LIVER

Open

Unreliable Estimation of Fibrosis Regression During Treatment by Liver Stiffness Measurement in Patients With Chronic Hepatitis B

Dong Ji, MD, PhD[1], Yan Chen, MD[1], Qinghua Shang, MD[2], Huabao Liu, MD[3], Lin Tan, MD[4], Jing Wang, MD[5], Yongping Chen, MD[6], Qin Li, MD[7], Qinghua Long, MD[8], Laicheng Song, MD[9], Li Jiang, MD[10], Guangming Xiao, MD[11], Zujiang Yu, MD[12], Liang Chen, MD[13], Xiaoyu Hu, MD[14], Xiaodong Wang, MD[6], Da Chen, MD[7], Zhiqin Li, MD[12], Zheng Dong, MD[1], Guofeng Chen, MD[1] and Yongping Yang, MD[1]

INTRODUCTION: **Little reliable evidence has been reported regarding usefulness of liver stiffness measurement (LSM) for monitoring the hepatic fibrosis changes during treatment. We aimed to assess the association between changes in LSM and histological outcomes in patients with chronic hepatitis B.**

METHODS: **In this prospective multicenter study, 727 treatment-naive patients receiving entecavir-based therapy, who underwent paired biopsies at treatment baseline and week 72, were analyzed. Changes in LSM were defined as ≥30% decrease, minor change, and ≥30% increase. Multivariate logistic regression was used to estimate odds ratios (ORs) of changes in LSM on clinical outcomes accounting for regression to the mean. A new on-treatment LSM threshold was established by receiver operating curve.**

RESULTS: **Overall regression of fibrosis, improvement of inflammation, significant histological response, virologic response, alanine aminotransferase normalization, and hepatitis B e antigen seroconversion were 51.2%, 74.4%, 22.0%, 86.0%, 83.5%, and 13.3%, respectively. The association between changes in LSM and improvement of inflammation was nonlinear ($P = 0.012$). LSM decrease ≥30% was associated with regression of fibrosis (OR 1.501, 95% confidence interval [CI] 1.073–2.099, $P = 0.018$), significant histological response (OR 1.726, 95% CI 1.124–2.652, $P = 0.013$), and alanine aminotransferase normalization (OR 2.149, 95% CI 1.229–3.757, $P = 0.007$). After adjusting for regression to the mean, LSM increase ≥30% became negatively associated with the above 3 outcomes. A new on-treatment LSM cutoff value of 5.4 kPa was established for indicating the significant histological response.**

DISCUSSION: **Changes in LSM are unreliable to estimate regression of fibrosis during treatment; the established cutoff value of on-treatment LSM can optimize monitoring strategy for histological outcomes in patients with chronic hepatitis B.**

Am J Gastroenterol 2021;116:1676–1685. https://doi.org/10.14309/ajg.0000000000001239

INTRODUCTION

Chronic infection of hepatitis B virus (HBV) has infected 350 to 400 million patients worldwide. Without treatment, it can cause progressive liver fibrosis and even hepatocellular carcinoma (1). To eliminate chronic hepatitis B (CHB) by 2030, both treating and assessing hepatic fibrosis need to be further strengthened.

Antifibrotic therapy is important to effectively prevent progression to cirrhosis. Although it has been proven that nucleos(t)ide analogs (NAs) treatment can lead to virologic, biochemical and histological benefits (2–6), current chemical drugs, aiming to eliminate the etiology, alleviate hepatocyte inflammation, inhibit the activation or promote the apoptosis of hepatic stellate cells,

[1]Department of Liver Diseases, Fifth Medical Center of Chinese PLA General Hospital, Beijing, China; [2]Department of Liver Diseases, the 960th Hospital of Chinese PLA Joint Logistics Support Force, Taian, Shandong Province, China; [3]Traditional Chinese Medicine Hospital of Chongqing, Chongqing, China; [4]Liver Disease Department, Fuyang 2nd People's Hospital, Fuyang, Anhui Province, China; [5]Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou, Sichuan Province, China; [6]Department of Infectious and Liver Diseases, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang Province, China; [7]Fuzhou Infectious Diseases Hospital, Fuzhou, Fujian Province, China; [8]Department of Infection and Liver Disease, Yichun People's Hospital, Yichun, Jiangxi Province, China; [9]Traditional Chinese Medicine Hospital of Taihe, Taihe, Anhui Province, China; [10]Department of Infectious Diseases, the First Affiliated Hospital (the Southwest Hospital) of the Third Military Medical University, Chongqing, China; [11]Guangzhou 8th People's Hospital, Guangzhou, Guangdong Province, China; [12]Department of Infectious Disease, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, China; [13]Department of Hepatic Diseases, Shanghai Public Health Clinical Center, Shanghai, China; [14]Department of Infectious Diseases, Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan Province, China. **Correspondence:** Yongping Yang, MD. E-mail: yongpingyang@hotmail.com. Guofeng Chen, MD. E-mail: guofengchen302@163.com.
Received August 20, 2020; accepted February 23, 2021; published online April 8, 2021

etc., often result in poor antifibrosis efficacy because of their single-targeted molecular mechanism (7,8). Over the past 2 decades, tremendous progress has been made in treating fibrosis with traditional Chinese medicine, which is more appealing and will provide new opportunities for future treatment (9–12).

Regarding fibrosis assessment, liver biopsy is still considered the gold standard; however, repeated biopsy is often unacceptable (13,14). Currently, liver stiffness measurement (LSM) has been recommended as the most accurate noninvasive method for this purpose (15,16). However, the correlation of the regression of fibrosis predicted by on-treatment LSM with histology has not been determined, and some studies have presented controversial results and even come to opposite conclusions (17–23).

Therefore, we reanalyzed the data from a prospective multicenter study evaluating the synergistic effect of Biejia-Ruangan (BJRG) on entecavir (ETV) therapy in treatment-naive CHB patients, so as to determine the correlation of changes in LSM with the histological outcomes and then to establish a new LSM threshold to optimize monitoring strategy for patients with CHB.

METHODS

Study design and patients

The study protocol was approved by the institutional review board of 14 participating hospitals (24). All participants provided written informed consent. Data included in this study were obtained from an ongoing randomized controlled clinical trial (NCT01965418) (11). Briefly, treatment-naive HBV-infected patients who presented to one of participating centers between October 2013 and October 2014 were recruited and randomly assigned in a 1:1 ratio to the treatment group (ETV 0.5 mg/d plus BJRG 2.0 g/time, 3 times daily) or the control group (ETV plus placebo). The primary endpoint was the regression of fibrosis. Liver biopsies were performed at both baseline and week 72. Demographic data and clinical laboratory tests were collected at baseline and every 12 weeks.

The inclusion criteria were as follows: (i) treatment-naive patients with chronic HBV infection; (ii) eligible for NA treatment (25); (iii) agreement to receive ETV treatment rather than any other anti-HBV agent; (iv) eligibility for LSM assessment (alanine aminotransferase [ALT] $<5 \times$ ULN, note: ULN = upper limit of normal; 40 U/L for ALT) and liver biopsy; and (v) Ishak fibrosis score ≥ 3 points. The exclusion criteria included (i) coinfection with other viruses; (ii) other liver diseases; (iii) decompensated cirrhosis or any cancer; or (iv) pregnancy or breastfeeding (see Supplementary Figure S1, Supplementary Digital Content 1, http://links.lww.com/AJG/B954).

