Abstract

AIM

To determine the diagnostic accuracy of two-dimensional shear wave elastography (2D-SWE) for the non-invasive assessment of liver fibrosis in patients with autoimmune liver diseases (AILD) using liver biopsy as the reference standard.

METHODS

Patients with AILD who underwent liver biopsy and 2D-SWE were consecutively enrolled. Receiver operating characteristic (ROC) curves were constructed to assess the overall accuracy and to identify optimal cut-off values.

RESULTS

The characteristics of the diagnostic performance were determined for 114 patients with AILD. The areas under the ROC curves for significant fibrosis, severe fibrosis, and cirrhosis were 0.85, 0.85, and 0.86, respectively, and the optimal cut-off values associated with significant fibrosis (≥ F2), severe fibrosis (≥ F3), and cirrhosis (F4) were 9.7 kPa, 13.2 kPa and 16.3 kPa, respectively. 2D-SWE showed sensitivity values of 81.7% for significant fibrosis, 83.0% for severe fibrosis, and 88.9% for cirrhosis.
Liver biopsy (LB) is still considered the reference standard for determining medical management and prognosis. The evaluation of the degree of liver fibrosis is important in chronic liver damage with fibrosis and cirrhosis. An autoimmune pattern of inflammation, and the clinical phenotype according to the focus of the autoimmune injury, the cholangitis (PBC), and primary sclerosing cholangitis (PSC) cirrhosis. Autoimmune hepatitis (AIH), primary biliary antibodies and a potential tendency to progress to chronic liver diseases characterized by an anomalous immune response that is directed at hepatocytes or bile ducts diseases.

Autoimmune liver diseases (AILD) are a group of autoimmune liver disease; Liver fibrosis; Two-dimensional shear wave elastography; Ultrasound; Liver stiffness

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Core tip: The study determined the diagnostic accuracy of two-dimensional shear wave elastography (2D-SWE) for the non-invasive assessment of liver fibrosis in patients with autoimmune liver diseases (AILD) using liver biopsy as the reference standard. The characteristics of the diagnostic performance were determined for 114 patients with AILD. The areas under the receiver operating characteristic curves for significant fibrosis, severe fibrosis, and cirrhosis were 0.85, 0.85 and 0.86, respectively, and the optimal cut-off values were 9.7 kPa, 13.2 kPa and 16.3 kPa, respectively. 2D-SWE showed promising diagnostic performance in assessing liver fibrosis stages and exhibited high cut-off values in patients with AILD.

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INTRODUCTION

Autoimmune liver diseases (AILD) are a group of diseases characterized by an anomalous immune response that is directed at hepatocytes or bile ducts along with the presence of serum antimitochondrial antibodies and a potential tendency to progress to cirrhosis. Autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and primary sclerosing cholangitis (PSC) are the three major forms of AILD, which differ according to the focus of the autoimmune injury, the pattern of inflammation, and the clinical phenotype[1-2]. These diseases are important because they may result in chronic liver damage with fibrosis and cirrhosis. An evaluation of the degree of liver fibrosis is important for determining medical management and prognosis. Liver biopsy (LB) is still considered the reference standard for the evaluation of liver fibrosis[3]. However, LB is an invasive procedure, limiting its use for screening and frequent follow-up. The demand for a non-invasive and reliable test to evaluate liver fibrosis in patients with AILD has increased.

Two-dimensional shear wave elastography (2D-SWE), an ultrasound elastography technique based on shear waves that is available on a clinical diagnostic ultrasound scanner, has been used to non-invasively measure liver fibrosis[4]. The 2D-SWE technique creates a real-time, two-dimensional quantitative map of liver tissue stiffness under the guidance of very-high-frame-rate B mode imaging[5]. This method has good diagnostic accuracy for the staging of liver fibrosis in patients with chronic liver diseases[6-11]. However, studies on the use of 2D-SWE to assess histologically confirmed liver fibrosis in patients with AILD have not been published. AILD are considered a relatively uncommon etiology in the Asia-Pacific region, where viral hepatitis is the primary diagnosis in the majority of patients with chronic liver diseases. However, based on recent findings, the prevalence of both AIH and PBC is increasing worldwide[12]. Despite advances in the understanding and treatment of AILD, areas of unmet need remain.

