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Original article

Diagnostic accuracy of liver stiffness measurement and serum hyaluronic acid for detecting liver fibrosis in chronic hepatitis B with respect to ALT levels

Prachya Kongtawelert[a], Theerawut Chanmee[a], Peraphan Pothacharoen[a], Naruemon Wisedopa[b], Pavanrat Kranokpiruk[c], Kittiyod Poovorawan[d], Yong Poovorawan[e], Pisit Tangkijvanich[f]

[a]Thailand Excellence Center for Tissue Engineering, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand, [b]Department of Pathology, [c]Department of Radiology, [d]Department of Medicine, [e]Center of Excellence in Clinical Virology, Department of Pediatrics, [f]Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Background: The assessment of liver fibrosis in chronic hepatitis B is crucial in clinical practice.

Objective: We compared the diagnostic accuracy of liver stiffness measurement (LSM) using transient elastography and serum hyaluronic acid (HA) in detecting liver fibrosis (METAVIR) in chronic hepatitis B, with respect to ALT levels.

Method: One hundred fifty-six Thai patients with chronic hepatitis B who had undergone a liver biopsy were enrolled, and included 112 (71.8%) men and 44 (28.2%) women. The mean age of the patients was 40.1±12.2 years. The predictive accuracy was analyzed by comparing the areas under the receiver-operating characteristic curves (AUROCs).

Result: LSM was superior to HA in predicting fibrosis stages of ≥F2 (AUROCs were 0.820 vs 0.727, p=0.009), ≥ F3 (0.910 vs 0.848, p=0.015) and F4 (0.938 vs 0.876, p=0.031). There was significant correlation between ALT level and LSM value, while such correlation between ALT and HA was not detected. Regarding the subgroup of patients with ALT levels >80 IU/L (2 × ULN), AUROCs of LSM and HA for predicting fibrosis stages of ≥F2 (0.733 vs 0.696), ≥F3 (0.892 vs 0.844) and F4 (0.934 vs 0.893) were not significantly different.

Conclusion: LSM was superior to HA in predicting liver fibrosis and cirrhosis in patients with chronic hepatitis B. However, in patients with ALT elevation, the diagnostic performance of LSM was reduced and its accuracy was comparable to that of HA. Thus, HA could be an alternative method in assessing liver fibrosis in patients with high ALT levels.

Keywords: ALT level, AUROC, cirrhosis, diagnostic accuracy, hepatitis B, hyaluronic acid, liver stiffness, transient elastography

Hepatitis B virus (HBV) infection is a major public health problem worldwide, with approximately 400 million people chronically infected. Chronic HBV infection is associated with a diverse clinical spectrum of liver damage ranging from mild chronic hepatitis to cirrhosis with hepatic decompensation and hepatocellular carcinoma (HCC) [1]. An accurate assessment of fibrosis stages is essential for predicting the prognosis and therapeutic decisions for patients with chronic hepatitis B. Although percutaneous liver biopsy has been a criterion standard to assess liver histopathology, this procedure has some limitations because of its invasive nature and risk of potentially life-threatening complications [2]. In addition, its accuracy is restricted as a consequence of sampling errors and variations in interpretation [3]. Therefore, this procedure is being gradually replaced by various noninvasive methods for the assessment of liver fibrosis.

Liver stiffness measurement (LSM) using transient elastography has recently been introduced as a new, noninvasive tool for assessing liver fibrosis with high reproducibility [4]. This ultrasound-based technique allows the assessment of approximately

Correspondence to: Pisit Tangkijvanich, MD, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. E-mail: pisittkvn@yahoo.com

1/500 of the liver's total mass, thus ensuring a reduction in the sampling error compared with liver biopsy. In patients with chronic hepatitis C virus (HCV) infection, several studies have shown significant positive correlation between LSM and the stage of hepatic fibrosis, as evaluated by the METAVIR score system [5]. Data from the use of LSM to assess the severity of liver fibrosis in patients with chronic hepatitis B are increasing. Recent studies have suggested that LSM exhibits comparable diagnostic performances in chronic hepatitis B compared with chronic hepatitis C [6-9]. However, one limitation is that LSM values can be increased significantly with higher alanine aminotransferase (ALT) levels regardless of fibrosis staging [8, 9]. Thus, additional studies are required to define the accuracy of LSM for predicting liver fibrosis and cirrhosis in patients with chronic hepatitis B, with respect to ALT levels.