Clinical and laboratory variables

Serum HBV DNA levels were measured by the COBAS TaqMan HBV Test (Roche, Branchburg, NJ). HBV serological markers were measured by chemiluminescent immunoassay (Abbott, Wiesbaden, Germany). LSM was performed by experienced operators using FibroScan (Echosens, Paris, France). The appropriate probe was selected according to body mass index (BMI) (M probe for BMI $\leq$ 30 kg/m^2, or XL probe for BMI $>$30 kg/m^2). Reliable measurement was defined as median values of 10 valid shots, with interquartile range (IQR) $\leq$30% and success rate $\geq$60% (26,27).

Histological assessment

Ultrasound-guided liver biopsy was performed according to the standard protocol (28). A minimum of 2.0 cm in length per liver specimen and at least 2 pieces were collected to ensure that there was an adequate specimen of at least 11 portal tracts for histological assessment. Biopsy specimens were examined independently by 2 pathologists who were blinded to the timing of biopsy and clinical data. When inconsistencies occurred, the samples were rereviewed by both pathologists together to reach a consensus. Hepatic inflammation was graded using the Ishak modified histologic activity index (HAI), and fibrosis was staged using the Ishak fibrosis (F) score (29,30).

Definitions of clinical outcomes

Regression of fibrosis was defined as a $\geq$1-point decrease in the F score; improvement of inflammation was defined as a $\geq$2-point decrease in the HAI score; significant histological response was defined as an F score $\leq$2 points plus HAI $\leq$4 points during antiviral treatment; virologic response was defined as a serum HBV DNA level $<$20 IU/mL after antiviral treatment initiation; ALT normalization was defined as the proportion of patients with ALT restored to normal in the subset with elevated ALT at baseline; hepatitis B e antigen (HBeAg) seroconversion was defined as a change in detectable anti-HBe from negative at baseline to positive during treatment in the subset with positive HBeAg at baseline. The clinically meaningful changes in LSM were defined as a decrease $\geq$30%, minor changes (decrease $<$30% to increase $<$30%) and an increase $\geq$30% compared with the baseline (31). This is also because an IQR less than 30% of the median LSM is one of the key factors in regard to a valid LSM, which means that a more than 30% change in LSM cannot be considered a variation.

Statistical analyses

Data are expressed as the mean $\pm$ SD, median (IQR), or n (%), as appropriate. The diagnostic performance of LSM was determined with receiver operating curve (ROC) and evaluated by the sensitivity, specificity, positive predictive value, and negative predictive value. Factors significantly associated with the clinical outcomes in the univariate analysis were entered into a multivariate logistic model. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated from the model. Linear trends across categories were tested by logistic regression. A possible nonlinear relationship between the continuous changes in LSM and clinical outcomes was examined by a restricted cubic spline regression model (32). Statistically, it is a fact that someone who has a high value at baseline will tend to have a lower value on a subsequent measurement and vice versa: so-called regression to the mean (RtM) (33,34). The grouping diagram of participants was according to baseline and percent changes in LSM to account for RtM (see Supplementary Figure S2, Supplementary Digital Content 2, http://links.lww.com/AJG/B955). We conducted sensitivity analyses in which the assessment of association between quartile of changes in LSM and the clinical outcomes was performed. $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using R software, version 3.6.1 (Vienna, Austria; http://www.r-project.org/). An experienced statistician verified all analyses independently.

RESULTS

Baseline characteristics

Totally, 727 treatment-naive CHB patients with paired liver biopsies were enrolled, the mean age was 42.2 years, 68.4% were male, and 57.1% were HBeAg-positive. Patients' median ALT was 51 U/L, median HBV DNA was 6.1 Log_{10} IU/mL, and median

Table 1. Baseline characteristics of study participants

	Overall (n = 727)	Nonregression (n = 355)	Regression (n = 372)	*P* value
Age (yr)	42.2 ± 9.8	42.9 ± 9.2	41.6 ± 10.4	0.060
Male sex	497 (68.4)	236 (66.5)	261 (70.2)	0.323
Drinker	90 (12.4)	51 (14.4)	39 (10.5)	0.143
BMI (kg/m^2)	23.5 ± 3.4	23.7 ± 3.7	23.3 ± 3.2	0.102
ETV plus BJRG	370 (50.9)	163 (45.9)	207 (55.6)	0.011
ALT (U/L)	51 (32–97)	48 (31–85)	55 (32–105)	0.130
AST(U/L)	43 (29–74)	44 (29–70)	42 (29–75)	0.968
TBIL (μmol/L)	14.0 (10.8–18.6)	14.0 (11.0–18.4)	13.9 (10.6–18.9)	0.711
PLT (×10^9/L)	157 (119–198)	153 (112–189)	164 (128–205)	0.001
HBeAg positive	415 (57.1)	194 (54.6)	221 (59.4)	0.222
HBV DNA level (Log$_{10}$ IU/mL)	6.1 ± 1.6	5.9 ± 1.6	6.3 ± 1.6	0.003
High HBV DNA[a]	375 (51.6)	166 (46.8)	209 (56.2)	0.014
LSM (kPa)	9.7 (6.8–16.1)	11.0 (7.3–17.6)	8.8 (6.5–14.3)	0.001
HAI score				0.512
1–4	129 (17.7)	70 (19.7)	59 (15.9)	
5–8	424 (58.3)	203 (57.2)	221 (59.4)	
9–12	161 (22.2)	77 (21.7)	84 (22.5)	
13–18	13 (1.8)	5 (1.4)	8 (2.2)	
F score				0.450
3	188 (25.9)	87 (24.5)	101 (27.1)	
4	134 (18.4)	60 (16.9)	74 (19.9)	
5	157 (21.6)	83 (23.4)	74 (19.9)	
6	248 (34.1)	125 (35.2)	123 (33.1)	

Mean ± SD is presented for normally distributed continuous variables.
Categorical variables are presented as numbers and percentages (n [%]). Median values (IQR) are presented for skewed distributed continuous variables.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BJRG, Biejia-Ruangan; BMI, body mass index; ETV, entecavir; HAI, histology activity index; HBeAg, hepatitis B e antigen; LSM, liver stiffness measurement; PLT, platelet; F, Ishak fibrosis; TBIL, total bilirubin.
[a]HBV DNA ≥ 6.0 Log$_{10}$ IU/mL.

LSM value was 9.7 kPa. Histologically, 25.9% of the patients had significant fibrosis (F3), 18.4% had severe fibrosis (F4) and 55.7% had cirrhosis (F5-6). We compared baseline characteristics according to whether the regression of fibrosis was achieved after 72 weeks of treatment, and results showed that treatment, platelet (PLT), HBV DNA level, and LSM were significantly different between the groups, and these would be used as adjusting factors for following analyses (Table 1). Moreover, for 272 patients with normal ALT, 27.6% had no or mild hepatic necroinflammation (HAI 0–4), 61.8% had significant necroinflammation (HAI 5–8), and 10.6% had severe necroinflammation (HAI 9–18).

