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Original paper

Transient elastography reliably estimates liver fibrosis in autoimmune hepatitis

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Abstract

Aim of the study: Autoimmune hepatitis (AIH) may result in liver fibrosis and cirrhosis. While the gold standard for staging fibrosis is biopsy, transient elastography (TE) represents a non-invasive alternative. TE has been validated in several chronic liver diseases, but no data exist to establish an association between histologic fibrosis on biopsy and TE liver stiffness measurements among a United States cohort of AIH patients.

Material and methods: We conducted a retrospective cohort study of 53 AIH patients who received TE assessment and liver biopsy. Histologic fibrosis was classified as advanced (F3-F4) or mild/moderate (F0-F2). Liver stiffness by TE was measured in kilopascals (kPa). We performed a score test for trend to test the association between histologic fibrosis stage and increasing TE kPa categories. Analyses incorporated probe type (medium or extra-large) and body mass index (BMI). Linear regression was used to generate predicted associations between median kPa and histologic fibrosis score with the medium probe.

Results: The cohort was primarily female (83%) with median age 56.3 years. Increasing kPa category was associated with worsening fibrosis stage when using the medium probe ($p = 0.04$), but not the extra-large probe ($p = 0.40$). BMI, however, differed between these groups (median 25.8 vs. 33.1, respectively, $p < 0.001$). In adjusted linear regression, increasing median kPa corresponded well to worsening fibrosis stage ($p = 0.003$).

Conclusions: In a United States AIH cohort, increasing TE kPa measurements are associated with worsening histologic fibrosis staging. While medium probe performance was superior to the extra-large probe, significant variation in BMI between groups may explain this difference.

Key words: transient elastography, autoimmune hepatitis, FibroScan, liver biopsy, fibrosis staging.

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Introduction

Autoimmune hepatitis (AIH) is a condition whereby chronic inflammation in the liver may result in progressive fibrosis and eventually cirrhosis. AIH is diagnosed through a combination of biochemical and serological markers, including increased levels of serum aminotransferases, high immunoglobulins, and the presence of circulating autoantibodies [1, 2]. Liver biopsy plays an important role in the diagnosis and follow-up of patients with AIH by allowing for disease staging and assessment of inflammation and fibrosis. While the gold standard for staging liver fibrosis is liver biopsy [3], this is an invasive procedure with an inherent risk of complications. It is also subject to sampling error and/or variations in technique that may result in incorrect staging of disease [4, 5]. Several technologies, such as transient elastography (TE), have therefore emerged as non-invasive means of estimating liver fibrosis.

TE is commonly used across the world and was approved for use in the United States by the Food and Drug Administration in April 2013 [4, 6]. The association between TE measurements and liver fibrosis has

been previously validated in several other chronic liver diseases, including alcoholic liver disease, non-alcoholic fatty liver disease, hepatitis B, and hepatitis C [4, 7-10]. However, data regarding the validity of TE in AIH patients remain limited, with existing data coming exclusively from Asian or German cohorts [11-15]. To date no studies have focused on a North American cohort, nor have they studied the impact of body mass index (BMI) on TE measurements for AIH patients. Thus, In this study, we aimed to determine the association between histologic liver fibrosis and TE measurements of liver stiffness in demographics reflective of the United States AIH population. The impact of probe type and BMI on this relationship was also evaluated.

Material and methods

Design and cohort creation

This was a retrospective cohort study performed at the Hospital of the University of Pennsylvania in order to determine the association between TE assessment of liver stiffness, as measured in kilopascals (kPa), and histologic fibrosis staging. We included patients age 18-85 who underwent TE for an indication of AIH between December 2014 and May 2017, and who received liver biopsy, through either the percutaneous or the transjugular approach. Diagnoses of AIH were confirmed through manual chart review of clinical documentation and laboratory data. In general, at our institution AIH is diagnosed according to American Association for the Study of Liver Disease guidelines [16]. In brief, patients are diagnosed with AIH if there is at least one elevation of an aminotransferase, typically at least 2 times the upper limit of the normal range, as well as elevation of an associated autoantibody (e.g., anti-smooth muscle antibody, antinuclear antibody, anti-liver/kidney microsomal-1 antibody). In patients suspected of having AIH with negative antibodies, liver biopsy is pursued to evaluate for histologic evidence of AIH. At our institution, in patients with established AIH, liver biopsy is also commonly performed prior to tapering therapy, as was done for many patients in our cohort. In this study, we excluded patients who had not received a liver biopsy. We also excluded patients who had concomitant liver disease in addition to AIH, or who had unreliable TE measurements (described below). Finally, this study met criteria for University of Pennsylvania Institutional Review Board exemption.

