Accuracy of Real-Time Tissue Elastography for the Evaluation of Hepatic Fibrosis in Patients with Chronic Hepatitis B: A Prospective Multicenter Study

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Key Words
Chronic hepatitis B · Hepatic fibrosis · Real-time tissue elastography

Abstract
Background: The prognosis and management of hepatic fibrosis are closely related to the stage of the disease. The limitations of liver biopsy, which is the gold standard for treatment, include its invasiveness and sampling error. Ultrasound elasticity might be the most promising imaging technology for the noninvasive and accurate assessment of hepatic fibrosis. Real-time tissue elastography (RTE) measures the relative stiffness of the tissue in the region of interest caused by the heartbeat. Many studies have verified that RTE is useful for the diagnosis of hepatic fibrosis in patients with chronic hepatitis C (CHC). Purpose: To determine the formula of the liver fibrosis index for chronic hepatitis B (BLFI) and to validate the diagnostic accuracy of the BLFI for hepatic fibrosis compared with the liver fibrosis index (LFI).

Materials and Methods: RTE was performed in 747 prospectively enrolled patients with chronic hepatitis B (CHB) or cirrhosis from 8 centers in China; 375 patients were analyzed as the training set, and 372 patients were evaluated as the validation set. The fibrosis stage was diagnosed from pathological specimens obtained by ultrasound-guided liver biopsy. Nine image features were measured from strain images, and the new formula for the BLFI was obtained by combining the nine imaging features of the RTE images using multiple regression analysis of the training set. The BLFI and LFI were compared with the pathological fibrosis stage at diagnosis, and the diagnostic performances of the indexes were compared. Results: The Spearman correlation coefficient between the BLFI and hepatic fibrosis stages was significantly positive ($r = 0.711$, $p < 0.001$), and significant differences were present between all disease stages. The areas

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under the receiver-operating characteristic (AUROC) curves of the BLFI and LFI for predicting significant fibrosis (S0–S1 vs. S2–S4) were 0.858 and 0.858, respectively. For cirrhosis (S0–S3 vs. S4), the AUROC curves of the BLFI and LFI were 0.868 and 0.862, respectively. **Conclusion:** The results of this large, multicenter study confirmed that RTE is valuable for the diagnosis of hepatic fibrosis in patients with CHB. However, the diagnostic efficiencies of the new BLFI and the original LFI, which were based on CHC, for the assessment of CHB hepatic fibrosis were similar; thus, the LFI has the potential to be used to directly evaluate the extent of hepatic fibrosis in patients with CHB.

**Introduction**

Chronic viral hepatitis infection increases liver fibrosis and stiffness and is an important cause of liver cirrhosis and hepatocellular carcinoma [1–6]. Liver biopsy remains the gold standard for the diagnosis of hepatic fibrosis; however, a biopsy is an invasive procedure associated with discomfort and adverse events [7]. Moreover, the evaluation of liver biopsies could be influenced by sampling errors and by intra- and interobserver variability [8, 9]. Thus, noninvasive diagnostic methods for the assessment of hepatic fibrosis are urgently needed. Ultrasound (US) elastography has been considered a promising noninvasive and accurate modality for the assessment of hepatic fibrosis in several studies; it includes many forms, such as sonographic transient elastography (FibroScan; EchoSens, London, UK) and acoustic radiation force impulse imaging. Results have shown that FibroScan measurements of the velocity of the shear wave correlated well with the stage of fibrosis [10, 11], and this method has been recommended for use by the European Association for the Study of the Liver [2, 12]. However, the FibroScan has its limitations, including the lack of 2D image guidance and difficulties in evaluating certain types of patients with thick, fat tissue under the skin and ascites [13, 14].