Clinical outcomes

After 72 weeks of treatment, the overall regression of fibrosis, improvement of inflammation, significant histological response, virologic response, ALT normalization, and HBeAg seroconversion were 51.2%, 74.4%, 22.0%, 86.0%, 83.5%, and 13.3%, respectively. The improvement of inflammation was significantly higher in patients who achieved regression of fibrosis than in those who did not achieve regression of fibrosis (83.6% vs 64.8%, $P < 0.001$). ALT normalization showed the same trend (87.8% vs 78.6%, $P = 0.012$). Virologic response and HBeAg seroconversion showed no difference between the regression and nonregression patients. The overall absolute decrease in LSM was 5.0 kPa; the percent changes in LSM showed no difference between the regression and nonregression patients ($P = 0.073$) (Table 2).

Moreover, for patients who achieved regression of fibrosis, the distribution of HAI scores showed a significant improvement of inflammation ($P < 0.001$), consistently, the median LSM value significantly decreased from 8.8 kPa at baseline to 5.4 kPa at week 72 ($P < 0.001$). Interestingly, for patients who did not achieve regression of fibrosis (actually, progression of fibrosis, $P = 0.013$), a significant improvement of inflammation was still achieved at week 72 ($P < 0.001$), and median LSM value significantly decreased from 11.0 kPa at baseline to 6.8 kPa at week 72 ($P < 0.001$) (Figure 1). In addition, for those who had an increase LSM of ≥30%, percentage of patients receiving ETV plus BJRG therapy was 40.9%, which was numerically lower than 50.9% of overall data ($P = 0.198$). The situation was similar with respect to the composition of cirrhosis. Other parameters (e.g., age, sex,

Table 2. Clinical outcomes at week 72

	Overall (n = 727)	Nonregression (n = 355)	Regression (n = 372)	*P* value
ALT (U/L)	24 (17–33)	25 (18–35)	23 (17–32)	0.015
AST(U/L)	25 (20–30)	26 (21–32)	24 (20–29)	0.001
TBIL (μmol/L)	13.2 (10.2–17.5)	13.4 (10.6–17.2)	13.0 (10.0–17.9)	0.636
PLT ($\times 10^9$/L)	176 (133–216)	168 (123–206)	186 (144–223)	<0.001
LSM (kPa)	6.1 (4.6–9.3)	6.8 (4.9–11.8)	5.4 (4.4–7.8)	<0.001
Decrease in LSM (kPa)	5.0 ± 7.4	5.0 ± 8.0	4.9 ± 6.8	0.965
Changes in LSM (percent)				0.073
Increased ≥30%	44 (6.0)	27 (7.6)	17 (4.6)	
Minor change	292 (40.2)	150 (42.3)	142 (38.2)	
Decreased ≥30%	391 (53.8)	178 (50.1)	213 (57.2)	
Histological liver fibrosis status				<0.001
Fibrosis progressed	77 (10.6)	77 (21.7)	0 (0.0)	
Fibrosis stable	278 (38.2)	278 (78.3)	0 (0.0)	
Fibrosis regressed	372 (51.2)	0 (0.0)	372 (100.0)	
Improvement of inflammation	541 (74.4)	230 (64.8)	311 (83.6)	<0.001
Significant histological response	160 (22.0)	0	160 (43.0)	<0.001
Virologic response	625 (86.0)	306 (86.2)	319 (85.8)	0.948
ALT normalization[a]	380 (83.5)	165 (78.6)	215 (87.8)	0.012
HBeAg seroconversion[b]	55 (13.3)	27 (13.9)	28 (12.7)	0.708

Mean ± SD is presented for normally distributed continuous variables. Categorical variables are presented as numbers and percentages (n [%]).
Median values (IQR) are presented for skewed distributed continuous variables.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen; LSM, liver stiffness measurement; PLT, platelet; TBIL, total bilirubin.
[a]The denominator is the number of patients who had elevated ALT at baseline.
[b]The denominator is the number of patients who had a positive HBeAg at baseline.

drinking history, and BMI) showed no differences between patients with LSM increased ≥30% and overall patients (see Supplementary Table S1, Supplementary Digital Content 4, http://links.lww.com/AJG/B957).

Consistency of LSM and histology

To evaluate whether a decrease in LSM might be useful in assessing the regression of fibrosis, we performed a consistency test. The results showed that 53.4% (388/727) were identified as having regression of fibrosis by a decrease in LSM ≥30%; however, among them, only 54.6% (212/388) were confirmed by histology, 45.4% (176/388) were false positive, and the kappa value was 0.074, indicating that the decrease in LSM ≥30% seemed to have poor consistency with the Ishak fibrosis assessment. Moreover, the percent changes in LSM by changes in the Ishak fibrosis score from baseline to week 72 were plotted and the Spearman rank correlation coefficient (rho) was 0.094, representing the percent changes in LSM had correlation with fibrosis change (1% of the change in LSM was associated with 0.094 change in the fibrosis score), but the correlation was extremely low (less than 10%) (Figure 2).

Restricted cubic spline regression analysis

The association between fold changes in LSM and clinical outcomes was modeled with restricted cubic spline regression after adjustment for important baseline confounders (treatment, PLT, HBV DNA level, and LSM). Linear associations between fold changes in LSM and regression of fibrosis ($P < 0.001$), significant histological response ($P = 0.005$), and ALT normalization ($P = 0.001$) were observed; however, the association between fold changes in LSM and improvement of inflammation was nonlinear (P for nonlinearity = 0.012). There was no relationship between fold changes in LSM and virologic response ($P = 0.817$) or HBeAg seroconversion ($P = 0.181$) (Figure 3).

Adjusted logistic regression analysis

As expected, there was strong evidence of RtM, which showed that the extremely higher or lower LSM on its first measurement tended to be closer to the average on its second measurement (see Supplementary Figure S3, Supplementary Digital Content 3, http://links.lww.com/AJG/B956).

Before adjustment for RtM, compared with patients who experienced a minor change in LSM, the likelihood for regression of fibrosis (OR 1.501, 95% CI 1.073–2.099, $P = 0.018$), improvement of inflammation (OR 2.107, 95% CI 1.440–3.083, $P = 0.001$), significant histological response (OR 1.726, 95% CI 1.124–3.083, $P = 0.013$), and ALT normalization (OR 2,149, 95% CI 1.229–3.757, $P = 0.007$) was significantly higher in those with a decrease in LSM of ≥30%, whereas LSM increase ≥30% was not significantly associated with a low probability of these outcomes ($P > 0.05$) (Figure 4a).

