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ULTRASOUND

Shear wave elastography for liver fibrosis in chronic hepatitis B: Adapting the cut-offs to alanine aminotransferase levels improves accuracy

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Abstract

Objectives To determine and validate alanine aminotransferase (ALT)-adapted dual cut-offs of liver stiffness measurements (LSMs) for assessing liver fibrosis with two-dimensional shear wave elastography (2D-SWE) in patients with chronic hepatitis B (CHB) infection.

Methods Patients with CHB infection who underwent liver biopsy to assess liver fibrosis were consecutively included. 2D-SWE confirmation thresholds with a positive likelihood ratio ≥10 and 2D-SWE exclusion thresholds with a negative likelihood ratio ≤0.1 were identified to rule in or rule out significant fibrosis and cirrhosis, respectively.

Results The first 515 patients (index cohort) and the next 421 patients (validation cohort) were included in the final analysis. The low and high cut-offs to rule out and rule in patients with significant fibrosis (≥ F2) were 5.4 kPa and 9.0 kPa, respectively, in patients with ALT levels ≤ 2 × the upper limit of normal (ULN) and 7.1 kPa and 11.2 kPa in patients with ALT levels > 2 × ULN. For cirrhosis (F4), the corresponding values were 8.1 kPa and 12.3 kPa in patients with ALT levels ≤ 2 × ULN and 11.9 kPa and 24.7 kPa in patients with ALT levels > 2 × ULN. The dual cut-off values showed an overall accuracy of more than 90% for diagnosis of the presence or absence of significant fibrosis and cirrhosis in the index and validation cohorts. There were no significant differences in the accuracy values between the cohorts (all p>0.05).

Conclusion The ALT-adapted dual cut-offs of LSMs showed high accuracy for diagnosis of the presence or absence of significant fibrosis and cirrhosis in patients with CHB infection.

Key Points

- *The ALT-adapted dual cut-off values of LSMs showed high accuracy for diagnosis of the presence or absence of significant fibrosis and cirrhosis.*
- *ALT levels did not influence the overall diagnostic accuracy for predicting significant fibrosis and cirrhosis.*
- *The ALT-adapted dual cut-offs in patients with ALT levels > 2 × ULN were markedly higher than those in patients with ALT levels ≤ 2 × ULN.*

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Keywords Elasticity imaging techniques · Ultrasonography · Hepatitis B · Alanine transaminase · Sensitivity and specificity

Abbreviations

2D-SWE	Two-dimensional shear wave elastography
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APRI	Aspartate aminotransferase-to-platelet index
AST	Aspartate aminotransferase
AUROCs	Areas under the ROC curves
BMI	Body mass index
CHB	Chronic hepatitis B
GGT	Gamma-glutamyl transpeptidase
kPa	Kilopascal
LB	Liver biopsy
LR+	Positive likelihood ratio
LR-	Negative likelihood ratio
LSM	Liver stiffness measurement
ROC	Receiver operating characteristic
ROI	Region of interest
SD	Standard deviation
TE	Transient elastography
ULN	Upper limit of normal

Introduction

The measurement of liver stiffness using ultrasound elastography (i.e., transient elastography (TE)) has been recommended for the non-invasive staging of liver fibrosis by the European and Asian-Pacific clinical practice guidelines on the management of chronic hepatitis B (CHB) [1, 2]. Two-dimensional shear wave elastography (2D-SWE) is an ultrasound elastography technique based on shear waves implemented on a diagnostic ultrasound system, with the advantage of liver stiffness imaging in real time and simultaneous anatomic B-mode ultrasound imaging [3–5]. The overall accuracy of 2D-SWE for staging liver fibrosis is high, and this technology is an excellent modality for the non-invasive evaluation of the severity of liver fibrosis [6–9].

The Society of Radiologists in an ultrasound consensus conference statement indicated that a single cut-off value is determined for each stage of fibrosis in most studies; however, there is substantial overlap of liver stiffness measurements (LSMs) between fibrosis stages, and considering stiffness values as a continuum may be more appropriate [10]. The EFSUMB guidelines and recommendations on the clinical use of liver ultrasound elastography, in the 2017 update, also indicated that LSMs show a substantial overlap among adjacent stages of fibrosis [11]. However, no published studies have determined two cut-off values for the staging of liver fibrosis by 2D-SWE. A high cut-off is employed to confirm a particular stage of fibrosis, and a low cut-off is employed for exclusion.