Several clinical studies have identified blood tests as surrogate markers of liver fibrosis, which would greatly reduce the necessity to perform liver biopsy. Indirect serological markers on the basis of common laboratory tests, including aspartate aminotransferase (AST)-to-platelet-ratio-index (APRI), FIB-4, Forns' index, Fibrotest, and FibroSpect have been used to stage chronic liver disease [10]. Additionally, several direct serum markers of liver fibrogenesis including hyaluronic acid (HA), serum collagenases and their inhibitors (tissue inhibitor of metalloproteinase [TIMP]), and profibrogenic cytokines such as transforming growth factor 1 have been investigated [10, 11]. Currently, there are few studies directly comparing the diagnostic accuracy between LSM and serum markers in patients with chronic hepatitis B.

HA, a high-molecular-weight glycosaminoglycan that is an essential component of the extracellular matrix, appears to be the most suitable test for the noninvasive assessment of liver fibrosis [11, 12]. In the liver, HA is mainly synthesized by hepatic stellate cells and degraded by hepatic sinusoidal endothelial cells [13]. It has been shown that serum HA levels are low in healthy subjects, but elevated levels occur in patients with various etiologies of fibrotic liver disease, including chronic viral hepatitis an alcohol induced liver disease[11]. Previous studies demonstrated that serum HA concentrations were significantly related to the histological degree of liver fibrosis, but there was no correlation between this marker and the histological activity of necroinflammation [12, 14]. However, the accuracy of HA for predicting liver fibrosis and cirrhosis in conjunction with ALT levels has never been investigated.

This study was aimed at comparing the diagnostic accuracy of LSM and serum HA in detecting liver fibrosis and cirrhosis in patients with chronic hepatitis B. In particular, we evaluated the impact of serum ALT elevations on the diagnostic accuracy of these noninvasive tests.

Patients and methods

Patients

This cross-sectional study included consecutive patients with chronic hepatitis B who had undergone a liver biopsy at King Chulalongkorn Memorial Hospital, Bangkok, Thailand between January 2010 and September 2011. Chronic hepatitis B was diagnosed based on hepatitis B surface antigen (HBsAg) in the patient's serum for at least 6 months and detectable serum HBV DNA.

A total of 156 patients were enrolled in this study, and included 112 (71.8%) men and 44 (28.2%) women. The mean age of the patients was 40.1±12.2 years. Patients with the following conditions were excluded from the study: presence of HCV-coinfection or other cause of liver disease, seropositive for anti-HIV, presence of decompensated cirrhosis and HCC and prior antiviral therapy. All patients gave written informed consent for the study and the protocol was approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University.

Laboratory tests

A serum sample was obtained from each patient for analysis at the time of performing TE. Liver biochemistry tests [AST, ALT, total bilirubin (TB), alkaline phosphatase (AP), albumin] were performed using commercially available assay kits in an automated analyzer (Hitachi 912). HBsAg and hepatitis B e-antigen (HBeAg) were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Abbott Laboratories, Chicago, IL, USA). Serum HBV DNA level was quantified using a commercially available kit (Amplicor HBV Monitor; Roche Diagnostics, Tokyo, Japan).

