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# Etiology-related determinants of liver stiffness values in chronic viral hepatitis B or C

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**Background & Aims**: Transient elastography (TE) has gained popularity for the non-invasive assessment of severity of chronic viral hepatitis, but a comprehensive evaluation of the factors that might account for discrepancy in diagnostic accuracy between TE and the standard of care liver biopsy (LB) is still needed.

**Methods**: Patients with chronic hepatitis-B (HBV, $n = 104$) or -C (HCV, $n = 453$) underwent percutaneous LB concomitantly with TE (FibroScan®; Echosens, Paris, France). Liver cell necroinflammatory activity (A) and fibrosis (F) were assessed by METAVIR. Perisinusoidal fibrosis was rated with a 0–3 score. Determinants of TE results were investigated by a linear regression model whereas discordance between TE and LB results was assessed by logistic regression.

**Results**: Fibrosis ($p < 0.0001$) and liver cell necroinflammatory activity ($p < 0.0001$) were independently associated with TE results in both HBV and HCV patients, whereas steatosis ($p < 0.0001$) was independently associated with TE in HCV only. Fibrosis overestimation was predicted by severe/moderate necroinflammatory activity in HBV and by older age (41–60 or >60 years vs. <40), >2 UNL AST and >2 UNL GGT, as well as severe/moderate necroinflammatory activity and severe/moderate steatosis in HCV. In the latter patients, however, moderate/severe necroinflammatory activity and steatosis were the only independent predictors of fibrosis overestimation.

**Conclusions**: Fibrosis and necroinflammatory activity are the main determinants of TE in chronic viral hepatitis. Since TE staging of fibrosis is influenced by necroinflammatory activity and steatosis, a diagnostic LB is deemed necessary for a reliable intra-patient TE monitoring of the course of viral hepatitis.



Keywords: Transient elastography; Liver biopsy; Liver fibrosis; Hepatitis B; Hepatitis C.

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*Abbreviations:* TE, transient elastography; LB, liver biopsy; CLD, chronic liver disease; A, necroinflammatory activity; F, fibrosis stage; BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminostransferase; GGT, gamma-glutamyltranspeptidase; HBsAg, hepatitis B surface antigen; US, ultrasound; kPa, kilopascal; IQR, interquartile range; UNL, upper normal limit; OR, odds ratio; 95% CI, 95% confidence interval.

## Introduction

Transient elastography (TE) is a simple, non-invasive approach for predicting liver disease severity, based upon a mechanical wave generated by vibration that provides an estimate of the liver stiffness, which in turn has been demonstrated to correlate with hepatic fibrosis [1]. Within a defined clinical context, non-invasive assessment of liver disease severity with TE is an attractive alternative for many patients with chronic liver disease (CLD), to avoid the risks inherent with invasive procedures like liver biopsy (LB). However, the relative advantages and disadvantages of TE need to be better elucidated, since increased liver stiffness values by TE do not reflect liver fibrosis only, but also the grade of necroinflammatory activity of the liver [2–8]. Indeed, inflammatory cell infiltrates, interstitial oedema, liver cell swelling and necrosis, cholangiocellular damage, pericellular fibrosis, and steatosis differently contribute to the liver damage caused by various etiologic agents. More recently, extrahepatic cholestasis and an increased venous pressure due to cardiac failure have added to the list of potential TE confounders [9,10].

Under defined circumstances, TE compared well with LB as the reference standard to accurately identify patients with CLD with significant liver fibrosis or cirrhosis [1–8], with a satisfactory inter- and intra-observer reproducibility [5]. However, despite an apparently high diagnostic accuracy, several studies reported incomplete concordance between TE and LB in patients with CLD, owing to a substantial overlap between diagnostic categories, especially in patients with pre-cirrhotic changes of the liver [1–8,11–14]. Due to the increasing role of TE in the management of patients with CLD, the variables related to the patient, the reference standard or TE technique that could favor discordant results between TE and LB, need to be comprehensively evaluated. This is particularly true in patients with chronic viral hepatitis, in whom TE is progressively replacing LB not only in the clinical surveillance programs, but also in the treatment decision making process [15]. In this study, 608 patients with CLD due to either hepatitis B (HBV) or hepatitis C (HCV) virus

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infection were concurrently investigated with TE and LB, with a particular focus on patients showing discordant results with these diagnostic techniques.