Real-time tissue elastography (RTE) belongs to the category of strain elastography, which is different from the FibroScan. RTE measures the relative stiffness of the tissue in the region of interest (ROI) caused by the heartbeat using a combined autocorrelation method [14–17]. RTE displays the stiffness by overlaying the B-mode image in the ROI with a color; the colors range from blue to red and indicate the relative softness and hardness of the area, respectively. This method is less affected by the body mass index and ascites than the FibroScan [18]. Studies have demonstrated that RTE is useful for the diagnosis of hepatic fibrosis. However, evaluation indexes for the diagnosis of liver fibrosis in current reports are different, and some indexes are calculated manually and subjectively [16–22]. Recently, Fujimoto et al. [16] reported a novel image analysis method using RTE for the evaluation of hepatic fibrosis in patients with chronic hepatitis C (CHC). The authors extracted 9 image features to quantify the patchy pattern of the RTE images and used these features to characterize the image; the authors correlated these features with fibrosis staging. To improve the accuracy of estimating the extent of hepatic fibrosis and to make the method more convenient, they performed a multiple regression analysis that used the 9 image features. Thus, they obtained an index of the liver fibrosis index (LFI) from a multiple regression equation which correlated highly with the stages of hepatic fibrosis and also reflected the underlying hepatic fibrosis accurately [16].

However, published studies have demonstrated different results regarding liver stiffness in patients with CHC and chronic hepatitis B (CHB) [23–25]. A meta-analysis of FibroScan data indicated that different liver diseases influence the diagnostic performance of significant fibrosis [10]. The diagnostic performance of hepatic fibrosis assessment in patients with CHC and CHB might be different using this impressive and quantitative image analysis method. Estimates have indicated that there are more individuals with CHB (approx. 120 millions) than individuals with CHC in China; approximately 300,000 CHB patients die annually of liver cirrhosis and hepatocellular carcinoma [1]. Thus, the aims of this large, prospective, multicenter study in patients with CHB were (1) to obtain a new liver fibrosis index (BLFI), which is calculated from the image features of RTE images using a new multiple regression equation to examine the clinical data of our cases with CHB rather than CHC and (2) to explore the effectiveness of this new BLFI in the diagnosis of hepatic fibrosis and to compare these results with the LFI computed from the original multiple regression equation based on the data from CHC patients.

**Patients and Methods**

**Patients**

The study protocol was approved by the independent ethics committees of our institutions, and written informed consent was obtained from the participants. This prospective, multicenter, cross-sectional study was conducted at eight hospitals in China: the Third
Liver Histology Assessment

A US-guided percutaneous liver biopsy (1.2-mm-diameter and 160-mm-long needle, cutting technique) was performed within 1 week prior to the RTE examination. If the liver biopsy samples were less than 12 mm long, a second liver biopsy was performed to obtain longer samples in order to avoid sampling error in the identification of liver fibrosis [8]. The liver biopsy samples were fixed in formalin and embedded in paraffin. Slices (3 μm thick) were stained with hematoxylin-eosin and argyrophilic proteins. Fibrosis was staged by a single pathologist, who had more than 15 years of experience and was blinded to all patient characteristics.

Liver fibrosis was staged on a four-point scale from S0 to S4 according to the Scheuer scoring system as follows [26]: S0, no fibrosis; S1, enlarged, fibrotic portal tracts; S2, perportal or portal-portal septa, but intact architecture; S3, fibrosis with architectural distortion, but no obvious cirrhosis, and S4, probable or definite cirrhosis.

Measurement of Liver Stiffness

All study patients underwent RTE examinations using ultrasonography (HI-VISION Ascendus; Hitachi Aloka Medical, Tokyo, Japan) and the EUP-L52 linear probe (3–7 MHz; Hitachi Aloka Medical). The patients were examined in the supine position with the right arm elevated above the head to widen the intercostal space. The examinations were performed on the right lobe of the liver through the intercostal spaces while holding the transducer lightly against the skin without vibration to obtain RTE images that were displayed in the direction of the heart. The appropriate position was selected where the B-mode images were devoid of artifacts, and the position in which the hepatic parenchyma moves in a lateral direction as a result of cardiac motion was not appropriate for this study. While the patient was holding his breath, we ensured that the strain images were shown periodically by cardiac motion. The ROI of the strain image was 2.5 × 2.5 cm and was placed more than 1 cm below the surface of the liver. Additionally, to obtain accurate and reliable images, the ROI should avoid large vessels and attenuation by the lungs or ribs. Regions deep inside the liver are not suitable because they often appear blue because of poor US penetration. The best RTE images were selected for the final analysis. An average of the best 3–5 images for each patient was used in the multiple regression analysis and to calculate the BLFI [16]. In July 2012, we provided RTE education and training regarding the operation procedure, which was combined with previous experience. The RTE images that included horizontal slipping by cardiac movements, images that contained artifacts and images with poor penetration of the US were rejected; when the number of suitable RTE images for the patient was not sufficient, the patient was excluded.