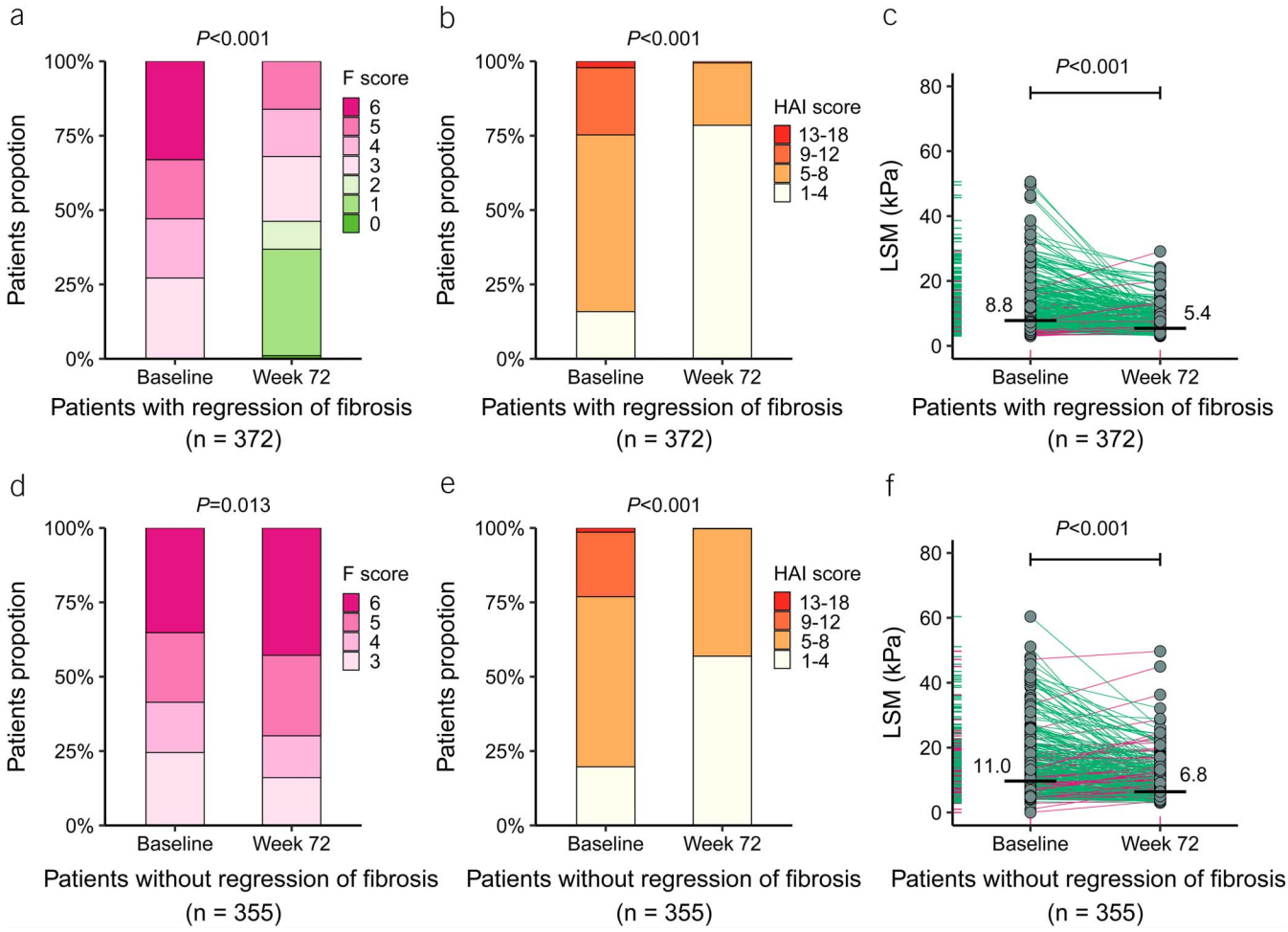

Figure 1. Histological assessment and LSM changes over 72-week treatment. (**a** and **d**) Distribution of the F score in patients with (without) regression of fibrosis. (**b** and **e**) Distribution of the HAI score in patients with (without) regression of fibrosis. (**c** and **f**) Changes in LSM values in patients with (without) regression of fibrosis. HAI, histology activity index; LSM, liver stiffness measurement; F, Ishak fibrosis.

After adjustment for RtM, the tendency became even more pronounced because both LSM of ≥30% decrease and increase had significant linear association with the abovementioned outcomes except for improvement of inflammation. Be consistent with restricted cubic spline regression analysis, changes in LSM could not indicate virologic response and HBeAg seroconversion, neither (Figure 4b).

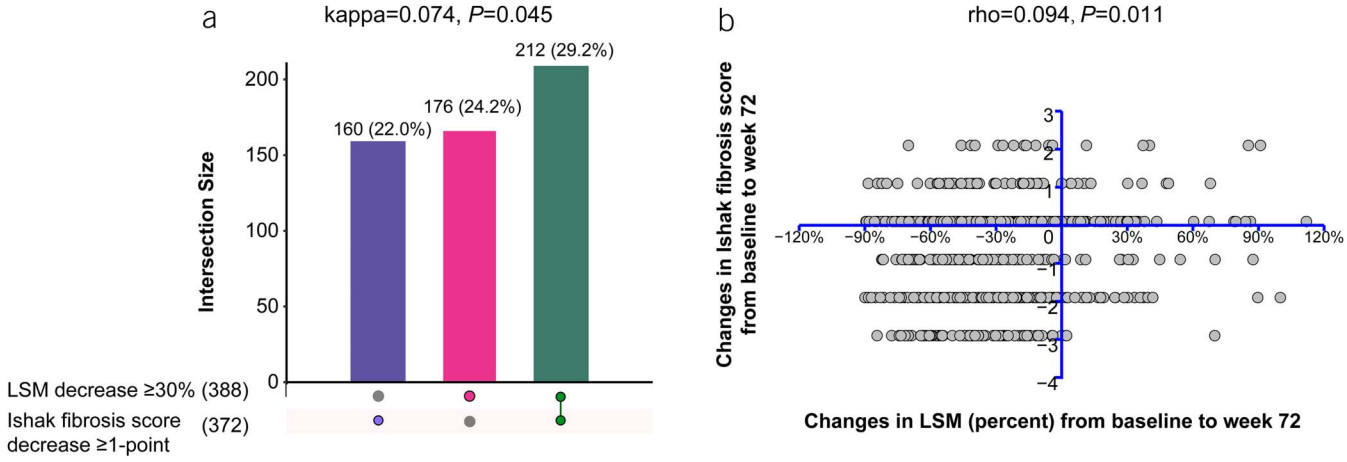

Figure 2. Changes in LSM against the changes in the Ishak fibrosis score in HBV-infected patients from baseline to week 72 of treatment. (**a**) The consistency test for estimation of regression of fibrosis between LSM decrease ≥30% and the Ishak fibrosis score decrease ≥1 point. (**b**) The correlation test for percent changes in LSM and the changes in the Ishak fibrosis score. HBV, hepatitis B virus; LSM, liver stiffness measurement.