## Patients and methods

*Patients*

From April 2006 to February 2008 all patients with chronic hepatitis B or C who consecutively underwent LB for diagnostic or therapeutic purposes at the A.M. & A. Migliavacca Center for Liver Disease, were concurrently examined by TE (FibroScan®; Echosens, Paris, France). Patients with clinically overt cirrhosis and/or ascites were excluded. Patients were enrolled after obtaining their written informed consent to the study protocol that was approved by the Ethics Committee of our hospital.

*Serum measurements*

Serum alanine aminotransferase (ALT), aspartate aminostransferase (AST) and gamma-glutamyltranspeptidase (GGT) activities were measured using an automatic method at 37 °C (normal value ALT, AST ⩽40 IU/L; GGT ⩽50 IU/L). Commercially available enzyme immunoassays were used to determine serum hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core antigen and anti-HCV. Serum HBV-DNA was detected by the Amplicor HBV Monitor® (Roche Diagnostics, Branchburg, NJ). Serum antibody to HCV (anti-HCV) was detected in-house by nested reverse transcription (RT)-PCR using primers of the 5′ non-coding region (minimum detectable level of 20 international units). The diagnosis of chronic viral hepatitis B was the co-presence markers of chronic virus infection (HBV-DNA and HCV-RNA) in seropositive patients for HbsAg and anti-HCV, selection only, for more than 12 months, in the absence of histological evidence of other etiologies.

Excessive drinking was defined as ethanol consumption in the five previous years of more than 20 g per day in women and more than 40 g per day in men. Patients with a BMI >25 kg/m$^2$ and >30 kg/m$^2$ were considered overweight and obese, respectively. Co-morbidities and drug exposure were retrieved from the patient's history.

*Transient elastography*

The procedures were performed by two independent investigators (M.F. and C.R.) who were blind to clinical, serologic, and histological data. TE was performed on the same day of LB, prior to the invasive procedure. The right lobe of the liver was targeted through an intercostal space access while the patient was lying in the dorsal decubitus position with the right arm in maximal abduction. By the ultrasound (US) guide of FibroScan®, a liver portion of at least 6 cm thickness, free of large vessels, was identified to carry out the examination. The rate of successful measurements was calculated as the ratio between the numbers of those validated on total measurements. The results were expressed as a median value of the total measurements in kiloPascal (kPa). Only the examinations with at least 10 validated measurements and a success rate of at least 60% were considered reliable. In addition, the median value of successful measurements was considered as a representative of the liver stiffness in a given patient only if the interquartile range (IQR) of all validated measurements was less than 30% of median values [1].

*Liver biopsy*

All candidates for the LB procedure underwent a standard US scan of the abdomen using standard equipment (iU22, Philips, Bothell, USA) with a detailed study of the liver, spleen, and main vessels. LB was performed by experienced hepatologists with a ***16 G Menghini needle (Biomol, Hospital Service, HS, Rome, Italy) under US guidance in the intercostal space on the right hepatic lobe. The liver tissue was fixed in formalin and paraffin embedded. Five-micron-thick sections of liver tissue were stained with hematoxylin–eosin and Masson trichrome and read by one expert liver pathologist (G.R.) blinded to TE results and clinical data. Only