We extracted the following 9 image features to quantify the variable pattern of the RTE images: mean relative strain value (MEAN); standard deviation of the relative strain value (SD); percentage of low strain area (percentage of blue color area: %AREA); complexity of low strain area (calculated as perimeter squared/area: COMP); skewness (SKEW); kurtosis (KURT); entropy (ENT); inverse difference moment (IDM) and angular second moment (ASM). A multiple regression analysis was performed to improve the diagnostic accuracy for hepatic fibrosis by combining these 9 image features rather than by a diagnosis using individual image features. The BLFI was assessed using these 9 image features as independent variables and the hepatic fibrosis stage as a dependent variable, and the multiple regression equation was estimated from the training set. The BLFI was applied to estimate the stage of hepatic fibrosis and to validate the diagnostic accuracy of the BLFI for hepatic fibrosis in the validation set. Moreover, the LFI was computed from the original multiple regression equation based on data from CHC patients as follows:

\[ LFI = -0.009 \times MEAN - 0.005 \times SD + 0.023 \times %AREA + 0.025 \times COMP + 0.775 \times SKEW - 0.281 \times KURT + 2.083 \times ENT + 3.042 \times IDM + 39.979 \times ASM - 5.542. \]

The effectiveness of the BLFI and LFI in the evaluation of fibrosis staging was compared.

Statistical Analysis

The BLFI equation was calculated using multiple regression analysis. The 9 image features comprised the independent variables, and the hepatic fibrosis stage comprised the dependent variable. For comparisons between included and excluded cases at the different times, the \( \chi^2 \) test was employed. The stiffness measurements were not normally distributed. Therefore, the measurements were compared with the categories of the consensus fibrosis staging using the nonparametric Mann-Whitney U test. Correlations

<table>
<thead>
<tr>
<th>Table 1. Clinical characteristics of and laboratory information on the study patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female ratio</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Body mass index</td>
</tr>
<tr>
<td>AST, IU/l</td>
</tr>
<tr>
<td>ALT, IU/l</td>
</tr>
<tr>
<td>Albumin, g/dl</td>
</tr>
<tr>
<td>Total bilirubin, mg/dl</td>
</tr>
<tr>
<td>GGT, IU/l</td>
</tr>
<tr>
<td>ALP, IU/l</td>
</tr>
<tr>
<td>Platelet count, × 10^4/l</td>
</tr>
<tr>
<td>Prothrombin time, %</td>
</tr>
</tbody>
</table>

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between the measurements and the histologic fibrosis stage were analyzed by Spearman’s correlation coefficients.

Receiver operating characteristic (ROC) curves were constructed, and the areas under the ROC (AUROC) curves were calculated using the trapezoidal rule. The optimal cutoff value for hepatic fibrosis was calculated according to the Youden index, which represents the best combination of sensitivity and specificity. The diagnostic performance for hepatic fibrosis was determined in terms of sensitivity, specificity and diagnostic accuracy using cutoff values obtained from the ROC curves. z scores were applied to compare AUROC curves between the BLFI and LFI [27].

p < 0.05 was considered statistically significant. Analyses were performed using SPSS Statistics 20 (IBM, Armonk, N.Y., USA) and MedCalc (version 12.7.0; MedCalc Software, Mariakerke, Belgium).