Because of the complex natural history of CHB infection, which frequently presents as fluctuating patterns associated with necro-inflammatory activity [2], the EFSUMB guidelines and recommendations on the clinical use of liver ultrasound elastography, updated in 2017, stated that LSM cut-offs should be adapted to alanine aminotransferase (ALT) levels since transaminase levels tend to influence LSMs in CHB, and hepatitis flares are often observed in CHB [11]. It has been reported that inflammation adapted liver stiffness cut-off values both for chronic hepatitis C and alcoholic liver disease improved the diagnostic accuracy and the agreement with histological fibrosis stages [12].

Therefore, the goal of this study was to determine and validate clinical methods for assessing liver fibrosis by 2D-SWE using dual cut-off values of LSMs and integrating ALT levels into the analysis in patients with CHB infection using the histological METAVIR scoring system as the reference standard.

Materials and methods

Patients

Between January 2012 and August 2016, patients with CHB infection who were consecutively admitted to our hospital to undergo liver biopsy (LB) for assessing liver fibrosis and necro-inflammation were enrolled in this study. CHB infection was diagnosed when the hepatitis B surface antigen and hepatitis B virus DNA were present in the serum for at least 6 months. Patients were excluded from enrolment using the following criteria: no consent for 2D-SWE examination; younger than 18 years; chronic hepatitis caused by another hepatitis virus or disease; a transplanted liver; biopsy samples less than 15 mm long or with fewer than six portal tracts under a microscope; and undergoing antiviral therapy. No patients with obstructive cholestasis were included in the study. Patients with focal liver lesions or obvious cirrhosis were not especially excluded from analyses. There were no patients with huge liver cancer. Patient characteristics, epidemiological data and biochemical test results were recorded. All participants provided informed written consent. The study protocol was approved by the clinical medical research ethics committee of our hospital.

2D-SWE

The 2D-SWE measurements were obtained using an Aixplorer US system (SuperSonic Imagine) with a broadband convex probe (SC6-1) within 3 days after LB. Three

radiologists (J. Zeng, J. Zheng and J.Y. Jin), each of whom were blinded to the patients' clinical information and pathology results, performed the procedures. All radiologists had at least 2 years of experience in B-mode ultrasound and 6 months of experience in 2D-SWE examinations. Patients had fasted for at least 6 h and rested for at least 10 min before undergoing examination. 2D-SWE measurements were performed on the right lobe of the liver, through intercostal spaces with the patient lying in the supine position and the right arm in maximal extension, during transient breath holding in a neutral position [11]. The colour-coded elasticity image box, approximately 4 × 3 cm, was placed 1–2 cm deeper than the liver capsule in a parenchyma-area free of large vessels. A circular region of interest (2 cm in diameter) was positioned inside the 2D-SWE box mainly in the centre of the box, and the mean elasticity displayed was recorded [13]. Five consecutive acquisitions (lasting 3–5 min) were performed in the same location of the right lobe, and each measurement was performed during a separate breath hold. Measurements were classified as failed when no or little signal was obtained in the 2D-SWE box for all acquisitions [13, 14]. The mean of five measurements in kilopascals was used for statistical analysis [13, 15].

Serum liver fibrosis index

The aspartate aminotransferase-to-platelet index (APRI) has previously been shown to be a simple and efficient index for the non-invasive assessment of liver fibrosis [16, 17]. The APRI were calculated as follows [18]:

$$\mathrm{APRI} = [(\mathrm{AST}/\text{upper limit of normal AST}) \times 100]/\text{platelet count } (10^9/\mathrm{L})$$

Liver histology assessment

Ultrasound-guided percutaneous biopsy was performed on the right lobe of the liver using a 16-gauge Magnum needle (Bard). Biopsy specimens were fixed in formalin and embedded in paraffin. Two liver pathologists with more than 10 years of experience separately analysed the biopsy specimens and were blinded to the 2D-SWE values but not to the clinical information. Fibrosis was staged on a 5-point scale (from 0 to 4) according to the METAVIR scoring system: stage F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis [19]. Significant fibrosis was defined as stage F2 or higher, and severe fibrosis was defined as stage F3 or higher. Necro-inflammatory activity was graded as follows: A0 = none; A1 = mild; A2 = moderate; and A3 = severe [19].