**Table 1. Main clinical and demographic characteristics of 453 consecutive patients with chronic hepatitis C and 104 with chronic hepatitis B, who concurrently underwent TE and liver biopsy according to the etiology of viral hepatitis.**

| Patients characteristics | HBV (n = 104) | HCV (n = 453) | *p* value |
|---|---|---|---|
| Males, Number | 76 (74%) | 252 (55%) | 0.0005° |
| Age, years * | 45 ± 11 (17-73) | 51 ± 12 (19-70) | <0.0001^ |
| BMI, kg/m$^2$ * | 24.3 ± 3.5 (17- 35) | 24.3 ± 3.3 (16-36) | 0.962^ |
| ALT (IU/L; n.v. <38 ) * | 106 ± 91.8 (22-711) | 97 + 91.9 (14-745) | 0.113§ |
| AST (IU/L; n.v. <38) * | 79 ± 10.7 (17-108) | 69 ± 64.4 (10-737) | 0.781§ |
| GGT IU/L; n.v. <50) * | 60 ± 56.4 (8-409) | 68 ± 84.6 (7-965) | 0.574§ |
| Bilirubin (mg/dl; n.v. <0.12-1.10 ) * | 1.25 ± 0.9 (0.1- 18.1) | 1.12 ± 1.0 (0.2-19.2) | 0.107 |
| Alkaline phosphatase (IU/L; n.v. <40-129 ) * | 212 ± 196 (76-675) | 202 ± 156 (83-775) | 0.007 |
| INR* | 1.10 ± 0.11 (0.84-1.54) | 1.05 ± 0.06 (1-1.19) | <0.0001§ |
| Platelet count, 10$^9$/L * | 193.368 ± 56.609 (50.000-378.000) | 210.481 ± 72.144 (51.000-628.000) | 0.040§ |
| Liver stiffness (kPa) * | 11.8 ± 10.4 (2.6-70) | 9.8 ± 8.6 (2.3-75) | 0.014§ |

*Mean + SD (range); n.v. normal values; °chi-square test; ^*t*-test; §Mann–Whitney test. *Abbreviations:* HBsAg, hepatitis B surface antigen; HCV-RNA, hepatitis C virus RNA; BMI, body mass index; UNL, upper normal limit; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltranspeptidase.

samples with at least 12 complete portal tracts were considered adequate. Liver necroinflammatory activity (A) and fibrosis (F) were semi-quantitatively evaluated by METAVIR [16–17]. Fibrosis was staged on a 0–4 scale according to METAVIR as follows: F0 – no fibrosis; F1 – portal fibrosis without septa; F2 – portal fibrosis and few septa; F3 – numerous septa without cirrhosis; F4 – cirrhosis. Activity was graded as follows: A0 – none; A1 – mild; A2 – moderate; A3 – severe. The length of each liver specimen (in millimeters) and the number of fragments were recorded. Steatosis in liver specimen was arbitrarily graded from 0 to 3 (0 ⩽5%, 1 = 6–24%; 2 = 25–49%; 3 ⩾ 50% of fatty hepatocytes).

All liver specimens were stained with Sirius red to assess perisinusoidal fibrosis [18], which was scored according to the modified system described by Mendler et al. [19]. This system evaluates perisinusoidal fibrosis with a score ranging from 0 to 3. 0: absent; 1: perivenular and/or periportal involvement of some lobules; 2: perivenular and/or periportal involvement of most lobules, without diffuse interstitial sinusoidal collagen deposition; 3: perivenular and/or periportal involvement of most or all lobules, with diffuse interstitial fibrosis involving some or most of the lobules).

*Statistical analysis*

The study was prospectively planned to evaluate patients not included in previous studies. All analyses were conducted separately by HCV and HBV etiology. Descriptive statistics (means and standard deviations, medians and interquartile ranges, proportions) were calculated, Chi-square or Fisher exact test was used to compare proportions. Mann–Whitney test or two samples *t*-test was used to compare continuous variables, as appropriate. Multiple linear regression [20] was used to assess the effect of histologic variables on log transformed TE stiffness values. Independent variables considered in the regression model were: (a) necroinflammatory activity (0–1 vs. 2–3); (b) steatosis (0–1 vs. 2–3) (c) fibrosis stage (0–1 vs. 2–3) and (d) perisinusoidal fibrosis (0–1 vs. 2–3).