Measurement of serum HA concentration

Serum HA was measured by a modified competitive ELISA-like method using an HA-test kit

(Allswell Singapore) according to the manufacturer's specifications as previously described[15]. Briefly, microtiter plates (Maxisorp, Nunc) were coated with umbilical cord HA (100 µl/ well) in coating buffer at 4°C overnight. Wells were blocked with 150 µl of 1% (w/v) BSA in phosphate-buffered saline (PBS) for 60 minutes at room temperature. After washing, 100 l of the mixture, either sample or standard competitor (HA Healon: range 39.06–10,000 ng/ml) in B-HABPs (1:100), were added. After incubation for 60 minutes at room temperature, plates were washed and then peroxidase-mouse monoclonal anti-biotin (100 µl/well; 1:2,000) was added and incubated for 60 minutes at room temperature. The plates were washed again and the peroxidase substrate (100 µl/well) was added and incubated at room temperature for 15 minutes to allow the color to develop. The reaction was stopped by the addition of 50 µl of 4 M H_2SO_4. The absorbance ratio at 492/690 nm was measured using the Titertek Multiskan M340 microplate reader.

Liver stiffness measurement

LSM values were obtained from each patient using transient elastography (FibroScan, Echosens, Paris, France) according to the manufacturer's instructions [4]. All patients underwent LSM within 30 days of their liver biopsy. Results were recorded in kilopascals (kPa) as the median value of all measurements. The procedure was based on at least 10 validated measurements: the success rate (ratio between numbers of validated and total measurements) was over 60% and interquartile range was less than 30%.

Histopathology assessment

Liver biopsy specimens were obtained using 16 G disposable needles (Hepafix; B. Braun, Melsungen, Germany) applying an ultrasound-guided technique. The specimens were fixed in formalin, embedded in paraffin blocks, and stained with hematoxylin and eosin, and Masson's trichrome stain. Histopathology was performed by an experienced pathologist, who was blinded to the patients' clinical data. A liver biopsy specimen was considered adequate if it was longer than 15 mm (except when cirrhosis was present). The stage of fibrosis was scored according to the METAVIR classification [16]: no fibrosis (F0), portal fibrosis without septa (F1), portal fibrosis with few septa (F2), numerous septa without cirrhosis (F3), and cirrhosis (F4). Significant liver fibrosis was defined as METAVIR fibrosis stage of at least F2 (≥F2), while advanced liver fibrosis was defined as METAVIR fibrosis stage of at least F3 (≥F3).

Statistical analyses

Data were expressed as mean standard deviation (SD) and percentages as appropriate. Comparisons between groups were analyzed using a χ^2 or Fisher's exact test for categorical variables and using a Mann–Whitney test or Student's *t* test when appropriate for quantitative variables. Pearson's correlation coefficient was used. Univariate and multiple regression analysis were used to determine variables that significantly correlated in the univariate analysis. The diagnostic performance of each test was assessed by using receiver operating characteristics (ROC) curves. The area under the ROC curves (AUROC) and 95% confidence intervals (CI) were used as indices of accuracy, with values close to 1.0 indicating high diagnostic accuracy. Optimal cutoff values for each test were chosen to maximize both sensitivity and specificity. A two-sided probability value of $p<0.05$ was considered statistically significant. Data were analyzed using SPSS software for Windows version 17.0 (SPSS, Chicago, IL).

Results

Characteristics of the patients

In this study, there were 19 (12.2%), 50 (32.0%), 45 (28.8%), 21 (13.5%), and 21 (13.5%) patients with METAVIR fibrosis stage F0, F1, F2, F3, and F4 respectively. Compared with patients with absent (F0) and mild fibrosis (F1), patients with significant liver fibrosis (≥F2) had higher average age. No significant difference between groups was observed in respect to sex, body mass index (BMI), AST, ALT, TB, AP, albumin, platelet count, HBeAg immunoreactivity, and HBV DNA level (**Table 1**).

LSM and HA according to liver fibrosis stages

LSM and HA values of all enrolled patients according to their fibrosis stages are shown in **Table 2**. The mean LSM value was 8.1 kPa (ranging from 3.3 to 31 kPa) and the mean HA level was 120.4 ng/mL (ranging from 8.3 to 1327.8 ng/mL). There were significant differences in the mean LSM between F0–F1 and F2–F4 fibrosis stages (5.7±1.9 kPa vs 9.9±5.7 kPa, $p<0.001$), between F0–F2 and F3–F4 (6.2±2.2 kPa vs 13.1±6.5 kPa, $p<0.001$), and between F0–F3 and F4 (6.9±3.1 kPa vs 15.7±7.1 kPa, $p<0.001$).

