Levels of Alanine Aminotransferase Confound Use of Transient Elastography to Diagnose Fibrosis in Patients With Chronic Hepatitis C Virus Infection

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BACKGROUND & AIMS: Hepatic elastography (HE) is a noninvasive technique that measures liver stiffness and is used to diagnose hepatic fibrosis. It can help patients who are thought to have early-stage disease avoid a staging liver biopsy, but only when confounding variables that increase liver stiffness are excluded. Chronic inflammation from hepatitis C virus (HCV) infection is not considered to be one of these variables. **METHODS:** We identified 684 patients with HCV and METAVIR fibrosis scores of 0–2 from a prospective, multi-institutional study of liver stiffness in 2880 patients with chronic liver disease. Patients were 49.6 ± 9.0 years old, 64.3% were male, and they had an average body mass index of 26.7 ± 4.1 kg/m^2. **RESULTS:** In a multivariate analysis, inflammation (based on histologic analysis) and level of alanine aminotransferase (ALT) were associated with liver stiffness. The chances of a patient having a level of stiffness that indicates cirrhosis increased with grade of inflammation and level of ALT. By using a conservative 14.5-kPa cutoff for the diagnosis of cirrhosis, grade 3 inflammation had an odds ratio of 9.10 (95% confidence interval, 2.49–33.4). Likewise, levels of ALT greater than 80 and 120 IU/L had odds ratios of 3.84 (95% confidence interval, 2.10–7.00) and 4.10 (95% confidence interval, 2.18–7.69), respectively. The effect of the level of ALT persisted when analysis was restricted to patients with fibrosis scores of F0 to F1. **CONCLUSIONS: In patients with HCV infection and early-stage fibrosis, increased levels of ALT correlate with liver stiffness among patients in the lowest strata of fibrosis (METAVIR scores 0–2). Patients without fibrosis but high levels of ALT could have liver stiffness within the range for cirrhosis. Inflammation should be considered a confounding variable in analysis of liver stiffness.**

Keywords: Fibroscan; Diagnostic Test; Imaging; Chronic HCV.

Hepatic elastography (HE) uses ultrasound and shear waves to measure stiffness of the liver. It is a validated and reproducible, noninvasive method for making the diagnosis of liver cirrhosis. At the opposite end of the fibrosis spectrum, however, the predictive value of HE is a matter of ongoing study. Of chief interest for the practicing hepatologist is how chronic inflammation, as in the case of hepatitis C, affects the predictive value of HE. It is already recognized that certain conditions intrinsic to liver pathophysiology such as acute hepatitis, sinusoidal congestion, and obstructive cholestasis increase liver stiffness independent of fibrosis stage.[1]

Hepatic inflammation, when acute and severe, has an effect on the viscoelasticity of the liver and results in an overestimation of liver fibrosis stage by HE, as is the case with hepatitis B flares and reemergent hepatitis C after liver transplantation.[2,3] In the setting of smoldering inflammation (eg, chronic hepatitis C or steatohepatitis), however, the impact of inflammation on the predictive value of HE is less certain. Castera et al[4] concluded that liver stiffness measurements (LSMs) did not correlate with varying degrees of necroinflammation in chronic hepatitis C; likewise in steatohepatitis, there are reports of no correlation between LSM and necroinflammatory activity or alanine aminotransferase (ALT).[5] However, Oliveri et al[2] concluded that necroinflammation was independently associated with liver stiffness, albeit in hepatitis B–infected individuals.

There is sufficient animal model evidence that viscoelasticity of the hepatic parenchyma is decreased before the deposition of collagen scar.[6] The etiology of this dynamic, physical property is poorly defined, although cellular edema and cytoskeletal changes are possible contributors. Herein we present the results of a large, prospective multi-institutional study of HE in a North American cohort with chronic hepatitis C. We excluded patients with advanced fibrosis (METAVIR F3-4) and focused specifically on the earliest stages of fibrosis (METAVIR F0-2) in an effort to determine whether histologic and biochemical inflammation confounds the predictive value of HE.

Methods

Study Design

A prospective, multi-institutional study of liver stiffness by transient elastography (TE) in patients with chronic liver

Abbreviations used in this paper: ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; HCV, hepatitis C virus; HE, hepatic elastography; HIV, human immunodeficiency virus; kPa, kilopascals; LSM, liver stiffness measurement; NASH, nonalcoholic steatohepatitis; OR, odds ratio; TE, transient elastography; ULN, upper limit of normal.