Univariate and multiple logistic regression models were fitted in order to analyze the association between patients' characteristics and the probability of discordance between TE and LB [20]. Discordance was defined as the lack of correspondence between the METAVIR fibrosis score and TE diagnostic cut-off (7.9 and 12 kPa for F ⩾2 and F = 4, respectively), identified in a previously published cohort [5]. In particular, discordance was analyzed in terms of both overestimation (TE >7.9 and F <2; TE >12 and F <4) and underestimation (TE ⩽7.9 and F ⩾2; TE ⩽12 and F = 4) of the fibrosis stage. The individual variables included in this model were gender (male vs. female); age (⩽40 vs. 41–60 vs. >60 years); BMI (<25 vs. ⩾25 kg/m$^2$); AST, ALT, and GGT levels (⩽2 vs. >2 ULN); IQR% (⩽20 vs. 20–30%); necroinflammatory activity (0–1 vs. 2–3); steatosis (0–1 vs. 2–3); fibrosis stage (0–1 vs. 2–3), length of liver biopsy (>20 vs. ⩽20 mm) and perisinusoidal fibrosis (0–1 vs. 2–3). Only variables statistically significant ($p$ <0.05) at univariate analysis were included in multiple logistic regression models. Odds ratios (OR) of discordance between TE and LB, with their 95% confidence intervals (95% CI), were calculated for each variable included in the models. SAS software (Release 9.1, SAS Institute, Inc., Cary, NC) was used for all statistical analyses.

## Results

*Patients*

Six hundred and eight patients consecutively underwent both TE scan and ultrasound guided LB during the same session. Overall, TE examination failed in 47 patients (8%) in terms of stiffness measurement failure ($n$ = 6) and unreliable examinations (i.e. the success rate was <60% and/or an IQR >30% of all validated measurements, $n$ = 41). Unreliable results were mainly related to obesity (i.e. BMI >30 kg/m$^2$, $n$ = 38) and narrow intercostal space ($n$ = 4), whereas in five other patients the reason of unsuccessful examination with TE was unknown. In four patients (0.6%) LB was inadequate in terms of number of portal spaces (<11). Overall, 557 patients fitted the criteria for comparison between LB and TE. The etiology was chronic HCV infection in 453 (81%) and chronic HBV infection in 104 (19%) patients, respectively. There were 327 males (250 in the HCV group and 77 in the HBV group) and 230 females (203 in HCV and 27 in HBV group) with a median age of 50 years (51 years in HCV and 45 years in HBV patients) (range 19–70) and a median BMI of 24.2 kg/m$^2$ (range 16–36). Patients' characteristics according to virus etiology are summarized in Table 1.

Excessive ethanol intake was reported in three patients only (all HCV). 139 (30%) HCV patients were overweight and 30 (6%) were obese. The corresponding figures for HBV patients were 32 (31%) and 8 (8%), respectively.

**Table 2. Results of liver histology.** Fibrosis stage and necroinflammatory activity by METAVIR, steatosis grade and perisinusoidal fibrosis in patients with chronic hepatitis B and chronic hepatitis C who concurrently underwent TE and a liver biopsy.

| Liver biopsy | Score | HBV (n = 104) | | HCV (n = 453) | | $p$ value |
|---|---|---|---|---|---|---|
| | | n. | % | n. | % | |
| Fibrosis stage (F) | 0-1 | 49 | (47) | 256 | (56) | 0.0759 |
| | 2 | 21 | (20) | 109 | (24) | |
| | 3 | 17 | (16) | 44 | (10) | |
| | 4 | 16 | (16) | 44 | (10) | |
| Necroinflammatory activity (A) | 0 | 14 | (13) | 44 | (10) | 0.0088 |
| | 1 | 32 | (31) | 212 | (47) | |
| | 2 | 32 | (31) | 129 | (28) | |
| | 3 | 26 | (25) | 68 | (15) | |
| Steatosis | 0 | 41 | (39) | 155 | (34) | 0.2274 |
| | 1 | 34 | (33) | 142 | (31) | |
| | 2 | 18 | (17) | 72 | (16) | |
| | 3 | 11 | (11) | 84 | (19) | |
| Perisinusoidal fibrosis | 0-1 | 48 | (46) | 182 | (41) | 0.314 |
| | 2-3 | 56 | (54) | 271 | (59) | |