Variable collection

Electronic medical records for included patients were reviewed in order to obtain demographic data (age, sex, race). BMI, laboratory data (sodium, creatinine, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase [AST], alanine aminotransferase [ALT], international normalized ratio, and platelet count), model for end-stage liver disease (MELD) computation, and comorbidities (hypertension, hyperlipidemia, diabetes, other concomitant liver disease) were obtained at the time of TE measurement. We also obtained AIH treatment data including active therapy with steroids, azathioprine, tacrolimus, or mycophenolate mofetil, and created a binary variable to indicate any active therapy versus no therapy. All liver biopsy pathology reports were manually reviewed, and degree of liver fibrosis was graded using the Batts-Ludwig scale (F0 to F4, where F0 – no fibrosis and F4 – severe fibrosis). For pooled analyses, subcategories of advanced fibrosis (F3-F4) and mild fibrosis (F0-F2) were also created. In all included cases, liver biopsy specimens were adequate for fibrosis staging.

Transient elastography measurement

TE measurements of liver stiffness were acquired using a FibroScan device (Echosens, Paris, France). Patients were positioned in the supine position with their right arm maximally abducted. The probe tip was positioned over an intercostal space in order to target the right lobe of the liver. The medium (M) probe (3.5 MHz) or extra-large (XL) probe (2.5 MHz) was selected per operator discretion. Using A-mode ultrasound image feedback from the FibroScan device, a region of liver parenchyma with at least 6 cm thickness was identified, and ten kPa stiffness measurements were subsequently acquired. Readings were regarded to be reliable if the interquartile range (IQR) to median liver stiffness ratio (IQR/M) was ≤ 30%, consistent with the Society of Radiologists in Ultrasound consensus and the most recent recommendations from the literature [17-20]. Of those with reliable results, the median kPa was used as the liver stiffness measure.

Statistical analysis

Descriptive statistics were calculated for patient characteristics, with medians and interquartile range (IQR) presented for continuous variables. Bar graphs for median TE kPa for each fibrosis category were created, stratified by probe type, in order to visualize the distribution of TE measurements relative to histologic fibrosis. These plots were used to guide creation of five kPa categories for further analysis (≤ 5.2, 5.3-6.1, 6.2-8.4, 8.5-14.6, ≥ 14.7), based on approximate correspondence to fibrosis stage. Score tests for trend were

Table 1. Patient characteristics

Variable	Value (*N* = 53)
Age, median (IQR)	56.3 (44.4, 63.5)
Female sex	44 (83%)
BMI, median (IQR)	27.7 (24.02, 31.65)
Race	
White	36 (68%)
Black	11 (21%)
Other	6 (11%)
Diabetes	7 (13%)
Hypertension	20 (38%)
Hyperlipidemia	15 (28%)
MELD	6.6 (6.4, 8.7)
Sodium (mEq/l), median (IQR)	139 (137, 141)
Creatinine (mg/dl), median (IQR)	0.79 (0.67, 0.94)
Albumin (g/dl), median (IQR)	4.2 (4, 4.4)
Total bilirubin (mg/dl), median (IQR)	0.6 (0.4, 0.82)
Alkaline phosphatase (U/l), median (IQR)	69 (60, 101)
AST (U/l), median (IQR)	26 (20, 36)
ALT (U/l), median (IQR)	21 (15, 32)
INR, median (IQR)	1 (1, 1.13)
Platelets (× 10^9 per l), median (IQR)	207 (158, 261)
On any AIH treatment	44 (83%)
Steroids	17 (32%)
Azathioprine	32 (60%)
Mycophenolate mofetil	10 (19%)
Tacrolimus	4 (8%)

BMI – body mass index, MELD – model for end-stage liver disease, AST – aspartate aminotransferase; ALT – alanine aminotransferase, INR – international normalized ratio, AIH – autoimmune hepatitis

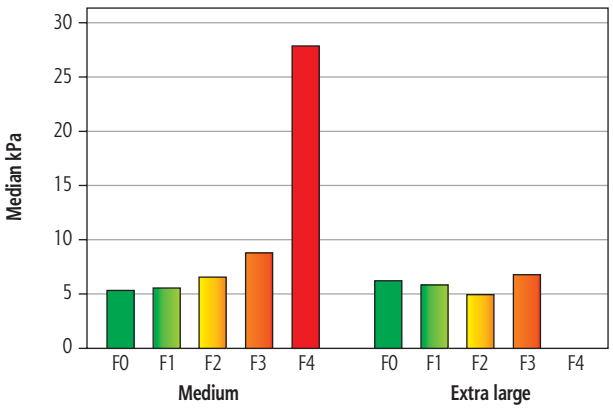

Fig. 1. Median kPa for fibrosis categories by probe size

Figure 1. Based on these data, the association between histologic fibrosis classification and kPa categories are presented in Table 2. When the M probe was used, we found a significant association between increasing kPa

performed to evaluate the association between histologic fibrosis stage and TE kPa categories for each probe type (M or XL) as well as pooled together. To assess for differences in BMI by probe type, we used the Wilcoxon rank-sum test.