**Results**

**Patients**

We performed RTE in 836 patients between June 2010 and July 2013, and we excluded 89 patients from our study. The majority of the exclusions were related to the

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**Fig. 1.** Relationships between hepatic fibrosis stages and the MEAN, SD, %AREA and COMP in the combined data set. There was a high correlation between the MEAN, SD, %AREA and COMP and the stages of hepatic fibrosis.

**Table 2.** Number of cases recruited during the study

<table>
<thead>
<tr>
<th>Study period</th>
<th>Eligible cases, n</th>
<th>Excluded cases, n</th>
<th>Total cases, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2010 to July 2012</td>
<td>420</td>
<td>73 (14.8%)</td>
<td>493</td>
</tr>
<tr>
<td>August 2012 to July 2013</td>
<td>327</td>
<td>16 (4.7%)</td>
<td>343</td>
</tr>
<tr>
<td>Total</td>
<td>747</td>
<td>89 (10.6%)</td>
<td>836</td>
</tr>
</tbody>
</table>

* The 2 exclusion rates differed significantly (p < 0.001).

**Table 3.** Stages of hepatic fibrosis in the training and validation sets

<table>
<thead>
<tr>
<th>Stage</th>
<th>Training set, n</th>
<th>Validation set, n</th>
<th>Total, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>86 (23%)</td>
<td>85 (23%)</td>
<td>171 (23%)</td>
</tr>
<tr>
<td>S1</td>
<td>126 (34%)</td>
<td>126 (34%)</td>
<td>252 (34%)</td>
</tr>
<tr>
<td>S2</td>
<td>70 (19%)</td>
<td>69 (19%)</td>
<td>139 (19%)</td>
</tr>
<tr>
<td>S3</td>
<td>58 (15%)</td>
<td>57 (15%)</td>
<td>115 (15%)</td>
</tr>
<tr>
<td>S4</td>
<td>35 (9%)</td>
<td>35 (9%)</td>
<td>70 (9%)</td>
</tr>
<tr>
<td>Total</td>
<td>375 (100%)</td>
<td>372 (100%)</td>
<td>747 (100%)</td>
</tr>
</tbody>
</table>
inexperienced RTE skill level of the radiologists. The exclusion rate was significantly reduced from 14.8 to 4.7% (p < 0.001) after RTE education and training sessions were provided in July 2012 (table 2). The clinical characteristics of and laboratory information on the 747 patients are shown in table 1. The hepatic fibrosis stages of the cases, which were confirmed by liver histology assessment, are displayed in table 3.

Correlation between Features and Pathological Hepatic Fibrosis in All Sets

In our study, we extracted 9 image features and the LFI to quantify the RTE images. Correlation coefficients between the quantitative parameters of the RTE images, such as the MEAN, SD, %AREA, COMP, SKEW, KURT, ENT, IDM and ASM, and the hepatic fibrosis stage in all included patients were –0.627, 0.607, 0.656, 0.615, 0.482, –0.180, 0.278, 0.123 and 0.221, respectively (fig. 1). The MEAN, SD, %AREA and COMP were highly correlated with the stage of hepatic fibrosis. Characteristic RTE images for each fibrosis stage are shown in figure 2.

Equation for BLFI in the Training Set

The BLFI was estimated by combining the 9 image features using the multiple regression analysis of the training set. The multiple regression equation of BLFI was calculated using multiple regression analysis:

\[
\text{BLFI} = -0.0475 \times \text{MEAN} + 0.0222 \times \text{SD} - 0.00555 \times \text{AREA} + 0.0151 \times \text{COMP} + 31.1 \times \text{ASM} + 4.27 \times \text{ENT} + 11 \times \text{IDM} - 0.0326 \times \text{SKEW} + 0.215 \times \text{KURT} - 12.9.
\]

There were some differences compared with the original multiple regression equation for the LFI based on CHC patients described in the report of Fujimoto et al. [16].