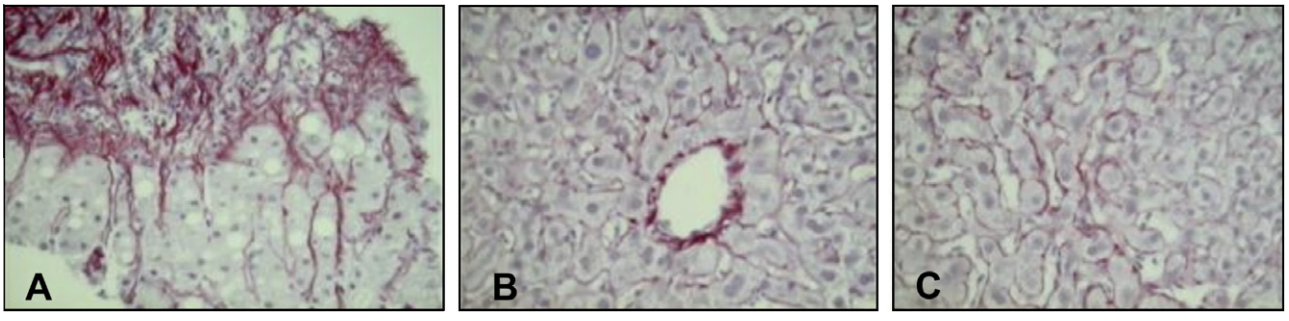


**Fig. 1. Picrosirius red staining demonstrating fibrous enlargement of portal tracts with periportal perisinusoidal fibrosis (A), perivenular fibrosis in zone 3 (B) and interstitial fibrosis in the lobule (C).**

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The median length of LB cores was 30 mm (15–55) with 416 (74%) specimens being longer than 20 mm. Histological features according to the two etiologies are detailed in Table 2: 49 HBV patients (47%) and 256 HCV patients (56%) had no or mild fibrosis (F0–F1), 21 (21%) and 109 (24%) had moderate fibrosis (F2), 17 (16%) and 44 (10%) had severe fibrosis (F3), and 17 (16%) and 44 (10%) had cirrhosis (F4), respectively. Forty-six HBV (44%) and 256 HCV (57%) patients showed no or mild inflammation (A <2) whereas 58 (56%) and 197 (43%) had moderate to severe necroinflammation (A ⩾2), respectively ($p = 0.008$). Seventy-five (72%) HBV and 297 HCV patients (65%) had absent or mild steatosis (i.e. <25% fatty liver cells); the remaining 29 HBV (27%) and 156 (35%) HCV patients had moderate to severe steatosis (i.e. >25% of fatty liver cells). Forty-eight (46%) patients in the HBV group and 182 (41%) patients in the HCV group had no or mild perisinusoidal fibrosis whereas the remaining 56 (54%) in the HBV group and 271 (59%) in the HCV one had perivenular and/or periportal involvement of most hepatic lobules (Fig. 1).

*Transient elastography (TE)*

The mean TE value was 11.8 ± SD 10.4 kPa (median 8.1 kPa, range 2.6–70) in HBV and 9.8 ± SD 8.6 kPa (median 7.4 kPa, range 2.3–75) in HCV ($p = 0.014$). At multivariate analysis, F ($p$ <0.0001) and A ($p$ <0.001) were independently associated with TE values in both HCV and HBV patients, whereas steatosis ($p$ <0.001) was independently associated with TE in HCV patients only.