Linear regression and correlation analysis were used to estimate projected associations between median kPa (as a continuous variable) and fibrosis categories when using the M probe. Univariate analysis of the variables in Table 1 was first performed to identify covariates potentially associated with median kPa scores for a given patient, using an α threshold = 0.10 for selection for subsequent multivariable analysis. Because there appeared to be an exponential rather than linear relationship between median kPa and fibrosis category, median kPa was log transformed at this stage. We then performed multivariable regression analysis using a clinician-driven modeling approach, using a minimum Bayesian information criterion value to select a final model. This model included the fibrosis score and alkaline phosphatase level at the time of TE measurement as variables significantly associated with log(median kPa). We then used this model to plot predicted median kPa values as a function of fibrosis score, with a 95% confidence band. Unless otherwise stated, an α level of 0.05 was used as the threshold for statistical significance in hypothesis testing. STATA/IC 15.1 (College Station, TX) was used for data management and analysis.

Results

Patient characteristics

A total of 60 patients with AIH were identified who received TE assessment and liver biopsy per the inclusion criteria. Of these, 2 had concomitant liver disease other than AIH, and an additional 5 had unreliable TE measurements, yielding an analytic cohort of 53 patients per the selection criteria. The cohort was predominantly female with a median age of 56.3 years (IQR 44.4-63.5 years) and median MELD of 6.6 (IQR 6.4-8.7; Table 1). Median transaminase values at the time of TE were within the normal range (median AST 26, IQR 20-36; median ALT 21, IQR 15-32), but the majority of patients (83%) were on active AIH treatment, with azathioprine being the most commonly prescribed medication (60%).

Association between transient elastography measurements and liver fibrosis

Median kPa values as a function of histologic fibrosis score, stratified by probe type, are shown in

Table 2. Fibrosis classification by probe type

Probe	Fibrosis	kPa Category					
		⩽ 5.2	5.3-6.1	6.2-8.4	8.5-14.6	⩾ 14.7	*p*-value
Pooled	Advanced	3	2	3	5	5	0.02*
	Mild/mod	10	10	7	5	3	
Medium	Advanced	2	2	1	3	5	0.04*
	Mild/mod	8	5	5	4	2	
Extra large	Advanced	1	0	2	2	0	0.40
	Mild/mod	2	5	2	1	1	

category and advanced fibrosis (p = 0.04). However, with use of the XL probe, there was no association between advanced fibrosis and increasing kPa category (p = 0.40). Median patient BMI, however, was significantly different between the M and XL probe groups (25.8, IQR 22.5-28.5 vs. 33.1, IQR 29.4-37.7; $p < 0.001$). Among patients where the M probe was used, there was a strong correlation between kPa and fibrosis score ($\rho = 0.42$, $p = 0.01$).

Linear regression analysis

In the final linear regression model, fibrosis score ($\beta = 0.27$, $p = 0.003$) and alkaline phosphatase ($\beta = 0.03$ per 10-unit change, p = 0.028) were retained as variables significantly associated with log(median kPa). The predicted association between fibrosis score and median kPa by TE, adjusted for alkaline phosphatase, is shown in Figure 2 and Table 3. Although median kPa increased with worsening fibrosis score, the increase was much more marked when fibrosis stage progressed from F3 to F4 (median kPa 10.1 to 18.8, respectively).

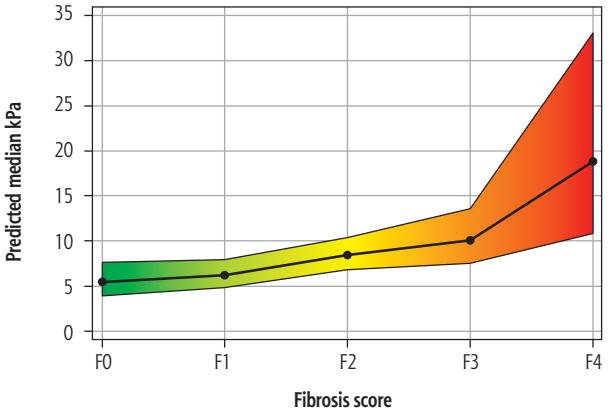

Fig. 2. Association between kPa and fibrosis stage with medium probe (with 95% confidence band)

Table 3. Predicted median kPa values by fibrosis score using the medium probe, with 95% confidence intervals

Fibrosis score	Median kPa	95% Confidence Interval
F0	5.4	3.8-7.7
F1	6.2	4.8-7.9
F2	8.4	6.8-10.4
F3	10.1	7.5-13.6
F4	18.8	10.7-33.1

Discussion

This retrospective study demonstrates a significant association between TE kPa values and histologic fibrosis staging on biopsy in a United States cohort, adding validity to the use of this non-invasive measure for assessing liver fibrosis in patients with AIH. Importantly, this significant association was only present when the M probe was used; there was no significant association with use of the XL probe. Significant differences in BMI between these two groups may explain these findings, which would be consistent with existing literature on TE inaccuracy in patients with increased BMI.