Fig. 2. RTE images for each fibrosis stage in patients with CHB. The blue areas (see online version for colors) of the RTE image gradually increased with the progression of liver fibrosis: stage 0 (a), stage 1 (b), stage 2 (c), stage 3 (d) and stage 4 (e).
Diagnostic Performance of Fibrosis Staging by the BLFI in the Validation Set

Figure 3 shows the box-and-whisker plots for the BLFI in the validation set calculated from the 9 image features using the multiple regression analysis of the training set for each fibrosis stage. When the histologic liver fibrosis stages and the BLFI were compared, there was a high correlation between increased BLFI and increased hepatic fibrosis stages. The Spearman correlation coefficient between the BLFI and hepatic fibrosis stages was significantly positive (r = 0.711, p < 0.001), and significant differences existed between the different stages. The ROC curve analysis identified cutoff values of the BLFI as high as 1.603 for ≥ stage 2 and 2.062 for stage 4 (fig. 4). The corresponding AUROC, sensitivity, specificity and accuracy data are shown in table 4.

Comparison of the BLFI and LFI in the Validation Set

In the validation set, assessments of diagnostic accuracy for each fibrosis stage were performed using the BLFI and LFI. Comparisons of the correlation coefficients of the BLFI and the LFI showed that there were no significant differences between the variables (p > 0.05). The AUROC curves of the BLFI and LFI for predicting significant fibrosis (S0–S1 vs. S2–S4) were 0.858 and 0.858, respectively. For cirrhosis (S0–S3 vs. S4), the AUROC curves of the BLFI and LFI were 0.868 and 0.862, respectively (table 4). Comparisons of the AUROC curves of the two index values indicated that there was no significant difference between the values (fig. 5; p > 0.05).
Discussion

In our study, we showed that the parameters measured by RTE are useful predictive features for the determination of the hepatic fibrosis stage in patients with CHB.

The MEAN, SD, %AREA and COMP showed a high correlation with the hepatic fibrosis stage; these results were similar to those of Fujimoto et al. [16]. The %AREA was the best indicator of the individual image features in all patients because the %AREA represents an area of low strain (blue) within the ROI; as hepatic fibrosis progresses, liver stiffness increases, with a corresponding increase in the area of low strain. Additionally, the BLFI is a comprehensive quantitative indicator of 9 image features that was developed using multiple regression analysis. It includes the most abundant information in the RTE images and highly correlates with the stages of hepatic fibrosis \( r = 0.711, p < 0.001 \): significant differences in the BLFI were identified between each stage. Moreover, the AUROC and accuracy for predicting significant fibrosis and cirrhosis were as high as 0.858 and 0.770 and 0.868 and 0.800, respectively.

We compared the diagnostic performance of the new BLFI in the validation set with the LFI obtained from patients with CHC [16]. The AUROC and accuracy of the BLFI for the diagnosis of significant fibrosis and cirrhosis were similar to the LFI (table 4), and there was no significant difference between the values; this similarity might have been caused by the similar basic morphologic changes that occur during the pathology of CHB and CHC [26]. However, the epidemiology is different between CHB and CHC [1], and the necroinflammatory activity observed during HBV infection may differ with time, which makes patients with CHB prone to transaminase fluctuations [24]. Transient elastography is affected by inflammation, which might overestimate the degree of hepatic fibrosis. Studies have shown that the correlation coefficient between liver stiffness measured by transient

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**Table 4. Diagnostic performance of the cutoff values of the BLFI and LFI for predicting significant fibrosis (S0–S1 vs. S2–S4) and cirrhosis (S0–S3 vs. S4)**

<table>
<thead>
<tr>
<th></th>
<th>BLFI</th>
<th>LFI</th>
<th>BLFI</th>
<th>LFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUROC</td>
<td>0.858</td>
<td>0.858</td>
<td>0.868</td>
<td>0.862</td>
</tr>
<tr>
<td>Cutoff value</td>
<td>1.603</td>
<td>2.099</td>
<td>2.062</td>
<td>2.511</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>77.0</td>
<td>77.0</td>
<td>80.0</td>
<td>74.3</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>77.3</td>
<td>76.8</td>
<td>77.4</td>
<td>79.8</td>
</tr>
<tr>
<td>Accuracy, %</td>
<td>77.2</td>
<td>76.9</td>
<td>77.7</td>
<td>79.3</td>
</tr>
</tbody>
</table>