Statistical analysis

The D'Agostino-Pearson test was used to test the normal distribution of quantitative variables. Correlations between LSMs and fibrosis stages or necro-inflammatory activity grades were calculated using the Spearman correlation coefficient. The Mann-Whitney test or independent samples t-test was used to compare continuous variables, as appropriate. The chi-square test or Fisher's exact test was used to compare proportions, as appropriate. The diagnostic performances of 2D-SWE and APRI for predicting significant fibrosis and cirrhosis were assessed with receiver operating characteristic (ROC) curves. 2D-SWE confirmation thresholds with a positive likelihood ratio (LR+) ≥10 and 2D-SWE exclusion thresholds with a negative likelihood ratio (LR-) ≤0.1 were identified to rule in or rule out significant fibrosis and cirrhosis, respectively [20–22]. The cohort was divided into an index cohort and a validation cohort. The upper limit of normal (ULN) for ALT is 40 U/L [2]. Serum ALT level is termed as minimally raised serum ALT if between ULN and 2 × ULN of ALT level; and as raised ALT if > 2 × ULN [2]. The patients in the index cohort were analysed according to different ALT levels. The areas under the ROC curves (AUROCs) were compared using the Delong test [23]. Statistical analyses were performed using SPSS software (version 13.0; SPSS) and MedCalc software (version 12.7; MedCalc Software bvba). For all tests, $p<0.05$ indicated a significant difference or correlation.

Results

In total, 1,113 patients were eligible for inclusion in the study during the recruitment period (Fig. 1). 160 patients were excluded from the study, including 28 patients younger than 18 years, eight patients who declined to provide consent, 89 patients with biopsy samples less than 15 mm long or with fewer than six portal tracts upon microscopic examination, three patients with hepatitis C virus coinfection, two patients with autoimmune hepatitis, 26 patients undergoing antiviral therapy and four patients with a transplanted liver. Thus, the final cohort consisted of 953 patients. The index cohort consisted of 524 patients enrolled between January 2012 and June 2014, and the validation cohort consisted of 429 patients enrolled between July 2014 and August 2016. The patient characteristics are summarised in Table 1. There were no significant differences between the two groups. The 2D-SWE examination failed in 17 patients. Therefore, a total of 515 patients in the index cohort and 421 patients in the validation cohort with valid LSMs were included in the final analysis.

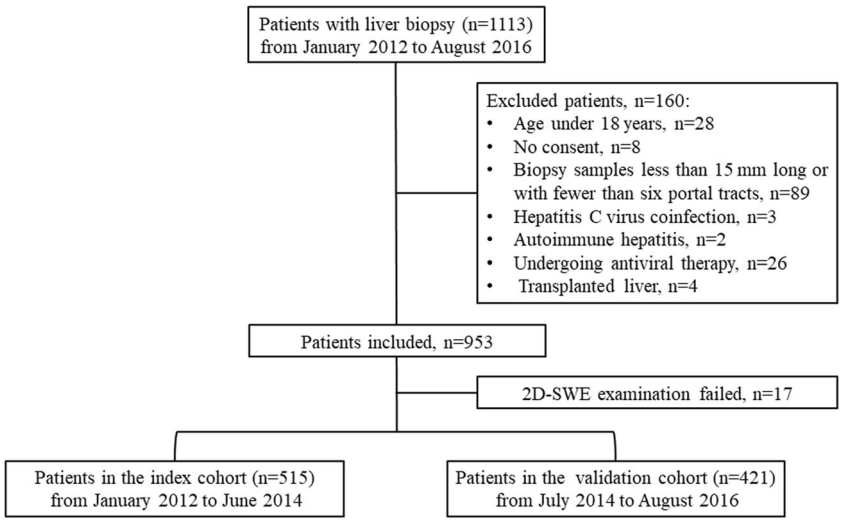

Fig. 1 Flowchart of study patients

Table 1 Demographic characteristics and biochemical and histological data of patients with chronic hepatitis B infection in the index and validation cohorts