Table 1. Demographic and clinical characteristics of the patients

Characteristics	All patients (n=156)	Patients with F0–F1 (n=69)	Patients with F2–F4 (n=87)	*p*
Age (years)	40.1±12.2	37.3±10.4	42.4±13.1	0.009
Male	112 (71.8)	49 (71.0)	63 (72.4)	NS
Female	44 (28.2)	20 (28.9)	24 (27.6)	NS
Body mass index (kg/m^2)	23.6±3.1	23.0±2.6	24.1±3.5	NS
Total bilirubin (mg/dL)	0.6±0.4	0.6±0.4	0.7±0.3	NS
AST (IU/L)	46.8±33.2	44.7±36.9	48.5±30.1	NS
ALT (IU/L)	75.4±55.4	69.8±59.5	79.8±51.9	NS
Alkaline phosphatase (IU/L)	69.7±21.8	65.0±14.7	72.7±25.0	NS
Albumin (g/dL))	4.4±0.4	4.5± 0.3	4.4±0.4	NS
Platelet count (10^9/L)	215.2±56.6	223.8±54.4	208.2±57.8	NS
HBeAg-positive	56 (35.9)	23 (33.3)	33 (37.9)	NS
HBeAg-negative	100 (64.1)	46 (66.7)	54 (62.1)	NS
HBV DNA ($\log_{10}$ IU/mL)	6.0±1.4	5.8±1.5	6.2±1.3	NS

Data are express as mean ± SD; no (%); NS = not significant

Table 2. Mean values of LSM and HA according to METAVIR fibrosis stages

Fibrosis stage	LSM (kPa)	HA (ng/mL)
F0 (n=19)	5.2±1.0	43.9±27.6
F1 (n=50)	5.9±2.1	64.9±48.9
F2 (n=45)	7.0±2.4	79.8±89.3
F3 (n=21)	10.4±4.8	181.8±146.6
F4 (n=21)	15.7±7.1	346.9±222.7

Data are express as mean ± SD; LSM, liver stiffness measurement; HA, hyaluronic acid

Likewise, there were significant differences in the mean HA levels between F0–F1 and F2–F4 stages (59.1±44.9 ng/mL vs 168.9±212.7 ng/mL, $p<0.001$), between F0–F2 and F3–F4 (67.3±66.5 ng/mL vs 264.4±261.3 ng/mL, $p<0.001$) and between F0–F3 and F4 (85.1±93.1 ng/mL vs 346.9±322.7 ng/mL, $p=0.001$).

ROC curve analyses for predicting the fibrosis stages

The AUROCs of LSM for predicting fibrosis stages of ≥F2, ≥F3, and F4 were 0.820 (95% CI, 0.752–0.888), 0.910 (0.851–0.968), and 0.938 (0.896–0.980), respectively. The AUROCs of HA for predicting fibrosis stages of ≥F2, ≥F3, and F4 were 0.727 (0.649–0.804), 0.848 (0.780–0.917) and 0.876 (0.806–0.947), respectively. The AUROCs of LSM for predicting fibrosis stages of ≥F2, ≥F3, and F4 were significantly higher than those of HA ($p=0.0.009$, $p=0.015$, and $p=0.031$, respectively) (**Figure 1**).

The optimal cutoff values of LSM for predicting stages of ≥F2, ≥F3, and F4 were 6.8 kPa, 8.5 kPa and 10.0 kPa, respectively. The optimal cutoff values of HA for predicting stages of ≥F2, ≥F3, and F4 were 65 ng/mL, 95 ng/mL, and 110 ng/mL, respectively. The cutoff values and the corresponding sensitivities and specificities are summarized in **Table 3**.