*Over and underestimation of liver fibrosis by TE*

119 patients showed discrepant results for the diagnosis of F ⩾2 with TE and LB (98 HCV and 23 HBV) and 53 (49 HCV and 14 HBV) had discrepant results for the diagnosis of F4. F ⩾2 discordances were attributable to underestimation of actual F by TE in 12 HBV patients (11.5%) and 49 HCV patients (10.8%), and to overestimation in 11 (10.5%) HBV patients and 47 (10.3%) HCV patients respectively. The results of univariate analysis and multivariate model according to clinical, biological, and histological characteristics of patients without and with discordant results are shown in Tables 3–5. Results are expressed both in terms of overestimation and underestimation of F by TE as compared to LB results. In HBV, no variables were identified to predict discordance between TE and LB for the diagnosis of significant fibrosis (F ⩾2) (Table 3). The only variable that at univariate analysis predicted an overestimation of F4 by TE was the presence of a moderate to severe histologic necroinflammation, with an OR of 11.7 (Table 4). At univariate analysis, the diagnosis of significant fibrosis (F ⩾2) in HCV (Table 5) was significantly affected by perive-

**Table 3. Univariate analysis in 104 HBV patients.** Patients' characteristics in discordant (overestimation) vs. concordant results for the diagnosis of significant fibrosis (F ⩾2).

| Patients characteristics | | Overestimation vs concordance Univariate | | Underestimation vs concordance Univariate | |
|---|---|---|---|---|---|
| | | OR (CI) | *p* value | OR (CI) | *p* value |
| Males | | 1 | 0.567 | 1 | 0.608 |
| Female | | 0.62 (0.12-3.13) | | 1.4 (0.38-5.15) | |
| Age | <40 years | 1 | | 1 | |
| | 41-60 | 0.30 (0.07-1.31) | 0.272 | 1.41 (0.34-5.94) | 0.866 |
| | >60 | 0.79 (0.14-4.53) | | 1.58 (0.23-10.78) | |
| BMI | ≤25 kg/m$^2$ | 1 | 0.282 | 1 | 0.462 |
| | >25 kg/m$^2$ | 0.46 (0.11-1.88) | | 0.62 (0.17-2.23) | |
| AST | ≤2 ULN | 1 | 0.515 | 1 | 0.856 |
| | >2 ULN | 0.59 (0.12-2.93) | | 0.88 (0.22-3.55) | |
| AST | ≤2 ULN | 1 | 0.322 | 1 | 0.486 |
| | >2 ULN | 0.52 (0.14-1.91) | | 0.65 (0.19-2.21) | |
| GGT | ≤2 ULN | - | | 1 | 0.943 |
| | >2 ULN | - | | 0.94 (0.19-4.78) | |
| Necroinflammation | 0-1 | 1 | 0.072 | 1 | 0.549 |
| | 2-3 | 0.28 (0.07-1.12) | | 1.48 (0.41-5.31) | |
| Steatosis | 0-1 | 1 | 0.987 | 1 | 0.676 |
| | 2-3 | 0.99 (0.24-4.07) | | 1.32 (0.36-4.82) | |
| IQR | <20% | 1 | 0.942 | 1 | 0.174 |
| | >20% | 0.95 (0.26-3.53) | | 0.33 (0.07-1.63) | |
| Liver biopsy length | ≤20 mm | 1 | 0.242 | 1 | 0.642 |
| | >20 mm | 0.44 (0.11-1.74) | | 1.46 (0.29-7.34) | |
| Perisinusoidal fibrosis | 0-1 | 1 | 0.221 | 1 | 0.532 |
| | 2-3 | 2.8 (0.54-14.55) | | 1.6 (0.37-6.97) | |

Multivariate analysis was not performed in this case as no significant variables were identified by the univariate model.

**Table 4. Univariate analysis in 104 HBV patients.** Patients' characteristics in discordant (overestimation) vs. concordant results for the diagnosis of liver cirrhosis (F = 4).