Characteristics	Index cohort (n = 524)	Validation cohort (n = 429)	*p*-value
Age, median (IQR) [range], years	36.0 (29.0–43.0) [18–67]	37.0 (31.0–43.0) [18–65]	0.11
Male, n (%)	403 (76.9)	339 (79.0)	0.49
BMI, median (IQR) [range], kg/m^2	21.9 (19.8–23.9) [14.5–33.5]	22.0 (19.9–24.4) [14.4–41.4]	0.38
AST, median (IQR) [range], U/L	32.0 (25.0–49.0) [13.0–465.0]	32.0 (25.0–48.0) [13.0–484.0]	0.55
ALT, median (IQR) [range], U/L	43.0 (28.0–72.5) [5.0–976.0]	38.0 (27.8–64.0) [7.0–736.0]	0.20
ALP, median (IQR) [range], U/L	70.0 (57.0–87.0) [27.0–324.0]	72.0 (61.0–89.0) [37.0–245.0]	0.07
GGT, median (IQR) [range], U/L	32.5 (21.0–70.5) [7.0–1615.0]	30.0 (20.0–54.0) [9.0–641.0]	0.13
Total bilirubin, median (IQR) [range], umol/L	14.0 (10.6–18.2) [3.5–241.6]	13.4 (10.7–18.5) [4.0–247.7]	0.68
Serum albumin, median (IQR) [range], g/L	43.7 (40.5–45.8) [18.3–53.7]	43.8 (41.1–46.1) [16.6–53.5]	0.29
Platelets count, median (IQR) [range], 10^9/L	186.0 (149.0–228.0) [47.0–380.0]	190.0 (154.0–227.5) [39.0–472.0]	0.44
Prothrombin activity, median (IQR) [range], %	97.0 (90.0–106.0) [57.0–149.0]	97.0 (87.0–105.0) [56.0–147.0]	0.09
METAVIR fibrosis stage*, n (%)			0.55
F0	95 (18.1)	73 (17.0)	
F1	156 (29.8)	146 (34.0)	
F2	122 (23.3)	86 (20.0)	
F3	79 (15.1)	61 (14.2)	
F4	72 (13.7)	63 (14.7)	
METAVIR activity grade†, n (%)			0.71
A0	14 (3.0)	16 (4.0)	
A1	229 (43.7)	184 (42.9)	
A2	168 (32.1)	130 (30.3)	
A3	113 (21.6)	99 (23.1)	

IQR inter quartile range, *BMI* body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma-glutamyl transpeptidase.

*F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis

† A0, none; A1, mild; A2, moderate; and A3, severe

Table 2 Median values, interquartile ranges, ranges and *p*-values of liver stiffness measurements in kilopascals obtained for each fibrosis stage with two-dimensional shear wave elastography in the index and validation cohorts

METAVIR Stage	Cohort	F0	F1	F2	F3	F4
Median value	Index cohort	5.8	6.4	8.3	10.2	17.6
	validation cohort	6.0	6.4	8.2	11.1	17.3
IQR	Index cohort	5.3–6.5	5.5–7.5	7.2–10.8	8.6–13.5	13.8–26.0
	validation cohort	5.3–6.5	5.8–7.6	6.8–10.7	9.4–15.4	12.3–22.6
Range	Index cohort	3.9–25.1	3.5–14.7	5.0–34.0	5.2–33.3	7.2–70.9
	validation cohort	4.3–15.4	3.9–20.5	4.7–26.7	5.5–26.0	7.5–41.4
p-value*	Index cohort		<0.001	<0.001	<0.001	<0.001
	validation cohort		<0.001	<0.001	<0.001	<0.001

IQR interquartile range

**p*-values refer to differences between consecutive fibrosis stages

Relationship between LSMs and histological liver analysis findings

Median values, interquartile ranges, ranges and *p*-values of LSMs obtained for each fibrosis stage with 2D-SWE are shown in Table 2. As the fibrosis stage increased, the median LSM of the fibrosis stage increased (Fig. 2). There were significant differences in LSMs between consecutive stages in both the index and validation cohorts (all $p<0.001$). The Spearman correlation coefficients for the correlations were 0.76 ($p<0.001$) between the LSMs and fibrosis stages and 0.65 ($p<0.001$) between the LSMs and necro-inflammatory activity grades in the index cohort.

LSMs in patients with different ALT levels

The patients in the index cohort were analysed according to different ALT levels: normal ALT levels (n=261), ALT levels 1–2 × ULN (n=141) or ALT levels > 2 × ULN (n=113) (Table 3). There were no significant differences between the LSMs of patients with normal ALT levels and ALT levels 1–2 × ULN within the same fibrosis stage (all $p>0.05$). Except for stage F0, the median LSMs of patients with ALT levels > 2 × ULN in each fibrosis stage were significantly higher than the median LSMs of patients with normal ALT levels and ALT levels 1–2 × ULN within the same fibrosis stage (all $p<0.05$).

Diagnostic accuracy of 2D-SWE and APRI for predicting significant fibrosis and cirrhosis, stratified by ALT level, in the index and validation cohorts

The AUROCs of 2D-SWE and APRI for predicting significant fibrosis and cirrhosis stratified by ALT level in the index and validation cohorts are shown in Fig. 3. The patients were analysed according to different ALT levels: ALT levels ≤ 2 × ULN (n=402) or > 2 × ULN (n=113) in the index cohort; ALT levels ≤ 2 × ULN (n=331) or > 2 × ULN (n=90) in the validation cohort (Table 4).

