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The combination of 5 serum markers compared to FibroScan to predict significant liver fibrosis in patients with chronic hepatitis B virus

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ABSTRACT

Background: We evaluated the performance of serum hyaluronan (HA), procollagen type III N-terminal peptide (PIIINP), type IV collagen (IVC), laminin (LN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), compared to transient elastography (FibroScan) in predicting significant liver fibrosis.

Methods: We therefore determined 4 serum fibrosis markers, FibroScan and liver biopsy in 70 consecutive adult patients with chronic hepatitis B. According to a modified Scheuer scoring system, significant fibrosis was defined as fibrosis stage ≥S2. We compared serum fibrosis markers to histological staging and FibroScan results using Spearman correlation analysis and area under receiver operating characteristic (ROC) curves (AUROCs).

Results: Of the 212 patients who had the results of FibroScans and four serum fibrosis markers for HBV, 70 had concurrent liver biopsy. Significant liver fibrosis was found in 24/70 patients. The serum levels of HA, PIIINP, IVC, LN, ALT, AST was all positively correlated with fibrosis stage of Liver biopsy. The coefficients with stages were respectively 0.468, 0.392, 0.538, 0.213, 0.350, 0.375. There was a significant difference between mild fibrosis (< S2) and significant fibrosis (≥S2), excluding LN, in the levels of these 5 serum makers ($P < .05$). AUROC for FibroScans and HA, PIIINP, IVC, LN, ALT, AST to correctly allocate patients to histological fibrosis stage ≥ S2 was 0.866, 0.784, 0.738, 0.827, 0.630, 0.713 and 0.728 respectively. Since LN shows the worst performance of the others. We decided to check the performance of the combination of HA, PIIINP, CIV, ALT, AST, excluding LN, to distinguish fibrosis stages. The index of the histological fibrosis stage ≥ S2, combining the 5 serum markers, significantly improved diagnostic performance (AUROC = 0.861) compared to the use of 5 serum markers alone in all HBV patients.

Conclusion: The combination of the 5 serum markers and FibroScan performed equally well in predicting significant fibrosis. The combination of the 5 serum markers is a reliable noninvasive method to predict significant liver fibrosis in patients with CHB. So, it provide another choice rather than FibroScan in predicting significant liver fibrosis.

1. Introduction

Chronic hepatitis B virus (CHB) infection remains a global public health issue, and it is estimated that about 93 million Chinese people who are chronically infected with HBV [1,2]. Current guidelines do not only recommend to monitor serial alanine aminotransferase (ALT) and hepatitis B virus (HBV) DNA replication quantity closely, but also to monitor liver fibrosis. As HBV infection can induce liver fibrosis that may subsequently lead to the development of liver cirrhosis and hepatocellular carcinoma, the long term monitoring of the degree of liver fibrosis in CHB patients is essential for clinicians to decide whether to initiate or stop antiviral therapy [3,4]. Therefore, to prevent liver fibrosis from progressing to cirrhosis and carcinoma is a key step in the treatment and prognostication of CHB.

Liver biopsy has been traditionally considered the gold standard for assessment of liver fibrosis. However, it is limited due to its invasiveness, cost, associated complications, sampling variability and potential risk in the clinical application [5–8]. Given these limitations, the development of noninvasive methods have been suggested to greatly facilitate the clinical management of CHB and reduce the need for liver biopsies [9–11].

FibroScan and serum markers are validated as noninvasive methods to assess the stage of liver fibrosis. Previous studies have already demonstrated a correlation between FibroScan and the stage of liver fibrosis. It has been widely studied to noninvasively assess the stage of liver fibrosis in CHB patients [12,13]. A recent meta-analysis showed that FibroScan had a good sensitivity, specificity and high accuracy for detecting liver cirrhosis [14], but these methods are not effective in the

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Table 1
Patient characteristics.

	Median (range)	Number (%)
Gender		
Male		49(70.00)
Female		21(30.00)
Age (y)	38.50(15–64)	
Biochemical tests		
ALT (U/l)	40.00(8–697)	
AST (U/l)	32.50(15–751)	
TBIL(μmol/l)	14.00(6–420)	
PLT(10^9/l)	207.00(112–342)	
GGT (U/l)	42.00(11–1666)	
ALP (U/l)	84.00(47–806)	
CHE (U/l)	7591.00(1876–11,436)	
ALB (g/l)	44.90(28.30–56.10)	
PT(s)	13.30(11.80–16.30)	
INR	1.02(0.89–1.31)	
HBV Serology		
HBeAg positive		25(35.71)
HBeAb positive		20(28.57)
HBV DNA positive		40(57.14)
Fibrosis markers tests		
HA (ng/ml)	39.46(14.85–1011.82)	
PIIINP (ng/ml)	6.82(3.07–43.90)	
CIV (ng/ml)	57.04(8.09–736.02)	
LN (ng/ml)	65.03(23.38–176.58)	