Factors associated with the performance of LSM and HA

Table 4 shows the results of the correlation of LSM and HA with various clinical, pathological, and laboratory parameters, including METAVIR fibrosis, BMI, platelet count, ALT, TB, albumin, AP, HBeAg, and HBV DNA. A multiple regression analysis was further performed on LSM and HA by comparison of all significant parameters. The data of multiple regression analysis showed that LSM was significantly correlated with serum ALT [odds ratio (OR), 2.116; 95% CI, 1.053–4.250; $p=0.008$) and METAVIR

fibrosis (OR, 3.929; 95% CI, 1.906–7.092; *p*=0.001), while HA was significantly correlated with METAVIR fibrosis (OR, 3.349; 95% CI, 1.678–6.681; *p*=0.001).

LSM and HA with respect to ALT levels

LSM values were significantly lower in patients with serum ALT levels ≤80 IU/L (2 × upper limit of normal; ULN) than in patients with serum ALT levels >80 IU/L in the subgroups of F0–F1 patients (5.2±1.0 kPa vs 6.8±2.7 kPa, *p*=0.001), but they did not differ significantly in the subgroup of F2 patients (6.5±1.5 kPa vs 7.5±3.1 kPa, *p*=0.155), F3 patients (8.5±2.2kPa vs 12.6±6.0 kPa, *p*=0.068) and F4 patients (15.0±7.6 kPa vs 17.4±5.9 kPa, *p*=0.504). In contrast, HA levels were not significantly different between patients with serum ALT levels ≤80 IU/L and levels >80 IU/L in subgroups of F0–F1 patients (59.1±41.4 ng/mL vs 59.2±25.5 ng/mL, *p*=0.993), F2 patients (94.4±116.2 ng/mL vs 63.2±38.4 ng/mL, *p*=0.247), F3 patients (171.1±119.6 ng/mL vs 193.5±177.6 ng/mL, *p*=0.736), and F4 patients (308.2±314.6 ng/mL vs 443.8±351.6 ng/mL, *p*=0.398).

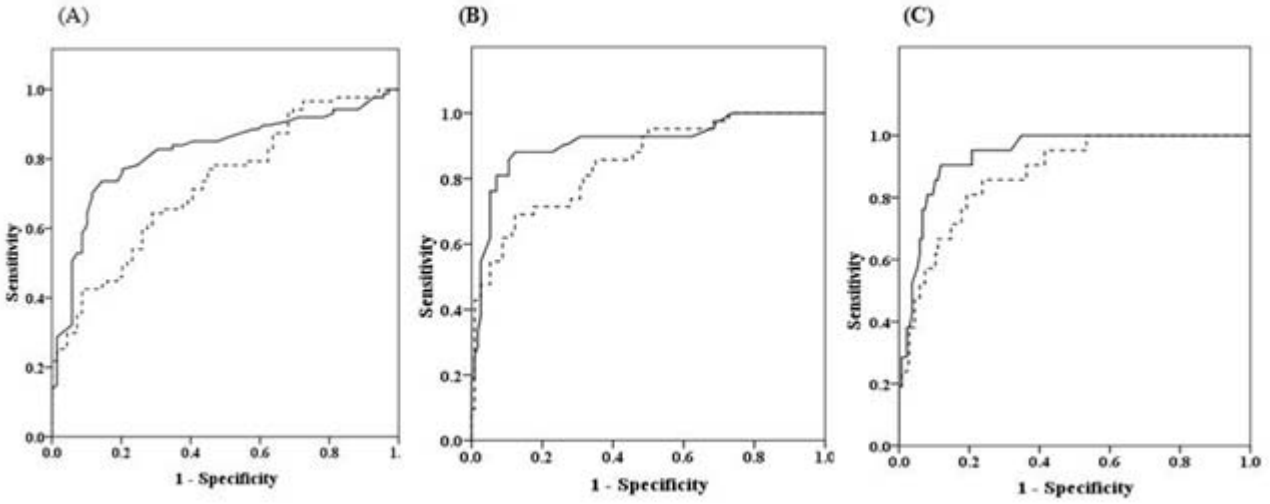

Figure 1. Receiver-operating characteristic (ROC) curves of liver stiffness measurement (LSM) and hyaluronic acid (HA) for predicting significant fibrosis (F0–F1 vs F2–F4), (B) advanced fibrosis (F0–F2 vs F3 or F4), and (C) cirrhosis (F0–F3 vs F4). –, LSM; —, HA