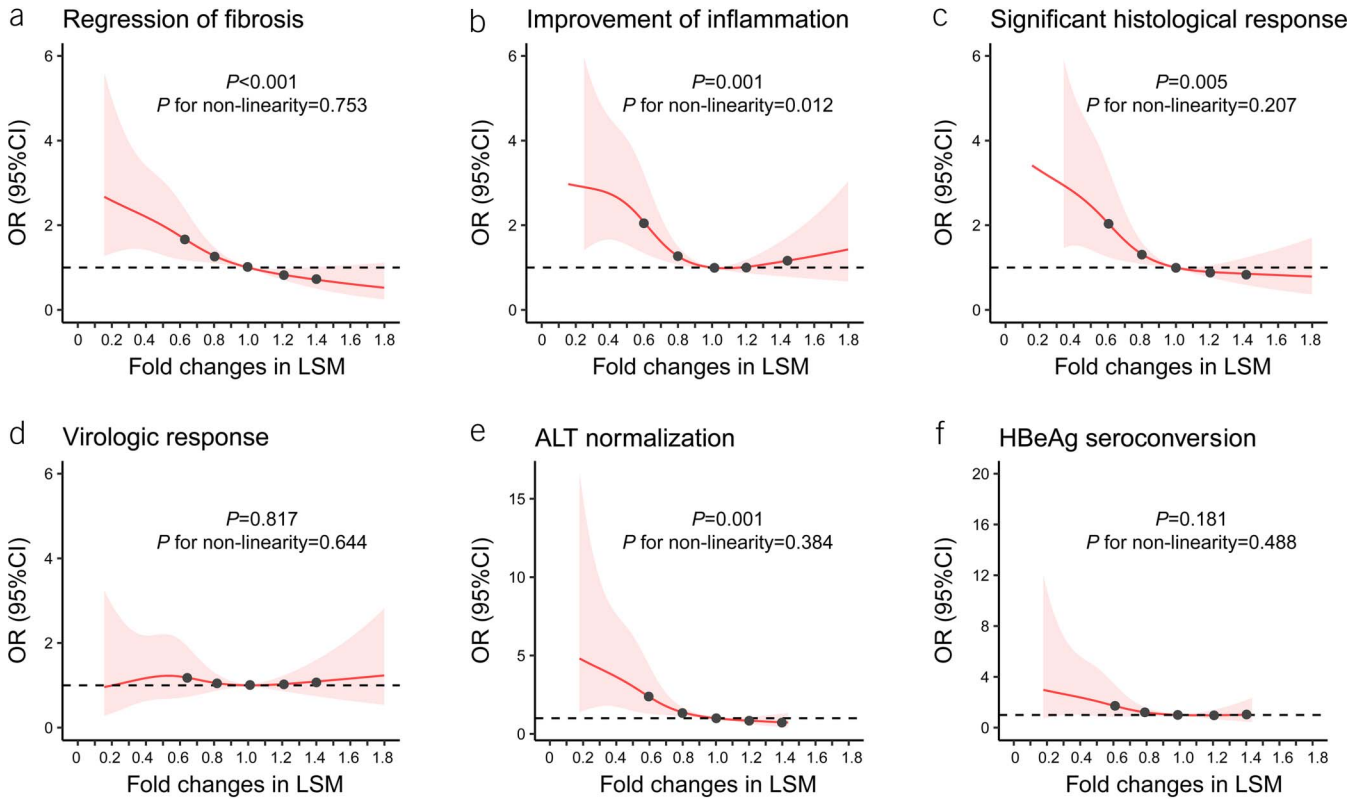

Figure 3. Predicted spline curves for the associations between fold changes in LSM and clinical outcomes. (**a**) Regression of fibrosis. (**b**) Improvement of inflammation. (**c**) Significant histological response. (**d**) Virologic response. (**e**) ALT normalization. (**f**) HBeAg seroconversion. The middle line shows the ORs adjusted for treatment, PLT, HBV DNA level, and LSM at baseline. The outer lines show the 95% CIs. ALT, alanine aminotransferase; CI, confidence interval; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LSM, liver stiffness measurement; OR, odds ratio; PLT, platelet.

In sensitivity analyses, patients were divided to 4 groups according to quartile of changes in LSM, and results showed the similar associations across the clinical outcomes assessed (Figure 4c).

On-treatment LSM threshold for histological outcomes

We further conducted univariate (Table 2 and see Supplementary Table S2, Supplementary Digital Content 5, http://links.lww.com/AJG/B958) and multivariate analyses to identify the predictors for histological outcomes. The results showed that ETV plus BJRG treatment and LSM at week 72 were independent predictors for regression of fibrosis, and baseline parameters (age, PLT, and LSM) and 72-week parameters (ALT and LSM) were predictors for significant histological response. Among them, the ORs of on-treatment LSM for indicating histological outcomes were the most meaningful, so we performed ROC analyses to establish the new cutoff values of on-treatment LSM, which showed that the accuracy to indicate regression of fibrosis was unsatisfactory (area under the ROC curve 0.625, 95% CI 0.585–0.666); however, the accuracy to indicate significant histological response was acceptable (area under the ROC curve 0.735, 95% CI 0.694–0.776), and of clinical significance, the cutoff value was 5.4 kPa (sensitivity 72.5%, specificity 69.1%, positive predictive value 39.9%, negative predictive value 89.9%), which might be further optimized when combined with other predictors (Figure 5).

DISCUSSION

This prospective multicenter study with large sample and paired biopsies provides direct histological evidence, rather than indirect deduction from a retrospective cross-sectional study, that a decrease in LSM to estimate the regression of fibrosis did not perform well in HBV-infected patients during treatment. In addition, we established a new on-treatment LSM threshold to assess whether significant histological response was achieved after therapy.

With regard to treating hepatic fibrosis, antiviral therapy is the most important, and the strategy for starting antiviral therapy is becoming increasingly progressive. The EASL and Chinese guidelines recommend that patients with elevated HBV DNA and elevated ALT should receive antiviral therapy (35,36). However, some studies have shown that 22.5%–49.4% of patients with persistently normal ALT have significant histological liver injury, and 8.4% of them have cirrhosis (37–39), which was consistent with our findings. The severity of disease in HBV-infected patients with normal ALT might be underrepresented by assessing ALT levels. Therefore, normal ALT should not be considered a rule-out indicator for anti-HBV treatment. On the other hand, it is widely accepted that drugs addressing multiple pathogenic pathways are usually more efficient than single specific pathway modulators in the treatment of liver fibrosis (40). From a clinical perspective, this study found that adding BJRG can increase the regression of fibrosis in HBV-infected patients receiving NA treatments.

In terms of assessing hepatic fibrosis, we demonstrated that a decrease in LSM does not clearly indicate the regression of fibrosis during treatment. Some investigations suggested that LSM could be applied for longitudinal monitoring of fibrosis status (15–19), which is the opposite of our findings; nevertheless, their sample