Therefore, the goal of this study was to assess the diagnostic accuracy of 2D-SWE for the non-invasive staging of liver fibrosis in patients with AILD. LB samples that were scored with the histology-based METAVIR staging system were used as the diagnostic reference standard.

MATERIALS AND METHODS

Study design and participants

Informed consent was obtained from all patients, and the study including the 2D-SWE examination was approved by the clinical medical research ethics committee of our hospital. One hundred and thirty-nine patients with AILD who underwent LB and 2D-SWE examinations were enrolled consecutively between April 2011 and March 2016. The diagnoses of AIH, PBC and PSC in all patients were confirmed by histological evidence[13-18]. All patients were not under immunosuppressive treatment at time of the LB and 2D-SWE examinations. The time intervals between LB and 2D-SWE were less than three days. The exclusion criteria included: patients younger than 18 years; a lack of consent for the 2D-SWE examination or LB; chronic liver disease accompanied by hepatitis virus infection or another disease; and LB samples less than 15 mm long or with fewer than 6 portal tracts under the microscopic examination. The following data were collected from all patients: liver stiffness measurements (LSM) obtained using 2D-SWE; fibrosis stages; necroinflammatory activity grades; age; gender; weight; height; alanine aminotransferase, aspartate aminotransferase, serum alkaline phosphatase (ALP), gamma-glutamyl transpeptidase, total bilirubin, and serum albumin con-
centrations; platelet count; and prothrombin activity. Body mass index was calculated as body weight (kg)/[height (m^2)].

**2D-SWE**

2D-SWE was performed within three days of LB. Two radiologists (Zeng J and Zheng J) performed the 2D-SWE examinations. Both radiologists had at least 6 mo of experience in performing 2D-SWE examinations. The radiologists were blinded to the patients’ clinical information and pathology results. 2D-SWE was performed using the Aixplorer US system (SuperSonic Imagine, France) with a convex broadband probe (SC6-1, 1-6 MHz). All patients had fasted for at least 6 h prior to the examination. 2D-SWE measurements were obtained from the right lobe of the liver through the intercostal spaces when the patient was lying in the supine position and the right arm was positioned in maximal abduction. The operator positioned the target area of the liver under the guidance of conventional, real-time B-mode imaging. When the target area was located, SWE was launched, and the patient was asked to hold his or her breath for approximately 5 s during a quiet breathing period. The elasticity image box, which was approximately 4 cm × 3 cm, was set 1-2 cm deeper than Glisson's capsule of the liver and in an area of the liver parenchyma that was free of large vessels.

A circular region of interest with a 2-cm diameter was drawn inside the elasticity image box, and the mean, minimum and maximum liver stiffness and SD were calculated (Figure 1). The mean value was used to represent the LSM for each 2D-SWE image. Five consecutive 2D-SWE images were obtained for each patient. Each measurement was performed during a separate breath hold. The mean value of the five 2D SWE measurements was calculated for statistical analysis. The entire 2D-SWE examination lasted three to five minutes for each patient. Five consecutive 2D-SWE images were obtained from each patient. Measurements were considered failures when no or little signal was obtained in the SWE box for any of the acquisitions.

**Analysis of liver histology**

Ultrasoundography-assisted percutaneous LB was performed in the right liver lobe using a 16-gauge Magnum needle (Bard, Tempe, AZ). The LB specimens were fixed in formalin and embedded in paraffin. The biopsy specimens were analyzed by two expert liver pathologists with more than 10 and 20 years of experience, respectively, and who were blinded to the results of 2D-SWE but not to the clinical and biochemical data for each patient. Liver fibrosis and necroinflammatory activity were semiquantitatively evaluated using the METAVIR scoring system. Liver fibrosis was staged on a 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. Significant fibrosis was defined as stage F2 or higher, and severe fibrosis was defined as stage F3 or higher. Necroinflammatory activity was graded as follows: A0 = none; A1 = mild; A2 = moderate; and A3 = severe.