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Table 1. Basic Characteristics by METAVIR Score

Total, N = 684	F0 fibrosis (n = 96)	F1 fibrosis (n = 323)	F2 fibrosis (n = 265)	*P* value
Age (*y*)	47.3 ± 9.2	49.1 ± .1	51.0 ± 8.5	.001
Sex (M; F)	54; 44	213; 110	174; 91	NS
BMI (kg/m^2)	26.0 ± 4.2	26.6 ± 4.1	27.0 ± 4.1	NS
ALT (*IU/L*)	62.9 ± 44.11	76.3 ± 88.7	82.0 ± 65.2	NS
Steatosis (yes; no)	56; 40	125; 198	119; 146	NS
Grade of inflammation	1.04 ± 0.48	1.31 ± 0.52	1.62 ± 0.54	<.001
LSM (*kPa*)	7.2 ± 7.1	7.42 ± 4.3	9.2 ± 7.4	.001

NOTE. Basic characteristics of study population divided by METAVIR score. Age, grade of histologic Inflammation, and liver stiffness differ significantly across strata of fibrosis.

disease undergoing liver biopsy has been in place since 2004, with correlation of LSM to clinical data and histologic grade and stage of disease by METAVIR score. The results of this study were pooled with those of a parallel study ongoing at the Liver Center of the Beth Israel Deaconess Medical Center. LSM was evaluated by using the FibroScan (Echosens, Paris, France) between December 2004 and September 2009. The total study population included men and women of at least 18 years of age with viral hepatitis. The inclusion criteria were confirmed diagnosis of chronic hepatitis C (detectable serum HCV RNA), successful LSMs (kilopascals [kPa]), and liver biopsy obtained within 3 months of each other. Patients with any of the following were excluded from study participation: active malignancy except for basal or squamous cell skin lesions, uninterpretable biopsy specimen, hepatitis C virus (HCV) therapy within last 6 months, other chronic liver disease (including Wilson's disease, $alpha_1$-antitrypsin deficiency, cholestatic liver disease, or hemochromatosis), clinical ascites, body mass index (BMI) ≥40 kg/m^2, pregnancy, or an implantable cardiac device. Steatosis without a diagnosis of nonalcoholic steatohepatitis (NASH) was included. We further restricted our analysis to those patients with F0–F2 fibrosis. All patients provided written informed consent.

Data Collection: History, Physical and Laboratory Examinations

Data collected were age, sex, race, BMI, alcohol consumption by history, stigmata of liver disease, ALT levels at the time of TE, human immunodeficiency virus (HIV) serology, and imaging results. Prior or current heavy alcohol use was defined as >50 g/d for 5 years or more. Hepatitis B virus coinfection was ruled out by routine serology. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Beth Israel Deaconess Medical Center Institutional Review Board, and written informed consent was obtained from all patients.

Primary Data Interpretation: Transient Elastography and Histology

LSMs (kPa) were determined as previously described. FibroScan was considered accurate if patients had at least 8 successful measurements, a minimum 60% success rate, and an interquartile range/median liver stiffness ratio of <0.3. The reproducibility of HE has been well-established in prior studies, with published intraoperator and interoperator agreement statistics of 0.98.[7] This finding is generalizable across studies that compare patients with the same underlying disease.[8] In this study, intraoperator and interoperator variability was evaluated at one center. Measurements taken by 2 different people on the same day (interoperator) and measurements taken by the same person 1 week apart (intraoperator) had coefficients of variability of 2.97% and 5.37%, respectively. Liver biopsies were obtained within 12 weeks of TE, and the METAVIR stage and grade were determined by one of two blinded, experienced hepatopathologists (T.C., I.N.). The length of each liver biopsy specimen was established in centimeters. Fibrosis was staged according to METAVIR scoring system, a 0–4 scale: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa or bridging fibrosis without cirrhosis; and F4, cirrhosis. Inflammatory activity was graded on a 0–3 score; 0, none; 1, mild; 2, moderate; and 3, severe. Steatosis was categorized as grade 0 (<5%), grade 1 (5%–32%), grade 2 (33%–65%), and grade 3 (>66%) on H&E staining.

General Patient Characteristics

These results are summarized in Table 1. We found 684 patients who met inclusion criteria from a total of 2880 patients with available LSMs. The principal reason for exclusion from our analysis was a stage of fibrosis on biopsy of F3 or F4 (1322 patients) (Supplementary Figure 1). The mean age of our cohort was 49.6 ± 9.0 years, with 440 men (64.3%) and 284 women (35.7%), and most patients had HCV genotype 1 infection (n = 553, 80.8%). The average BMI of our cohort was 26.7 ± 4.1 kg/m^2, which is considered overweight. The mean biopsy size was 1.74 ± 0.6 cm. Ninety-six patients had stage F0 fibrosis (14.0%), 323 had F1 (47.2%), and 265 had F2 (38.7%). Sixteen patients had grade 0 histologic inflammation (2.3%), 393 had grade 1 (57.4%), 265 had grade 2 (38.7%), and 10 had grade 3 (1.5%). One hundred sixty-seven patients (25.4%) had ALT <40 IU/L, 300 (45.6%) had ALT between 40 and 80 IU/L, 95 (14.4%) had ALT between 80 and 120 IU/L, and 96 (14.6%) had ALT >120 IU/L. Two hundred ninety-nine patients had ≥grade 1 steatosis (43.7%). There were 44 patients with diabetes (6.4%) and 28 patients with HIV coinfection (4.1%). There were 6 centers involved in this study (see Supplementary Table 1 for basic demographic data by institution). The age, BMI, proportion of men, length of biopsy, successful measurement rate, and interquartile range (kPa) were not significantly different between the centers.

Statistical Analysis and Database Management

Statistical analysis was tailored to the nature of the variable. Patient demographics are given as mean ±1 standard deviation as appropriate. For Table 2, for ease of interpretation,