To the best of our knowledge, this study represents the first TE validation study in AIH patients conducted in a United States population. Prior cohort studies corroborate these findings in Chinese and German populations. In a study of 30 patients by Wang *et al.*, a significant positive correlation was found between degree of liver stiffness as assessed by TE and Scheuer fibrosis stage by biopsy. One patient had a failed attempt at TE measurement due to high BMI [10]. In a larger study of 108 patients by Guo *et al.*, TE was found to be superior to other non-invasive markers, including the aspartate transaminase-to-platelet index (APRI) and a fibrosis index based on four factors (FIB-4), in detecting the stages of fibrosis in AIH patients previously determined through liver biopsy [12]. A study of 60 German patients with AIH demonstrated a strong positive correlation between TE measurements and

histologic fibrosis stage ($\rho = 0.78$, $p < 0.001$) and identified an optimal kPa cutoff of 16 to identify patients with F4 fibrosis [13]. Finally, another Chinese study of 100 patients reported an optimal kPa of 12.5 in classifying F4 fibrosis [14]. Our results mirror those of these studies, as we found that a transition from F3 fibrosis to F4 fibrosis would be marked by approximate median kPa values of 10.1 to 18.8, respectively. However, in contrast to the above work, our cohort included a mixed Caucasian and black population, which is more reflective of the United States AIH population [16], and explored the role of probe type and BMI in the analysis (discussed below).

The second major finding in our study was that TE measurements correlated strongly with fibrosis when using the M probe but were unreliable when using the XL probe. Based on existing literature, we believe that BMI differences between groups are likely to explain this observation, where reduced penetration of TE shear waves into the intrahepatic tissue reduces performance of the test [21]. Indeed, a prospective study of more than 10,000 patients indicated that liver stiffness measurements are unreliable in nearly one in five cases, often due to obesity [22]. Unreliable measurements ranged from 12% in patients with BMI < 25 to more than 50% in patients with BMI ≥ 40. This included patients with chronic hepatitis B/C, nonalcoholic fatty liver disease, alcoholic liver disease, and a miscellaneous category where etiology of liver disease was not specified. In our study, we observed poor performance beginning at a lower BMI range of approximately > 30. This suggests that the impact of BMI may vary among different etiologies of liver disease, although this premise would need to be explored in future studies.

There are several limitations that we acknowledge in this study. First, this study includes a relatively small sample size and therefore significant differences may have been missed due to insufficient power. Second, there is a possibility of misclassification of exposure in our study. Although we restricted study inclusion to patients with AIH and no documented concomitant liver disease, it is possible that some patients carry additional undiagnosed chronic liver diseases such as alcoholic liver disease or non-alcoholic fatty liver disease, which could impact the results of this study. To address this, we performed detailed chart reviews and only identified two patients with concomitant liver disease, suggesting that this impact is likely minimal. Third, there is a possible misclassification of fibrosis by biopsy, as errors in staging may result from sampling or pathologist variation [5]. However, this limitation is shared with similar studies, would not be expected to bias the results in a systematic fashion, and the impact is somewhat reduced by pooling fibrosis groups. Fourth, the transaminase levels in our cohort were generally in the normal range (i.e. biochemical stability), indicating that there was minimal active hepatitis in this study. Indeed, the vast majority of patients were on maintenance therapy with azathioprine with long-established diagnoses of AIH. As such, the results may not generalize well to populations with high degrees of active inflammation (as might occur during an AIH flare), and we could not explore the impact of hepatic inflammation on TE measurement, which could theoretically impact the results [23]. However, this question has been addressed in prior literature [12], without a suggestion that hepatic inflammation substantially impacts TE readings in AIH patients. Furthermore, AST and ALT in our study were not retained as covariates significantly associated with median kPa measurements. Finally, as our study population primarily included middle-aged, female, Caucasian and black patients, the results may not be generalizable to all patients with AIH.

In conclusion, our results support the use of TE as a reliable and non-invasive means of assessing liver fibrosis in patients with AIH. However, the reliability of TE may diminish in patients with increased BMI, even with use of the XL probe. Hence, caution should be exercised when assessing obese patients and further evaluation with biochemical and serological testing, imaging, and biopsy should be considered in the appropriate clinical context.

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This study received Institutional Review Board approval from the University of Pennsylvania with a waiver of informed consent.

Disclosure

Authors report no conflict of interest.

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