**Statistical analysis**

The demographic, clinical, and laboratory values are summarized using descriptive statistics. The data were first tested for normality using the one-sample Kolmogorov-Smirnov test. Spearman’s rank coefficient was used to determine the correlation between two study variables. Receiver operating characteristic (ROC) curves were constructed to assess the overall accuracy of the LSMs and to identify optimal cut-off values. Areas under the ROC curves (AUROCs) were compared using the method described by DeLong et al for correlated data. The optimal cut-off values were the points with the highest Youden’s index. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated from the same data. The positive likelihood ratio (LR+ and negative likelihood ratio (LR–) were calculated from the respective sensitivity and specificity values. All factors collected from patients with AILD were entered in a multivariate logistic model to analyze the disagreement between LSMs obtained using 2D-SWE and significant fibrosis or cirrhosis. ORs were estimated from the model and are presented with their 95% CIs. Continuous variables, such as age, were dichotomized around the median (46 years), unless a cut-off was considered clinically relevant.

All statistical tests were two-sided, and the alpha value was set at 0.05. Statistical analyses were performed using SPSS software for Windows, version 13.0 (SPSS, Chicago, IL, United States), and MedCalc software, version 12.7.0 (MedCalc Software, Mariakerke, Belgium).

**RESULTS**

**Patients**

One hundred and thirty-nine patients were enrolled in the study. Twenty-two patients were not included based on the exclusion criteria, including 2 patients who were younger than 18 years, 14 patients with biopsy samples less than 15 mm long or with fewer than six portal tracts under the microscope, 1 patient with a hepatitis A virus coinfection, 3 patients with hepatitis B virus coinfections, and 2 patients with hepatitis C virus coinfections. Therefore, 117 patients with AILD defined by a reliable reference standard were consecutively enrolled in the study. The 2D-SWE examination failed in three patients. Therefore, 114 patients with reliable LSMs obtained using 2D-SWE were used to assess diagnostic accuracy. The characteristics of the 114 patients are summarized in Table 1. Fibrosis conditions that were scored as significant fibrosis (≥ F2), severe fibrosis (≥ F3), severe fibrosis or cirrhosis (≥ F4), and F4: cirrhosis. Significant fibrosis was defined as stage F2 or higher, and severe fibrosis was defined as stage F3 or higher. Necroinflammatory activity was graded as follows: A0 = none; A1 = mild; A2 = moderate; and A3 = severe.
by 2D-SWE at a given fibrosis stage had significantly higher median LSMs obtained using 2D­SWE than patients with less fibrosis ($P < 0.05$). Spearman's correlation coefficients for LSMs and fibrosis stages were 0.68 ($P < 0.001$) (95%CI: 0.57-0.77).

Diagnostic performance of 2D-SWE for predicting significant fibrosis, severe fibrosis and cirrhosis

The AUROCs of 2D-SWE for significant fibrosis, severe fibrosis, and cirrhosis were 0.85 (95%CI: 0.77-0.91), 0.85 (95%CI: 0.77-0.92), and 0.86 (95%CI: 0.78-0.92), respectively (Figure 3). The optimal cut-off values for the different stages of fibrosis were determined by analyzing the ROCs. The optimal cut-off values associated with significant fibrosis ($\geq F2$), severe fibrosis ($\geq F3$), and cirrhosis (F4) were 9.7 kPa, 13.2 kPa and 16.3 kPa, respectively.

Figure 1  Two-dimensional shear wave elastography measurements in the liver. A: Fibrosis stage F1; B: Fibrosis stage F4.