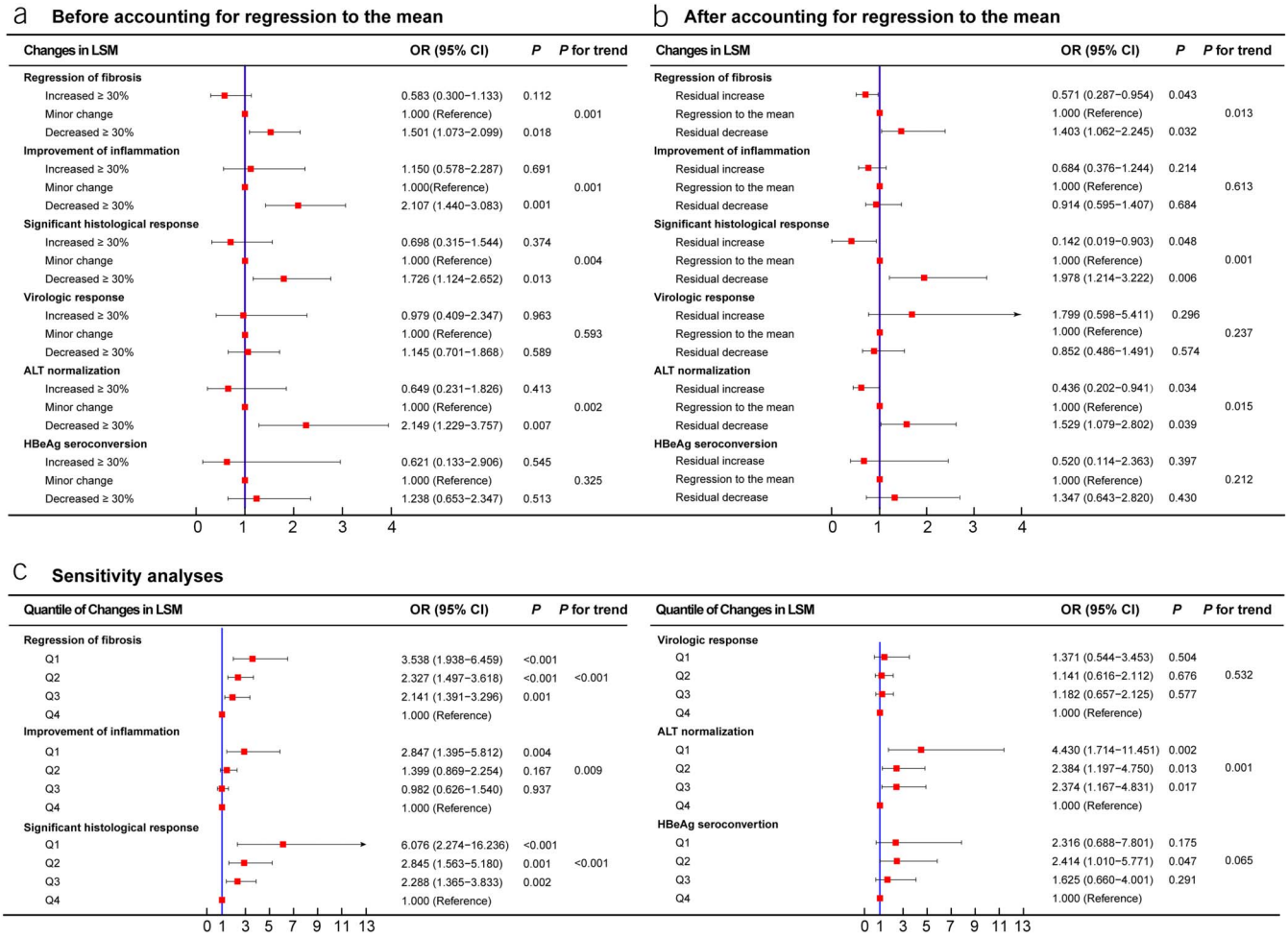

Figure 4. Adjusted ORs and 95% CIs for clinical outcomes according to changes in LSM. (**a**) Logistic regression analysis before accounting for RtM. (**b**) Logistic regression analysis after accounting for RtM. (**c**) Sensitivity analyses on quartile of changes in LSM. Adjustments were for treatment, PLT, HBV DNA level, and LSM at baseline. ORs are shown (red solid boxes) with 95% CIs (black line segments). ALT, alanine aminotransferase; CI, confidence interval; HBeAg, hepatitis B e antigen; LSM, liver stiffness measurement; OR, odds ratio; RtM, regression to the mean. Q1: first quartile (−45.2 to −7.0 kPa); Q2: second quartile (−7.0 to −3.0 kPa); Q3: third quartile (−3.0 to −0.8 kPa); Q4: fourth quartile (−0.8 to 16.3 kPa).

sizes were small. Although other researches draw the similar conclusions like ours. Wang et al. (41) observed 1,417 biopsy-proven HBV-infected patients with an unknown second biopsy rate and implicated the diagnostic potential of LSM to evaluate the severity of liver necroinflammation. Liang et al. (42) included 164 patients with paired liver biopsies and indicated that an LSM decline may reflect both regression of fibrosis and improvement of inflammation. Wong et al. (31) studied 71 patients and concluded that an LSM decrease could be related to ALT normalization.

Meanwhile, we wondered whether improvement of inflammation partially accounted for the decrease in LSM. After analysis of the HAI score distribution and association between changes in LSM and clinical outcomes, we concluded that the abovementioned assumption was reasonable. A reduction in ALT occurred early in the course of treatment, which was accompanied by a commensurate reduction in LSM values. These values change rapidly, presumably as a result of a reduction in hepatic inflammation rather than regression of fibrosis. Liver fibrosis results from excessive accumulation of extracellular matrix, which goes hand in hand with altered angiogenesis and the architectural changes of cirrhosis. Above all, the core driving factor for fibrosis progression lies in the context of inflammation. Therefore, improvement of inflammation is an essential precondition for the regression of fibrosis (43,44). The decrease in LSM observed in our study must be partly caused by ALT normalization and subsequent improvement of inflammation regardless of whether regression of fibrosis was achieved. This could partially explain why some CHB patients with other concurrent underlying liver diseases always have persistent or even progressive fibrosis after both virologic response and LSM value decline.

Furthermore, we also noticed that changes in LSM were not associated with either the regression of fibrosis or ALT normalization in many patients. What was the reason for their LSM decline? RtM, resulting from random measurement error, always occurs when unusually large or small measurement values are followed by values that are closer to the population mean. With regard to pre-post intervention studies that target high-risk factors, RtM should be given special consideration because it often makes the changes in repeated measures look like more meaningful because of the treatment (overestimation) (45). This study design and inherent characteristics of LSM meet the description of above setting. Therefore, taking RtM into account, our results