There were no significant differences in the AUROCs of 2D-SWE for predicting significant fibrosis and cirrhosis between the cohorts of patients with different ALT levels (all $p>0.05$). There were also no significant differences in the AUROCs of 2D-SWE between patients with ALT levels ≤ 2 × ULN and patients with ALT levels > 2 × ULN in both the index and validation cohorts (all $p>0.05$). The AUROCs of

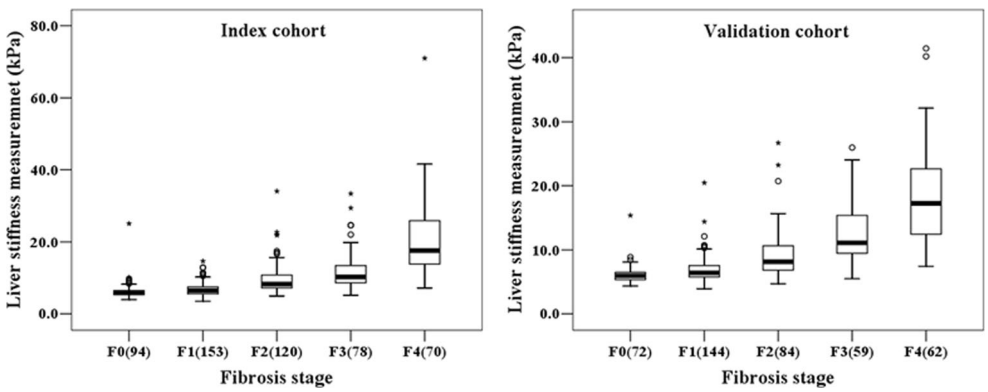

Fig. 2 Box plots of liver stiffness measurements obtained using two-dimensional shear wave elastography for different fibrosis stages in patients with chronic hepatitis B infection in the index and validation cohorts. The central box represents the interquartile range. The line through each box represents the median. Error bars show minimum and maximum non-extreme values

Table 3 Median values and interquartile ranges of liver stiffness measurements in kilopascals obtained using two-dimensional shear wave elastography for each fibrosis stage in patients with different alanine aminotransferase (ALT) levels in the index cohort

ALT level	F0	F1	F2	F3	F4
Normal	5.8 (5.3–6.1) (n=58)	6.1 (5.3–6.8) (n=85)	7.8 (6.7–10.4) (n=57)	10.2 (8.7–12.3) (n=38)	15.7 (11.6–22.2) (n=23)
1–2 × ULN	5.7 (5.1–7.1) (n=24)	6.4 (5.7–7.8) (n=39)	8.4 (7.5–9.6) (n=32)	9.9 (8.4–11.5) (n=20)	16.0 (13.8–25.3) (n=26)
> 2 × ULN	6.0 (5.8–7.1) (n=12)	8.0 (6.7–9.4) (n=29)	9.7 (8.1–13.8) (n=31)	13.3 (9.0–21.0) (n=20)	21.6 (17.4–29.7) (n=21)

ULN upper limit of normal

2D-SWE were significantly higher than those of APRI in both the index and validation cohorts in patients with different ALT levels (all $p<0.05$).

ALT-adapted dual cut-offs of LSMs obtained using 2D-SWE for determining the presence or absence of significant fibrosis and cirrhosis

We used LR threshold values of 10.0 and 0.1 for defining the presence ("high" result value) or absence ("low" result value), respectively, of significant fibrosis and cirrhosis via analysis of the ROCs for 2D-SWE in the index cohort. The sensitivity, specificity, accuracy, LR+, LR- and patients in the determinate ranges of the ALT-adapted dual cut-off values for significant fibrosis and cirrhosis are shown in Table 5.

The cut-off values of LSMs in patients with ALT levels >2×ULN were markedly higher than those in patients with ALT levels ≤2×ULN. The ALT-adapted dual cut-off values of LSMs showed an overall accuracy of more than 90% for diagnosis of the presence or absence of significant fibrosis and cirrhosis. There were no significant differences in the accuracy values between the index and validation cohorts in patients with different ALT levels (all $p>0.05$). The ALT-adapted dual cut-off values allowed diagnosis of the presence or absence cirrhosis in approximately 70% patients. However, there were only more than 40% of the patients with ALT levels ≤2×ULN in the determinate range for diagnosis of the presence or absence of significant fibrosis.