There was no significant difference in the AUROC between the BLFI and LFI \( p > 0.05 \).
elastography and the stages of hepatic fibrosis in patients with CHC was higher compared with patients with CHB [10, 24]. However, our study showed that the diagnostic performances of the BLFI and LFI were similar, perhaps because the RTE might reflect the actual degree of hepatic fibrosis not affected by inflammation. The diagnostic performance of the BLFI for the assessment of hepatic fibrosis in CHB patients was not significantly better than the LFI; the BLFI did not achieve its expected purpose. Therefore, it might be possible to continue to use the LFI to evaluate hepatic fibrosis, as is done for CHB patients. The LFI is automatically calculated by the machine, which is convenient and widely accepted by clinicians.

In our study, we excluded 89 patients from our analysis, which accounted for 10.6% of the total cases; however, the exclusion rate was significantly reduced after completion of the RTE education and training sessions. The main reason for the exclusions was the examiners’ lack of skill and experience, which might have an impact on the final outcome. It is necessary to further discuss the methodology of RTE in the future and to propose unified operational procedures for the training of examiners; these training procedures would be conducive to improving the acquisition rate and popularizing the applications of this technology.

Previous studies regarding RTE have shown that RTE is a useful and valuable tool for the assessment of hepatic fibrosis [16–22]. The subjects of these studies were primarily patients with CHC or chronic viral hepatitis, and there were only two reports regarding patients with CHB. Xie et al. [21] and Wang et al. [22] included patients with CHB; however, their evaluation indicators included the elastic strain ratio and elastic index, respectively. The LFI used in the study of Fujimoto et al. [16] contained abundant image information regarding the RTE, and the LFI was simple to use, which made it easier for clinicians to accept and further popularize and apply this technology. Our study was a large, multicenter prospective study of patients with CHB in China; we challenged a new index for evaluating hepatic fibrosis, as is done for CHB patients with CHB.

Moreover, according to the diagnostic test method, we divided the patients into two groups: a training set for obtaining the new formula of the BLFI and a validation set for verifying the accuracy of the formula. This approach could make the results more convincing. Furthermore, we compared the results of the new BLFI with the LFI computed from the original multiple regression equation based on data from CHC patients, which has not been previously reported in any study.

We used the Scheuer scoring system as the gold standard of hepatic fibrosis in our study because this system has been generally applied and accepted, recommended by the Chinese Society of Hepatology and used in previous studies [21]. Furthermore, the staging criteria of the Scheuer scoring system are similar to the METAVIR System [26]. For these reasons, the Scheuer scoring system would not affect the judgment of our results. In our study, the number of CHB patients in S2–S4 was low and only accounted for 43% of the total cases (324/747), whereas in the study by Fujimoto et al. [16], CHC patients with F2–F4 accounted for 71% of the total cases (211/295), which had a more balanced distribution. To obtain a more accurate diagnostic threshold of hepatic fibrosis for patients with CHB, it is necessary to increase the number of S2–S4 cases to reduce the offset. Some of our study results were obtained only by comparisons with the previous literature [16]. The best method may be based on a comparison of two patient groups with different causes of fibrosis, which would increase the reliability of the results. Because the inflammation stage of some cases was missing during the data collection, we are unable to discuss the related research regarding inflammation in our study.

Conclusion

The results of this large, multicenter study confirmed that RTE is a valuable tool for the diagnosis of hepatic fibrosis in patients with CHB. However, the diagnostic efficiencies of the new BLFI and the original LFI, which was based on CHC patients, in assessing CHB hepatic fibrosis were similar. Thus, we might be able to use the LFI directly to evaluate the extent of hepatic fibrosis in patients with CHB.

Acknowledgments

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