Table 1  Demographic data, blood tests and histological results in patients with autoimmune liver diseases $n$ (%)

| Characteristic | $n$ = 114 |
|---------------|----------|
| Age, yr (SD; range) | 45.6 (12.5; 18-74) |
| Gender, male, $n$ (%) | 21 (18.4) |
| BMI, kg/m$^2$ (SD; range) | 21.6 (3.0; 15.4-36.4) |
| AST, IU/L (IQR; range) | 84.0 (51.3-148.0; 16.0-473.0) |
| ALT, IU/L (IQR; range) | 78.5 (49.5-142.0; 9.0-920.0) |
| Alkaline phosphatase, IU/L (IQR; range) | 208 (110.0-353.0; 47.0-873.0) |
| Gamma-glutamyl transferase, IU/L (IQR; range) | 252.0 (111.0-523.8; 16.0-1535.0) |
| Total bilirubin, umol/L (IQR; range) | 20.3 (13.6-43.6; 3.6-375.8) |

Table 2  Number of patients with autoimmune liver diseases at different fibrosis stages

| METAVIR stage | FO-F1 ($n$) | F2 ($n$) | F3 ($n$) | F4 ($n$) |
|---------------|-------------|----------|----------|----------|
| F0-F1 | 19 | 18 | 9 | 16 |
| F2 | 8 | 10 | 8 | 4 |
| F3 | 1 | 1 | 0 | 1 |
| F4 | 4 | 6 | 7 | 2 |
| Total | 32 | 35 | 24 | 23 |

AHE: Autoimmune hepatitis; PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; PBC-AIH: PBC-AIH overlap syndrome.

Table 3  Median values, inter quartile range, ranges, and $P$ values of measurements obtained for each fibrosis stage using two-dimensional shear wave elastography

| METAVIR stage | FO-F1 | F2 | F3 | F4 |
|---------------|-------|----|----|----|
| Median value, kPa | 7.7 | 11.7 | 14.7 | 22.0 |
| IQR | 6.5-9.2 | 8.3-15.4 | 12.3-22.3 | 18.8-33.3 |
| Range | 4.9-25.8 | 4.6-51.7 | 8.7-67.2 | 10.1-45.3 |
| $P$ value$^1$ | < 0.001 | 0.009 | 0.011 |

$^1$P values refer to differences between consecutive fibrosis stages. IQR: Inter quartile range.

Liver stiffness measured by 2D-SWE at each stage of fibrosis

The median values, interquartile ranges, ranges, and $P$ values of the measurements obtained for each fibrosis stage with 2D-SWE are shown in Table 3. As the fibrosis stage progressed, the median LSM of the fibrosis stage increased on 2D-SWE (Figure 2). The LSMs of patients and cirrhosis (F4) were detected in 71.9%, 41.2%, and 20.2% of patients, respectively. The numbers of patients with different fibrosis stages of AILDs are reported in Table 2.

Table 4  Two-dimensional shear wave elastography measurements in the liver.

| Characteristic | FO-F1 | F2 | F3 | F4 |
|---------------|-------|----|----|----|
| Median value, kPa | 7.7 | 11.7 | 14.7 | 22.0 |
| IQR | 6.5-9.2 | 8.3-15.4 | 12.3-22.3 | 18.8-33.3 |
| Range | 4.9-25.8 | 4.6-51.7 | 8.7-67.2 | 10.1-45.3 |
| $P$ value | < 0.001 | 0.009 | 0.011 |
The sensitivity, specificity, PPV, NPV, LR+, and LR- for each METAVIR stage are shown in Table 4. Using the optimal cut-off values, 2D-SWE showed sensitivity and specificity values of 81.7% and 81.3% for significant fibrosis, 83.0% and 74.6% for severe fibrosis, and 87.0% and 80.2% for cirrhosis, respectively. The corresponding optimal cut-off values for patients with chronic hepatitis B (CHB) were 7.2 kPa, 9.1 kPa and 11.7 kPa, and the corresponding values for patients with chronic hepatitis C (CHC) were 7.1 kPa, 8.7 kPa and 9.1 kPa, respectively. Using the cut-off values for CHB and CHC, the sensitivity values for assessing the stages of fibrosis were greater than 90%. However, trade-offs were observed: the accompanying specificity values for predicting significant fibrosis were less than 85%, and the accompanying specificity values for predicting severe fibrosis and cirrhosis fibrosis were approximately 50%.