diagnosis of mild or significant liver fibrosis [15]. What's more, several factors such as fasting, operator bias, and anthropometric characteristics can influence the results of FibroScan of liver fibrosis evaluation and invalid assessments rates have been quoted to range between 2.4% and 9.4% [16,17]. So currently, the noninvasive diagnostic methods can not completely substitute liver biopsy. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) has been widely used for assessing hepatitis activity. However, patients with normal or mildly increased serum ALT and AST are not necessarily free from liver damage. In recent years, the levels of serum markers of extracellular matrix (ECM) has been introduced as a noninvasive method for the assessment of liver fibrosis. Hyaluronan (HA), procollagen type III N-terminal peptide (PIIINP), type IV collagen (IVC) and laminin (LN) are 4 major serum markers in the ECM. Numerous studies have confirmed their diagnostic Performance. They were found the correlation with liver fibrosis in patients with chronic hepatitis [18–21]. However, few studies have focused on assessing the diagnostic value of the 4 major serum markers for differentiating between mild fibrosis and significant fibrosis in CHB patients.

2. Materials and methods

2.1. Patients

Seventy consecutive patients with CHB were screened for enrollment in this study between January 2017 and December 2017, who had been referred for serum collection at admission to the Third Affiliated Hospital of Sun Yat-sen University. Inclusion criteria were: 1) age between 18 and 65 y; 2) HBV surface antigen (HBsAg)-positive for > 6 months without having received antiviral treatment before this study; 3) a liver biopsy test; 4) the FibroScan (Echosens) examination; 5) written informed consent. Exclusion criteria were: 1) HBV surface antigen (HBsAg)-negative; 2) asymptomatic carriers since not candidate for biopsy; 3) HAV, HCV, HDV and HIV co-infection; 4) alcoholic or nonalcoholic fatty liver disease; 5) autoimmune liver disease, 6) decompensated cirrhosis; 7) hepatocellular carcinoma 8) pregnancy. Samples were anonymously coded in accordance with local ethical guidelines which conformed to the Declaration of Helsinki, and written informed consent was obtained from patients and healthy volunteers. The work was conducted in strict accordance with the study design as approved by the Clinical Research Ethics Committee of the Third Affiliated Hospital and Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China.

2.2. Clinical laboratory parameters

clinical laboratory parameters were measured and recorded on admission, including age, gender, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), platelet count (PLT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), cholinesterase (CHE), albumin (ALB), prothrombin time (PT), international normalized ratio (INR), HBeAg, HBeAb and HBV DNA. Biochemical tests were performed using routine automated analyzers (HIitachi 7180). The 4 serum fibrosis markers concentration of PIIINP, CIV, LN, and HA were determined by electrochemiluminescence immunoassay (Mindray CL-i2000). Serum HBV DNA was measured by COBAS AmpliPrep/COBAS TaqMan. HBV other serum markers were also performed using Roche Elecsys assays according to the manufacturer's instructions.

2.3. Liver biopsy

Percutaneous Liver biopsy was performed using ultrasound localization. Liver samples were fixed in formalin and embedded in paraffin and stained with Hematoxylin–eosin and reticular fiber staining or Masson's staining on each section. The inflammation grade (G0–G4)

Table 2
The 4 serum fibrosis markers levels of PIIINP, CIV, LN, HA and FibroScan in fibrosis stage < S2 vs. ≥S2 (median ± interquartile ranges).

Fibrosis stages	n	HA	PIIINP	IVC	LN
< S2	46	37.02 ± 27.56	6.33 ± 4.86	41.56 ± 46.74	63.82 ± 35.72
≥S2	24	77.75 ± 106.45	11.63 ± 15.95	87.74 ± 91.92	74.20 ± 58.54
r		0.468	0.392	0.538	0.213
P		< 0.05	< 0.05	< 0.05	> 0.05

Fibrosis stages	n	ALT	AST	FibroScan
< S2	46	38.50 ± 56.75	26.00 ± 27.25	6.00 ± 4.00
≥S2	24	63.00 ± 131.75	51.00 ± 56.00	13.00 ± 15.50
r		0.350	0.375	0.606
P		< 0.05	< 0.05	< 0.05

HA: hyaluronan; PIIINP: procollagen type III N-terminal peptide; IVC: type IV collagen; LN: laminin; FibroScan: transient elastography.