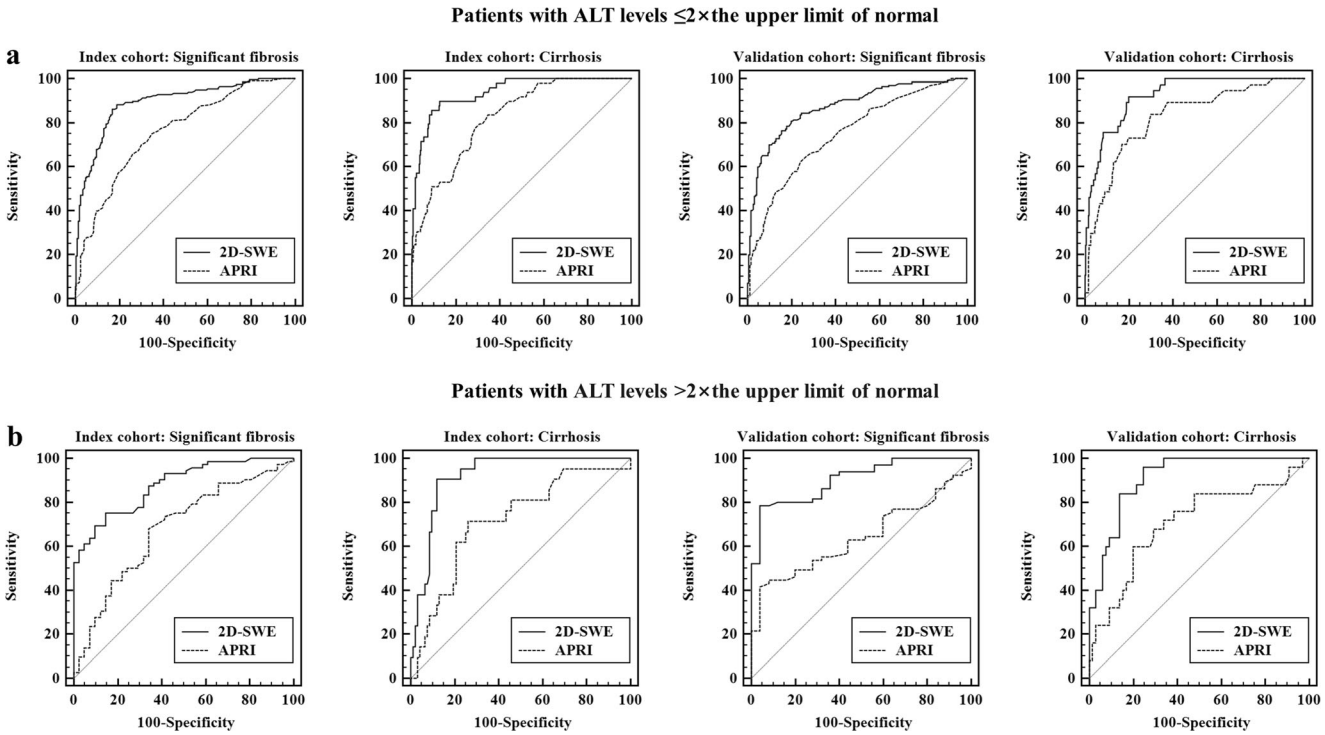

Fig. 3 Areas under the receiver operating characteristic curves (AUROCs) of two-dimensional shear wave elastography (2D-SWE) and the aspartate aminotransferase-to-platelet ratio index (APRI) for predicting significant fibrosis and cirrhosis in patients with (**a**) ALT levels ≤ 2 × the upper limit of normal (ULN) or (**b**) ALT levels >2 × ULN. The AUROCs of 2D-SWE were significantly higher than those of APRI for predicting significant fibrosis and cirrhosis in the index and validation cohorts in patients with different ALT levels. There were no significant differences in the AUROCs of 2D-SWE for predicting significant fibrosis and cirrhosis between the cohorts in patients with different ALT levels

Table 4 Areas under the receiver-operating characteristic curves of two-dimensional shear wave elastography (2D-SWE) and the aspartate aminotransferase-to-platelet ratio index (APRI) for predicting significant fibrosis (≥F2) and cirrhosis (F4) in patients with different alanine aminotransferase (ALT) levels in the index and validation cohorts

