cirrhosis due to factors of alcohol (2), auto immune hepatitis (1), primary biliary cirrhosis (1), and unknown cause (1). The patients were divided into four groups according to clinical diagnostic standards;<sup>[2]</sup> light chronic hepatitis (LCH) (65 patients), moderate chronic hepatitis (MCH) (124), severe chronic hepatitis (SCH) (26), and liver cirrhosis (LC) (38).

### Blood tests

The values of hemoglobin, lymphocyte, platelet, cholesterol, creatinine, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin,  $\gamma$ -globulin, prothrombin were analysed. Viral hepatitis markers were tested by ELISA. The serum concentrations of HA, PCIII, CIV were measured by radioimmunoassay (RIA-HA and CIV kit, Shanghai, China; RIA-PC III kit, Chongqing, China). Blood samples for HA, PCIII, CIV determination were collected at the same time of liver biopsy.

### Liver biopsy

Automatic cut liver biopsies using the 16G needle guided by B type ultrasound were performed in all patients. The biopsy specimens were fixed in Bouin's solution and embedded in paraffin. Sections in 4  $\mu$ m thickness were stained with hematoxylin and eosin and Masson's trichrome for histological evaluation. Liver was assessed histologically by a pathologist according to the scoring system.<sup>[2]</sup>

### Statistical analysis

Quantitative variables expressed as  $\bar{x} \pm s$  were compared using ANOVA. After the histological assessment (stage  $\geq S_2$  for extensive hepatic fibrosis; and stage  $S_4$  for liver cirrhosis), the predictive values of serum HA, PCIII, CIV for the diagnostic accuracy of hepatic fibrosis were calculated using constructed receiver operating curve (ROC). According to the ROC, the screening cutoff values of serum HA, PCIII and CIV for the diagnosis of patients with hepatic fibrosis ( $\geq S_2$ ) or cirrhosis ( $S_4$ ) were determined. A *P* value of 0.05 was considered significant. The computer software STATA was used.

### Results

The serum levels of HA, PCIII, CIV were compared between different stages of fibrosis (Table 1).

Combined with the results of liver pathological diagnosis, we constructed the ROC curve of HA, PCIII, CIV for screening liver fibrosis and liver cirrhosis; the cutoff value was determined (Tables 2 and 3).

**Table 1.** The serum levels of HA, PCIII, CIV in different stages of fibrosis ( $\bar{x} \pm s$ )

Stage(S)	n	HA ( $\mu$ g/L)	PCIII ( $\mu$ g/L)	CIV ( $\mu$ g/L)
S <sub>0</sub>	15	78.3 $\pm$ 56.4	100.3 $\pm$ 85.0	47.13 $\pm$ 33.3
S <sub>1</sub>	59	78.9 $\pm$ 57.9	98.9 $\pm$ 51.6	59.0 $\pm$ 45.3
S <sub>2</sub>	68	147.7 $\pm$ 128.4*	160.9 $\pm$ 126.2*	98.0 $\pm$ 113.1*
S <sub>3</sub>	56	198.2 $\pm$ 106.9	154.8 $\pm$ 92.7	109.2 $\pm$ 118.9
S <sub>4</sub>	55	453.3 $\pm$ 204.5**	236.4 $\pm$ 141.4**	187.0 $\pm$ 144.4**

\* compared with S<sub>0</sub>, S<sub>1</sub>, *P* value 5.34–3.28 (*P* < 0.05); \*\* compared with other stages, *P* value 7.15–4.31 (*P* < 0.05).



not satisfactory in clinical use because their Se, Spe, PPV values were low in our study. In contrast, the NPV of PCIII, CIV for screening cirrhosis were 91.3%–93.2%, which were clinically significant in ruling out cirrhosis if the concentrations of PCIII, CIV were lower than the cutoff value.

Our study showed that the determination of serum HA, PCIII and CIV can more accurately detect various stages of hepatic fibrosis. HA is of highly diagnostic accuracy in screening cirrhosis, hence the best for the diagnosis of liver cirrhosis.

### Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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*Received April 2, 2002*

*Accepted after revision August 18, 2002*