Concordance rates of the LSMs obtained using 2D-SWE vs METAVIR stages

Table 5 shows the concordance rates of the LSMs obtained using 2D-SWE vs METAVIR stages. Overall, 2D-SWE correctly classified 61 of 114 (53.5%) patients. 2D-SWE returned the highest rates of correctly classified patients at the F0-1 stage at 63.4%. 2D-SWE showed lower rates of correctly classified patients at other stages, particularly the F2 and F3 stages. The concordance rates of the F2 and F3 stages were less than 50%.

Analysis of factors associated with the disagreement between LSMs obtained using 2D-SWE and significant fibrosis or cirrhosis

According to the multivariate logistic regression analysis, the disagreement between LSMs obtained using 2D-SWE and cirrhosis was independently associated with the following factors: age greater than 40 years (OR = 7.5, 95%CI: 1.7-33.3, P = 0.016), abnormal ALP levels (OR = 4.0, 95%CI: 1.5-12.1, P = 0.02) and abnormal serum albumin levels (OR = 11.6, 95%CI: 3.2-42.0, P < 0.001). No factors were significantly associated with the disagreement between LSMs obtained using 2D-SWE and significant fibrosis.

DISCUSSION

This study included a cohort of patients with AILD and aimed to evaluate the diagnostic accuracy of 2D-SWE for the non-invasive staging of hepatic fibrosis using LB as the reference standard. AILD are divided into two groups; the first group is predominantly characterized by hepatocellular damage and its prototype is...
AIH. The second group is characterized by cholestasis and includes PBC and PSC\(^{[23]}\). AIH, PBC and PSC represent complex disorders, as they result from interactions between genetic and environmental factors. In AIH, autoimmune injury affects hepatocytes, leading to the histological manifestation of interface hepatitis. In PBC, autoimmune injury affects the small, interlobular bile ducts, causing the typical appearance of non-suppurative, destructive cholangitis. In PSC, autoimmune or immune-mediated injury affects the medium-sized intra- and extrahepatic bile ducts, causing concentric and obliterative fibrosis and multifocal bile duct structuring\(^{[24]}\). However, AIH, PBC and PSC share pathophysiologic mechanisms\(^{[24,25]}\). Overlap syndromes encompass a small group of patients within the spectrum of AILD that may have characteristics of cholestasis (PBC or PSC) in combination with AIH\(^{[17]}\). In our study, 62 patients were diagnosed with AIH and 30 patients diagnosed with PBC. Overlap syndromes of AIH and PBC were diagnosed in 19 (16.7%) of 114 patients. Therefore, we used the patients with AILD as the study cohort.

Although 2D-SWE has been widely recognized as a reliable method to assess liver fibrosis in recent years, the diagnostic performance of 2D-SWE for assessing liver fibrosis stages in patients with AILD remains unclear. The 2D-SWE examinations performed in our study were successful in all but 3 of the 117 patients. We concluded that 2D-SWE provides a very high percentage of interpretable test results. Our results regarding the rate of successful 2D-SWE measurement in patients with AILD were similar to those reported in a previous study using 2D-SWE in patients with CHB\(^{[22]}\).

Previous studies reported the diagnostic accuracy of 2D-SWE for the detection of fibrosis stage in patients with CHB or CHC when the histology-based METAVIR staging system was used as the diagnostic reference standard\(^{[8,11]}\). The METAVIR staging system is simple and practical and is widely used for liver fibrosis staging in patients with chronic liver diseases. In our study, we also used the METAVIR staging system as the diagnostic reference standard. AUROCs for the identification of significant fibrosis and cirrhosis were approximately 0.85 and 0.86, respectively. Stage F2 or greater indicates the beginning of progressive liver disease and therefore suggests a stronger indication to initiate treatment; thus, 2D-SWE may serve as a screening tool to differentiate patients with significant fibrosis from patients without. Cirrhosis is the end stage of chronic liver disease. 2D-SWE may also be used to differentiate patients with cirrhosis from those without. According to previous studies, AUROCs of 2D-SWE for predicting significant fibrosis, severe fibrosis, and cirrhosis were 0.88, 0.93 and 0.98 in patients with CHB and 0.92, 0.98 and 0.98 in patients with CHC, respectively\(^{[8,11]}\). In our study, moderate diagnostic performance for assessing liver fibrosis stages was observed in patients with AILD, with AUROCs of approximately 0.85. The AUROCs in patients with AILD were lower than those in patients with CHB or CHC. In our series, 2D-SWE correctly classified 53.5% of patients with AILD. The concordance rates were also lower for 2D-SWE vs METAVIR stages in patients with CHB or CHC\(^{[8,22]}\).