Table 3. Area under the receiver-operating characteristic curve (AUROCs) and cutoff values for predicting fibrosis stages

		LSM	HA
≥F2	AUROCs (95% CI)	0.820 (0.752–0.888)	0.727 (0.649–0.804)
	Cutoff values	6.8 kPa	65 ng/mL
	Sensitivity (%)	73.6	64.4
	Specificity (%)	85.5	71.0
≥F3	AUROCs (95% CI)	0.910 (0.851–0.968)	0.848 (0.780–0.917)
	Cutoff values	8.5kPa	95ng/mL
	Sensitivity (%)	88.1	71.4
	Specificity (%)	87.7	82.5
F4	AUROCs (95% CI)	0.938 (0.896–0.980)	0.876 (0.806–0.947)
	Cutoff values	10.0 kPa, 110 ng/mL	
	Sensitivity (%)	90.5	81.0
	Specificity (%)	88.1	80.7

LSM = liver stiffness measurement, HA = hyaluronic acid, PPV = positive predictive value, NPV = negative predictive value

Table 4. Parameters correlated with LSM and HA

Parameter	LSM		HA	
	r	*p*	r	*p*
Body mass index (kg/m^2)	0.218	0.030	0.001	0.990
METAVIR fibrosis	0.636	<0.001	0.516	<0.001
Total bilirubin (mg/dL)	0.056	0.504	0.242	0.004
ALT level (IU/L)	0.553	<0.001	–0.013	0.871
Alkaline phosphatase (IU/L)	0.467	<0.001	0.248	0.007
Albumin (g/dL))	–0.292	<0.001	–0.431	<0.001
Platelet count (10^9/L)	–0.245	0.002	–0.268	0.001
HBeAg	–0.078	0.330	–0.119	0.140
HBV DNA ($\log_{10}$ IU/mL)	0.092	0.255	0.031	0.704

ROC curve analyses of LSM and HA with respect to ALT levels

The AUROCs of LSM for predicting fibrosis stage of ≥F2 were significantly higher in patients with serum ALT levels ≤80 IU/L than in patients with serum ALT levels >80 IU/L [0.864 (0.788–0.941) vs 0.733 (0.602–0.864), *p*=0.040]. However, there were no statistically significant differences for predicting fibrosis stages of ≥F3 [0.936 (0.872–1.000) vs 0.892 (0.786–0.999), *p*=0.121] and F4 [0.967 (0.925–1.000) vs 0.934 (0.869–1.000), *p*=0.458]. By contrast, the AUROCs of HA values for predicting fibrosis stages of ≥F2, ≥F3, and F4 were not significantly different between patients with serum ALT levels ≤80 IU/L and patients with serum ALT levels >80 IU/L [0.757 (0.663–0.851) vs 0.686 (0.548–0.823), *p*=0.167; 0.845 (0.755–0.935) vs 0.844 (0.732–0.956), *p*=0.844 and 0.862 (0.780–0.958) vs 0.893 (0.744–1.000), *p*=0.741, respectively].

Regarding only patients with serum ALT levels ≤80 IU/L, the AUROCs of LSM for predicting fibrosis stages of ≥F2, ≥F3, and F4 were significantly higher than those of HA values with the same fibrosis stages. By contrast, in patients with serum ALT levels >80 IU/L, the AUROCs for predicting fibrosis stages of ≥F2, ≥F3, and F4 did not differ significantly between LSM and HA values (**Table 5**).