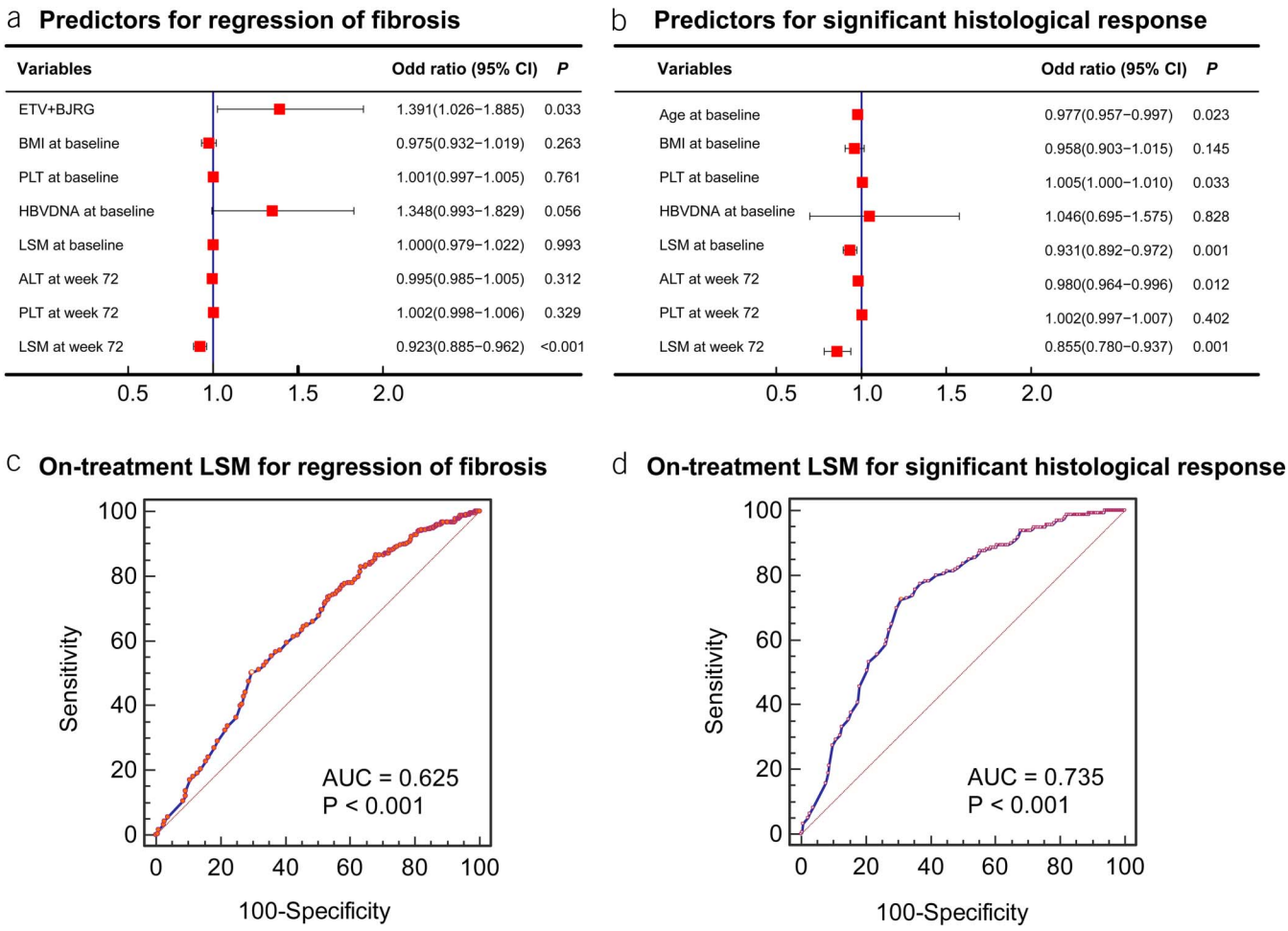

Figure 5. The predictors for regression of fibrosis or significant histological response. (**a** and **b**) Predictors for regression of fibrosis (significant histological response). (**c** and **d**) ROC analysis for on-treatment LSM and regression of fibrosis (significant histological response). ALT, alanine aminotransferase; BJRG, Biejia-Ruangan; BMI, body mass index; ETV, entecavir; LSM, liver stiffness measurement; PLT, platelet.

indicated that the unreliable estimation of the regression of fibrosis during treatment by a decrease in LSM partially resulted from random measurement error. To the best of our knowledge, no previous study has comprehensively accounted for RtM, and this may explain some situations in which a decrease in LSM did not lead to regression of fibrosis.

Finally, the limitations of LSM for gauging histological outcomes after therapy may also be due to the proposed cutoff values being derived from treatment-naive studies. Hence, our study established a new LSM cutoff value derived from on-treatment CHB patients, and strongly recommended this threshold for assessing whether the significant histological response is achieved, so as to avoid discontinuation of NA treatment or loss of follow-up.

The strengths of our study include (i) the assessment of the relationship between changes in LSM and clinical outcomes in a large sample with 100% paired biopsy data, derived from a multicenter randomized trial, and (ii) analyses from multiple perspectives, biochemical and histological, clinical and statistical, which can provide high-quality evidence for an increased understanding of LSM limitations in the monitoring process of anti-HBV treatment.

However, our study has several limitations. First, sampling errors of biopsies may exist. However, some other favorable factors in the current study decreased this potential influence to the lowest level; e.g., 2 pieces of specimens were collected to assure histological evaluation, and 2 experienced pathologists involving in the evaluation were independent and blinded to the clinical data, so as to decrease the interobserver and intraobserver variation. Second, the enrolled patients lacked non-Asian patients, and patients with mild fibrosis were excluded, which might limit the conclusion's generalizability to broader populations.

In summary, a decrease in LSM during antiviral treatment can be caused by regression of fibrosis, ALT normalization, improvement of inflammation, or random measurement error, which results in unreliable or even overestimation of the regression of fibrosis. However, a newly established cutoff value of on-treatment LSM can identify the significant histological response. This new understanding can help to correctly interpret LSM assessments and optimize monitoring strategies for histological outcomes in patients with chronic HBV infection.

CONFLICTS OF INTEREST

Guarantor of the article: Yongping Yang, MD.

Specific author contributions: Dong Ji, MD, PhD, Yan Chen, MD, and Qinghua Shang, MD, contributed equally. Y.Y. and G.C. were responsible for the study design, study supervision, and critical

revision of the manuscript. D.J., Y.C., and Q.S. were responsible for statistical analyses, drafting of the manuscript, and interpretation of the data. H.L., L.T., J.W., Y.C., Q. L., Q. L., L.S., L.J., G.X., Z.Y., L.C., X.H., X.W., D.C., Z.L., and Z.D. were responsible for management of patients and data collection. All authors approved the final submission.

Financial support: This study was supported by National Major Science and Technology Special Project of China (2018ZX10725-506) and Beijing Municipal Science and Technology Commission (Z181100001718034). The funding sources had no role in study design, data collection, data analysis and interpretation, preparation of the manuscript, or decision to publish.

Potential competing interests: None to report.

The name of RCT: A Clinical Evaluation on Traditional Chinese Medicine Diagnosis and Treatment Program Blocking and Reversing Hepatitis B-related Liver Fibrosis—a Randomized, Controlled, Double-blind, Multi-center Clinical Trial. RCT No.: NCT01965418.

Study Highlights

WHAT IS KNOWN

- ✓ Accurate assessment of liver fibrosis is important for patients with chronic hepatitis B.
- ✓ Liver stiffness measurement (LSM) has been recommended as the most accurate noninvasive method assessing liver fibrosis for treatment-naive chronic hepatitis B patients.
- ✓ The usefulness of LSM for indicating histological outcomes during antiviral treatment has not been determined.

WHAT IS NEW HERE

- ✓ A decrease in LSM after initiating of antiviral treatment can be caused by regression of fibrosis, improvement of inflammation, alanine aminotransferase normalization, and random measurement error.
- ✓ Decreases in LSM might lead to unreliable or even over estimation of regression of fibrosis, which should be interpreted cautiously.
- ✓ An on-treatment LSM cutoff value of 5.4 kPa was established to identify the significant histological response.