Method	ALT level	Index cohort (n=515)		Validation cohort (n=421)	
		≥F2 (95% CI)	F=4 (95% CI)	≥F2 (95% CI)	F=4 (95% CI)
2D–SWE	ALT ≤2 × ULN	0.89 (0.86–0.92)	0.94 (0.91–0.96)	0.87 (0.83–0.91)	0.92 (0.89–0.95)
	ALT >2 × ULN	0.88 (0.80–0.93)	0.92 (0.85–0.96)	0.90 (0.82–0.96)	0.91 (0.83–0.96)
APRI	ALT ≤2 × ULN	0.76 (0.72–0.80)	0.82 (0.78–0.86)	0.75 (0.70–0.80)	0.82 (0.78–0.86)
	ALT >2 × ULN	0.68 (0.59–0.76)	0.72 (0.62–0.80)	0.64 (0.53–0.74)	0.71 (0.60–0.80)

ULN upper limit of normal, *CI* confidence interval

Discussion

Both the WFUMB and EFSUMB guidelines and recommendations on the clinical use of liver ultrasound elastography indicate that hepatic inflammation associated with high ALT levels elevates LSMs, independently of fibrosis, and is an important confounding factor in the use of ultrasound elastography for non-invasive staging of liver fibrosis [11, 24]. The patients with ALT levels > 2 × ULN showed significantly increased LSMs. The results of our study support the opinion provided in these guidelines that LSMs show higher values when aminotransferase levels are elevated.

The AUROCs obtained in our study were similar to those in patients with CHB infection reported in the EFSUMB guidelines [11]. Furthermore, the AUROCs in patients with ALT levels > 2 × ULN were similar to those in patients with ALT levels ≤ 2 × ULN in both the index and validation cohorts. The results of our study indicated and verified that although the patients with ALT levels > 2 × ULN showed significantly increased LSMs, ALT levels did not influence the overall diagnostic performance. The increased LSMs mean that baseline levels are increased in patients with ALT levels > 2 × ULN, but this did not affect the overall diagnostic accuracy of 2D-SWE for assessing liver fibrosis stages in these patients.

Table 5 Performance characteristics of two-dimensional shear wave elastography for diagnosis of the presence or absence of significant fibrosis and cirrhosis in patients with chronic hepatitis B infection. The characteristics are based on dual cut-off values of liver stiffness measurements. Pathologic analysis was the diagnostic reference standard

	Cut-off *	Index cohort	Validation cohort
Patients with ALT ≤2 × ULN			
Sensitivity to exclude significant fibrosis	≤5.4	97.4% (LR- = 0.10)	98.6% (LR- = 0.06)
Specificity to confirm significant fibrosis	>9.0	94.7% (LR+ = 10.4)	95.3% (LR+ = 12.7)
Overall accuracy to exclude and confirm significant fibrosis	≤5.4 and >9.0	92.0%	91.4%
Patients in the determinate range of significant fibrosis		44.8% (180/402)	42.0% (139/331)
Sensitivity to exclude cirrhosis	≤8.1	93.9% (LR- = 0.09)	91.9% (LR- = 0.11)
Specificity to confirm cirrhosis	>12.3	92.9% (LR+ = 10.4)	93.9% (LR+ =10.2)
Overall accuracy to exclude and confirm cirrhosis	≤8.1 and >12.3	90.7%	91.3%
Patients in the determinate range of cirrhosis		74.9% (301/402)	76.7% (254/331)
Patients with ALT >2 × ULN			
Sensitivity to exclude significant fibrosis	≤7.1	95.8% (LR- = 0.09)	93.8% (LR- = 0.10)
Specificity to confirm significant fibrosis	>11.2	95.1% (LR+ = 12.5)	96.0% (LR+ = 17.3)
Overall accuracy to exclude and confirm significant fibrosis	≤7.1 and >11.2	92.6%	96.9%
Patients in the determinate range of significant fibrosis		60.2% (68/113)	72.2% (65/90)
Sensitivity to exclude cirrhosis	≤11.9	95.2% (LR- = 0.06)	96.0% (LR- = 0.06)
Specificity to confirm cirrhosis	>24.7	96.7% (LR+ = 11.7)	96.9% (LR+ = 10.4)
Overall accuracy to exclude and confirm cirrhosis	≤11.9 and >24.7	95.2%	94.9%
Patients in the determinate range of cirrhosis		73.5% (83/113)	65.6% (59/90)