Discussion

The discrimination between absent/mild fibrosis (F0–F1) and significant fibrosis to cirrhosis (F2–F4) in chronic viral hepatitis has essential clinical implications for clinicians in deciding therapeutic options, monitoring disease progression, and to determine prognosis of the patients. Our study demonstrates that LSM is an accurate noninvasive technique for the assessment of fibrosis in patients with chronic hepatitis B. For example, LSM was able to accurately discriminate between patients with METAVIR F0–F1 and F2–F4 (AUROC 0.82) and even better between patients with F0–F2 and F3–F4 (AUROC 0.91) and between patients with F0–F3, and F4 (AUROC 0.94). These data are consistent with recent studies conducted on Asian and Caucasian

Table 5. Area under the receiver-operating characteristic curve (AUROCs) with respect to serum ALT levels

		LSM	HA	*p*
ALT ≤80 IU/L	≥F2	0.864 (0.788–0.941)	0.757 (0.663–0.851)	0.009
	≥F3	0.936 (0.872–1.000)	0.845 (0.755–0.935)	0.002
	F4	0.967 (0.925–1.000)	0.862 (0.780–0.958)	0.006
ALT >80 IU/L	≥F2	0.733 (0.602–0.864)	0.696 (0.558–0.823)	0.626
	≥F3	0.892 (0.786–0.999)	0.844 (0.732–0.956)	0.778
	F4	0.934 (0.869–0.999)	0.893 (0.744–1.000)	0.907

Data are expressed as AUROCs (95% confidence intervals), LSM = liver stiffness measurement, HA = hyaluronic acid.

patients with chronic hepatitis B[6-8, 17-21]. In a French multicenter study, Marcellin et al. found that the AUROCs in differentiating F0–F1 vs F2–F4, F0–F2 vs F3–F4 and F0–F3 vs F4 were 0.81, 0.93, and 0.93, respectively[6]. Similarly, a study conducted in Taiwan by Wang et al. showed that the AUROCs in differentiating METAVIR F0–F1 vs F2–F4, F0–F2 vs F3–F4, and F0–F3 vs F4 were 0.86, 0.88 and 0.89, respectively[18]. A meta-analysis of 50 studies evaluating LSM in chronic liver disease of various etiologies has shown that the mean AUROCs for predicting significant fibrosis, advanced fibrosis and cirrhosis were 0.84, 0.89, and 0.94, respectively [22].

By maximizing sensitivity and specificity, the optimal cutoff values of LSM for ≥F2 (6.8 kPa), ≥F3 (8.5 kPa) and F4 (10.0 kPa) in this study were also comparable with most previous reports, ranging from 6.0–8.0 pKa, 8.1–8.8 pKa and 9.0–14.0 pKa, respectively[6-8, 17-21]. Using a cutoff level of 10.0 kPa, our data showed that LSM exhibited high sensitivity and specificity (90.5% and 88.1%, respectively) for estimating the presence of cirrhosis. This was in agreement with the French study in which the sensitivity and specificity for diagnosing cirrhosis using a cutoff of 11.0 kPa were 93% and 87%, respectively[6]. However, it should be mentioned that the cutoff points for estimating the presence of cirrhosis vary significantly among different studies (9.0–14.0 pKa). This discrepancy might be related to several factors including the different populations studied and different study design methodology.

Previous studies have demonstrated that acute severe flares of hepatitis, as defined by ALT>10 × ULN, could significantly affect LSM values [23-25]. Subsequent studies have also shown that even milder degrees of ALT elevation are associated with significantly higher values of LSM, and therefore might reduce the diagnostic accuracy of the test[8, 19, 26-28]. In the current study, there was a significant correlation between serum ALT levels and LSM scores. Moreover, ALT level was associated with LSM in addition to histological fibrosis in multiple regression analysis. Our results were in accordance with previous data that ALT elevation (>2 × ULN) significantly reduced the AUROCs of LSM, particularly in patients with F0–F1 fibrosis stages, while the diagnostic accuracy did not differ significantly in the subgroups of patients with F3–F4. In this study, the influence of biochemical activity on LSM was noticeable in the subset of patients with F0–F1 fibrosis in whom mean LSM values were significantly lower than in patients with ALT elevation, but with the same histological stages (5.2±1.0 kPa vs 6.8±2.7 kPa, p=0.001). These results indicate that patients with mild degree of fibrosis might more likely be overestimated by LSM to the extent of advanced fibrosis or cirrhosis because of their high ALT levels.