The optimal cut-off values associated with significant fibrosis, severe fibrosis and cirrhosis in patients with AILD determined by the ROC analysis were 9.7 kPa, 13.2 kPa and 16.3 kPa, respectively. The corresponding optimal cut-off values in patients with CHB were 7.2 kPa, 9.1 kPa and 11.7 kPa, and the corresponding values for patients with CHC were 7.1 kPa, 8.7 kPa and 10.4 kPa, respectively\(^{[8,22]}\). Thus, the optimal cut-off values in patients with AILD were markedly higher than the values in patients with CHB or CHC. Using the optimal cut-off values calculated from the ROC curves in our study, 2D-SWE showed sensitivity values of greater than 80% and the corresponding specificity values were approximately 80%. Using the cut-off values for CHB or CHC, the sensitivity values for assessing the stages of fibrosis were greater than 90%, but the accompanying specificity values

![Figure 3](image-url)
were very low. Fibrosis stages would be overestimated using the cut-off values for CHB or CHC in patients with AILD. Therefore, the cut-off values for CHB and CHC were not appropriate for patients with AILD. The lower diagnostic accuracy and higher cut-off values might be characteristics of 2D-SWE for assessing liver fibrosis in patients with AILD, and differences in patient populations and etiology might explain the differences in these results.

This study has certain limitations that warrant discussion. First, our study used patients with AILD as its cohort. Patients with AIH, PBC and PSC were not separately analyzed. Second, the distribution of patients across all fibrosis stages was not uniform, as a very low percentage of enrolled patients were at stage F0. Third, the LB did not meet the American Society for the Study of Liver Diseases criteria of being at least 2-3 cm in length with at least 11 portal tracts; also, agreement between the readers of the liver biopsies was not assessed in our study. Fourth, although other ultrasound techniques have been used to evaluate liver fibrosis, such as transient elastography (FibroScan; Echosens) and acoustic radiation force impulse (ARFI; Siemens Healthcare), we only evaluated 2D-SWE.

In conclusion, 2D-SWE showed promising diagnostic performance for assessing the liver fibrosis stages and exhibited high cut-off values in patients with AILD; however, the concordance rates of the LSMs obtained using 2D-SWE vs METAVIR stages were low. Our study opens possibilities for a non-invasive assessment of fibrosis progression in patients suffering from AILD.

**COMMENTS**

**Background**

Autoimmune liver diseases (AILD) are a group of diseases characterized by an anomalous immune response that is directed at hepatocytes or bile ducts along with the presence of serum antimitochondrial antibodies and a potential tendency to progress to cirrhosis. These diseases are important because they may result in chronic liver damage with fibrosis and cirrhosis. An evaluation of the degree of liver fibrosis is important for determining medical management and prognosis. The demand for a non-invasive and reliable test to evaluate liver fibrosis in patients with AILD has increased.

**Research frontiers**

Two-dimensional shear wave elastography (2D-SWE) has been used to non-invasively measure liver fibrosis. This method has good diagnostic accuracy for the staging of liver fibrosis in patients with chronic liver diseases. However, studies on the use of 2D-SWE to assess histologically confirmed liver fibrosis in patients with AILD have not been published.

**Innovations and breakthroughs**

2D-SWE showed promising diagnostic performance for assessing liver fibrosis stages and exhibited high cut-off values in patients with AILD; however, the concordance rates of the liver stiffness measurements obtained using 2D-SWE vs METAVIR stages were low.

**Applications**

2D-SWE is beneficial for assessing liver fibrosis stages in patients with AILD as this method is non-invasive and reproducible in the clinical setting. The optimal cut-off values are high but appropriate for patients with AILD.

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