ULN upper limit of normal, *ALT* alanine aminotransferase, *LR* likelihood ratio

*Cut-off values were calculated from the index cohort and cited in kilopascals

Due to the increased baseline levels of LSMs in patients with elevated ALT levels, overestimation of liver fibrosis might occur in this cohort. Correspondingly increased cut-off values might be appropriate for this cohort to avoid overestimation of liver fibrosis. The cut-off values of LSMs in patients with ALT levels > 2 × ULN were markedly higher than those in patients with ALT levels ≤ 2 × ULN in our study. Although ALT levels did not influence the overall diagnostic accuracy, they affected the LSM cut-off values. Different cut-offs were defined for different ALT levels in the present study, which could help to prevent inappropriate overestimation of liver fibrosis stages in those patients with increased baseline LSMs caused by inflammation. The results of our study support the statement of the EFSUMB guidelines and recommendations on the clinical use of liver ultrasound elastography in that LSM cut-offs should be adapted to ALT levels [11].

Because of the substantial overlap between fibrosis stages, the single cut-off values of LSMs for each stage of fibrosis obtained using 2D-SWE correctly classified only 64.4% of patients with CHB infection in the validation cohort in our previous study [25]. The most important question in a patient with chronic liver disease is whether or not the patient has cirrhosis [10]. The positive predictive value of the single cut-off value for cirrhosis was 60.0% in the validation cohort in our previous study [25]. The low positive predictive value indicated that the single cut-off value was not adequate for confirming cirrhosis. Therefore, it might be necessary to develop dual cut-off values for excluding or confirming cirrhosis in clinical use of 2D-SWE. And in order to avoid the overestimation of liver fibrosis in patients with elevated ALT levels, we identified novel ALT-adapted dual cut-off values both for significant fibrosis and cirrhosis. The ALT-adapted dual cut-off values were validated to be useful for the clinical application of 2D-SWE for diagnosing the presence or absence of significant fibrosis and cirrhosis. The most important goal of non-invasive diagnostic tools is to diagnose compensated cirrhosis, which would benefit from treatment regardless of the transaminase level [11]. In approximately 70% of patients, the dual cut-off values helped to define the results in reaching or rejecting a diagnosis of cirrhosis. There is substantial overlap of fibrosis stages when values are in the indeterminate range, and additional tests (blood tests, liver biopsy, or MR elastography) and clinical evaluation will be needed to determine the liver fibrosis stages. Both the EFSUMB and WFUMB guidelines suggest that cut-off values of elastography techniques used to evaluate the presence and severity of liver fibrosis depend upon the aetiology of the underlying liver disease [11, 24]. Therefore, the ALT-adapted dual cut-offs of LSMs are only suitable for patients with CHB infection.

This study has certain limitations that warrant discussion. First, we only studied the cut-off values for significant fibrosis and cirrhosis, whereas the cut-off values for severe fibrosis were not calculated. Significant fibrosis is a strong indication to initiate treatment. The most important question for a patient with chronic liver disease is whether the patient has cirrhosis. Therefore, we studied the two important fibrosis stages. Second, the distribution of patients across different ALT levels was not uniform. The number of patients with ALT levels > 2 × ULN was less than one-third the number of the patients with ALT levels ≤ 2 × ULN. Third, the LBs did not meet the American Society for the Study of Liver Diseases criteria of being at least 2–3 cm in length and having at least 11 portal tracts [26]. The agreement between the readers of the LBs was not assessed in our study. Fourth, there were more than 50% of the patients with ALT levels ≤ 2 × ULN in the indeterminate range of dual cut-off values for diagnosis of the presence or absence of significant fibrosis. Our study did not investigate methods for assessing liver fibrosis when values were in the indeterminate range. Fifth, there were no significant differences in the patient characteristics between the index and validation cohorts. Therefore, there was no systematic variation between the two cohorts.

In conclusion, the ALT-adapted dual cut-offs of LSMs showed high accuracy for diagnosis of the presence or absence of significant fibrosis and cirrhosis in patients with CHB infection.

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Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Rong-Qin Zheng.

Conflict of interest The authors of this manuscript declare no relationships with any companies, whose products or services may be related to the subject matter of the article.

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Informed consent Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval Institutional Review Board approval was obtained.

Study subjects or cohorts overlap Some study subjects or cohorts have been previously reported in two articles entitled "Comparison of 2-D shear wave elastography and transient elastography for assessing liver fibrosis in chronic hepatitis B" and "Diagnostic accuracy of two-dimensional shear wave elastography for the non-invasive staging of

hepatic fibrosis in chronic hepatitis B: a cohort study with internal validation".

Methodology
- prospective
- diagnostic or prognostic study
- performed at one institution

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