REFERENCES

1. Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: A modelling study. Lancet Gastroenterol Hepatol 2018;3:383–403.
2. Chang TT, Liaw YF, Wu SS, et al. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology 2010; 52:886–93.
3. Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: A 5-year open-label follow-up study. Lancet 2013;381:468–75.
4. Campana L, Iredale JP. Regression of liver fibrosis. Semin Liver Dis 2017; 37:1–10.
5. Lok AS. Hepatitis: Long-term therapy of chronic hepatitis reverses cirrhosis. Nat Rev Gastroenterol Hepatol 2013;10:199–200.
6. Li ZB, Li L, Niu XX, et al. Switching from entecavir to tenofovir alafenamide for chronic hepatitis B patients with low-level viremia. Liver Int 2021 (doi: 10.1111/liv.14786).
7. Schuppan D. Liver fibrosis: Common mechanisms and antifibrotic therapies. Clin Res Hepatol Gastroenterol 2015;39:S51–9.
8. Friedman SL. Hepatic fibrosis: Emerging therapies. Dig Dis 2015;33: 504–7.
9. Zhang L, Schuppan D. Traditional Chinese medicine (TCM) for fibrotic liver disease: Hope and hype. J Hepatol 2014;61:166–8.
10. Li H. Advances in anti hepatic fibrotic therapy with Traditional Chinese Medicine herbal formula. J Ethnopharmacol 2020;251:112442.
11. Rong G, Chen Y, Yu Z, et al. Synergistic effect of Biejia-Ruangan on fibrosis regression in patients with chronic hepatitis B treated with entecavir: A multicenter, randomized, double-blinded, placebo-controlled trial. J Infect Dis 2020:jiaa266 (doi: 10.1093/infdis/jiaa266).
12. Chen J, Hu Y, Chen L, et al. The effect and mechanisms of Fuzheng Huayu formula against chronic liver diseases. Biomed Pharmacother 2019;114: 108846.
13. Gill US, Pallett LJ, Kennedy PTF, et al. Liver sampling: A vital window into HBV pathogenesis on the path to functional cure. Gut 2018;67:767–75.
14. Lo RC, Kim H. Histopathological evaluation of liver fibrosis and cirrhosis regression. Clin Mol Hepatol 2017;23:302–7.
15. Stasi C, Salomoni E, Arena U, et al. Non-invasive assessment of liver fibrosis in patients with HBV-related chronic liver disease undergoing antiviral treatment: A preliminary study. Eur J Pharmacol 2017;806:105–9.
16. Chon YE, Park JY, Myoung SM, et al. Improvement of liver fibrosis after long-term antiviral therapy assessed by Fibroscan in chronic hepatitis B patients with advanced fibrosis. Am J Gastroenterol 2017;112:882–91.
17. Enomoto M, Mori M, Ogawa T, et al. Usefulness of transient elastography for assessment of liver fibrosis in chronic hepatitis B: Regression of liver stiffness during entecavir therapy. Hepatol Res 2010;40:853–61.
18. Kong Y, Sun Y, Zhou J, et al. Early steep decline of liver stiffness predicts histological reversal of fibrosis in chronic hepatitis B patients treated with entecavir. J Viral Hepat 2019;26:576–85.
19. Wu SD, Liu LL, Cheng JL, et al. Longitudinal monitoring of liver fibrosis status by transient elastography in chronic hepatitis B patients during long-term entecavir treatment. Clin Exp Med 2018;18:433–43.
20. Dong XQ, Wu Z, Li J, et al. China HepB-Related Fibrosis Assessment Research Group. Declining in liver stiffness cannot indicate fibrosis regression in patients with chronic hepatitis B: A 78-week prospective study. J Gastroenterol Hepatol 2019;34:755–63.
21. Huang R, Yan X, Liu Y, et al. Can transient elastography predict fibrosis regression in patients with chronic hepatitis B during long-term antiviral therapy? Am J Gastroenterol 2017;112:1477–8.
22. Gaia S, Carenzi S, Barilli AL, et al. Reliability of transient elastography for the detection of fibrosis in non-alcoholic fatty liver disease and chronic viral hepatitis. J Hepatol 2011;54:64–71.
23. Rigamonti C, Fraquelli M. Do not trivialize the Fibroscan examination, value its accuracy. J Hepatol 2007;46:1149.
24. Qu J, Yu Z, Li Q, et al. Blocking and reversing hepatic fibrosis in patients with chronic hepatitis B treated by traditional Chinese medicine (tablets of Biejia Ruangan or RGT): Study protocol for a randomized controlled trial. Trials 2014;15:438.
25. European Association for the Study of the Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus. J Hepatol 2012;57: 167–85.
26. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008;48:835–47.
27. European Association for Study of Liver. Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol 2015;63:237–64.
28. Rockey DC, Caldwell SH, Goodman ZD, et al; American Association for the Study of Liver Diseases. Liver biopsy. Hepatology 2009;49:1017–44.
29. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–9.
30. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431–5.
31. Wong GL, Wong VW, Choi PC, et al. On-treatment monitoring of liver fibrosis with transient elastography in chronic hepatitis B patients. Antivir Ther 2011;16:165–72.
32. Harrell FE Jr, Lee KL, Pollock BG. Regression models in clinical studies: Determining relationships between predictors and response. J Natl Cancer Inst 1988;80:1198–202.
33. MacMahon S, Peto R, Cutler J, et al. Blood pressure, stroke, and coronary heart disease. Part 1, prolonged differences in blood pressure: Prospective

observational studies corrected for the regression dilution bias. Lancet 1990;335:765–74.
34. Jun M, Ohkuma T, Zoungas S, et al. Changes in albuminuria and the risk of major clinical outcomes in diabetes: Results from ADVANCE-ON. Diabetes Care 2018;41:163–70.
35. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67:370–98.
36. Chinese Society of Infectious Diseases, Chinese Medical Association; Chinese Society of Hepatology, Chinese Medical Association. The guidelines of prevention and treatment for chronic hepatitis B (2019 version). Zhonghua Gan Zang Bing Za Zhi 2019;27: 938–61. Chinese.
37. Wang H, Ru GQ, Yan R, et al. Histologic disease in Chinese chronic hepatitis B patients with low viral loads and persistently normal alanine aminotransferase levels. J Clin Gastroenterol 2016;50:790–6.
38. Wu Z, Ma AL, Xie Q, et al. Significant histological changes and satisfying antiviral efficacy in chronic hepatitis B virus infection patients with normal alanine aminotransferase. Antiviral therapy decision in chronic HBV patients with normal ALT. Clin Res Hepatol Gastroenterol 2020 (doi: 10.1016/j.clinre.2020.05.011).
39. Nguyen MH, Garcia RT, Trinh HN, et al. Histological disease in Asian-Americans with chronic hepatitis B, high hepatitis B virus DNA, and normal alanine aminotransferase levels. Am J Gastroenterol 2009;104: 2206–13.
40. Schuppan D, Kim YO. Evolving therapies for liver fibrosis. J Clin Invest 2013;123:1887–901.
41. Wang L, Zhu M, Cao L, et al. Liver stiffness measurement can reflect the active liver necroinflammation in population with chronic liver disease: A real-world evidence study. J Clin Transl Hepatol 2019;7:313–21.
42. Liang X, Xie Q, Tan D, et al. Interpretation of liver stiffness measurement-based approach for the monitoring of hepatitis B patients with antiviral therapy: A 2-year prospective study. J Viral Hepat 2018;25:296–305.
43. Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: A translational success story. Gut 2015;64:830–41.
44. Trautwein C, Friedman SL, Schuppan D, et al. Hepatic fibrosis: Concept to treatment. J Hepatol 2015;62:S15–24.
45. Linden A. Assessing regression to the mean effects in health care initiatives. BMC Med Res Methodol 2013;13:119.