To minimize the risk of overestimating fibrosis by LSM during hepatitis flares, different strategies have been proposed. First, it is recommended to perform or repeat LSM after ALT normalization is achieved. By delaying LSM until ALT is normal or near normal, the false-positive results in diagnosing cirrhosis would be greatly reduced [19, 27]. In cases where ALT is persistently elevated, use of an algorithm that will stratify different cutoff LSM scores according to ALT levels is another option. In this respect, a recently published ALT-based algorithm has been developed for patients with chronic hepatitis B, using higher LSM values for optimal cutoffs in those with elevated ALT levels[8]. An alternative approach would be to utilize serum markers of liver fibrosis alone or in combination with LSM values to improve the diagnostic accuracy [29-32]. However, until now, only limited studies have taken into consideration the ALT levels in the assessment of available serum markers.

Several serum markers have been developed in recent years, including APRI, FIB-4, Forns' index, Fibrotest, and FibroSpect. However, the clinical applicability of Forns' index, Fibrotest, and FibroSpect is rather limited because these markers involve complex mathematical calculations. Likewise, although APRI and FIB-4 can be easily calculated from simple biochemical parameters, these tests take serum aminotransferase into account and are likely be affected by ALT elevation. In this study, we chose to compare the accuracy of LSM against serum HA in conjunction with ALT levels for two reasons. First, HA is generally considered to be the best individual serum marker available. Second, previous studies demonstrated that HA concentrations were not confounded by the grade of necroinflammation activity [12, 14]. Thus, the advantage of HA over the other simple noninvasive markers was that HA might not be affected by an increase in ALT level. This anticipation was supported by our current data showing that ALT levels were not correlated with HA concentrations and, as a result, did not affect its accuracy.

In the current study, determination of HA levels was accurate in predicting significant fibrosis, advanced fibrosis, and cirrhosis, with AUROCs of 0.73, 0.85, and 0.88, respectively.

These results are consistent with another study conducted in patients with chronic hepatitis C in which the AUROCs of the same fibrotic stages were 0.75, 0.82, and 0.89, respectively [33]. Our results are also comparable to another recent study in patients with chronic viral hepatitis (54% were chronic hepatitis B) showing the AUROCs of 0.72, 0.81 and 0.86, respectively[29]. Although HA was diagnostically inferior to LSM at identifying significant and advanced fibrosis, the performance of HA and LSM was not statistically significant if considering only the subgroup of patients with high ALT levels. HA yielded AUROCs of 0.84 and 0.89 for predicting advanced fibrosis and cirrhosis, respectively, which were considered satisfactory and comparable to those of LSM (AUROCs of 0.89 and 0.93, respectively). These data suggest that the sole measurement of HA may be appropriate and adequate to predict advanced fibrosis and cirrhosis in patients with high ALT levels. Thus, this method can be adopted without delay during ALT elevation and facilitates the clinical management of patients when LSM is not applicable.

In conclusion, our data showed that LSM was superior to HA in predicting liver fibrosis and cirrhosis in patients with chronic hepatitis B. There was significant correlation between ALT level and LSM value, while such correlation between ALT and HA was not found. As a consequence, the performance of LSM was reduced in patients with ALT elevation and its diagnostic accuracy was rather comparable to that of HA in this subgroup of patients. Thus, HA could be an alternative and accurate method for assessing liver fibrosis in patients with ALT elevation.

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