| Patients characteristics | | Overestimation vs concordance | |
|---|---|---|---|
| | | Univariate analysis | |
| | | OR (CI) | *p* value |
| Males | | 1 | 0.831 |
| Female | | 0.86 (0.22-3.4) | |
| Age | <40 years | 1 | |
| | 41-60 | 2.57 (0.5-13.15) | 0.154 |
| | >60 | 6.00 (0.96-37.37) | |
| BMI | ≤25 kg/m$^2$ | 1 | 0.131 |
| | >25 kg/m$^2$ | 2.52 (0.76-8.37) | |
| AST | ≤2 ULN | 1 | 0.300 |
| | >2 ULN | 1.90 (0.56-6.43) | |
| AST | ≤2 ULN | 1 | 0.767 |
| | >2 ULN | 1.19 (0.37-3.83) | |
| GGT | ≤2 ULN | 1 | 0.121 |
| | >2 ULN | 2.85 (0.76-10.74) | |
| Necroinflammation | 0-1 | 1 | 0.020 |
| | 2-3 | 11.73 (1.46-94.1) | |
| Steatosis | 0-1 | 1 | 0.138 |
| | 2-3 | 2.46 (0.75-8.08) | |
| IQR | <20% | 1 | 0.340 |
| | >20% | 1.77 (0.55-5.75) | |
| Liver biopsy length | ≤20 mm | 1 | 0.936 |
| | >20 mm | 1.06 (0.26-4.23) | |
| Perisinusoidal fibrosis | 0-1 | 1 | 0.666 |
| | 2-3 | 1.38 (0.32-5.97) | |

nular and/or periportal fibrosis (F2 or 3) with a 2-fold increased risk of overestimating the fibrosis stage by TE (OR 2.25). The risk of discordance was attenuated in females (OR 0.50).

Increased AST (OR 3.14) or GGT levels (OR 5.08), a necroinflammatory activity ⩾2 (OR 6.26) and a >2 grade of steatosis (OR 3.52) were significantly associated with an increased likelihood of overestimating F4 by TE (Table 6) in patients aged 41–60 years (OR 1.56) or >60 years (OR 3.38). Increased GGT levels (OR 2.74), necroinflammatory activity ⩾2 (OR 3.84) and >2 grade steatosis (OR 2.13) were the independent predictors.

*Correlation with published TE cut-offs*

We analyzed our series applying TE cut-offs previously reported by others to predict significant fibrosis (F ⩾2) and cirrhosis (F = 4), i.e. 7.2 kPa for HBV [21] and 8.8 kPa for HCV [2] for the diagnosis of F >2 and 13.4 kPa [22] for HBV patients and 14.6 kPa for HCV [2] for F = 4. None of these variables appeared to be significantly associated with an underestimation of fibrosis stage by TE, considering both the cut off of 7.2 kPa for HBV and that of 8.8 kPa for HCV. In HBV patients, the only variable predicting the overestimation of fibrosis stage was a moderate to severe necroinflammatory activity both using the Marcellin cut off value (OR = 2.3) [21] and the Chang cut off for cirrhosis (OR = 3.1) [22]. In HCV patients examined using the Ziol's cut off [2], perivenular and/or periportal F2 and F3 were also significantly related to an overestimation of hepatic fibrosis by TE (OR = 2.31, for the diagnosis of F >2 at univariate analysis). Considering the cut off of 14.6 kPa [2] to diagnose cirrhosis (F = 4), predictors of overestimation of fibrosis by TE were increased AST (OR = 2.31) and GGT (OR 4.5) levels, a moderate/severe necroinflammation (OR 6.9) and a moderate/severe steatosis at histology (OR 3.1). The latter two variables were also independent predictors of discordance at multivariate analysis (OR 4.2 and 1.9, respectively).

## Discussion

This study in patients with chronic viral hepatitis confirms that while hepatic fibrosis is the relevant determinant of liver stiffness assessed by TE, other histologic features, like liver necroinflammation and, in HCV patients, hepatic steatosis, are important independent confounders, too. Indeed, TE correctly predicted liver fibrosis stage in two-thirds of all patients with viral CLD, yet was more accurate in predicting advanced fibrosis rather than mild/moderate fibrosis, where it showed the greater rates of overlap between F1 and F2 stages. These observations, therefore, confirm previous reports in HCV patients investigated by TE [2,4–8,11–12], whereas they significantly add to the scant data on TE accuracy generated in patients with chronic hepatitis B [3,12,21–23].

It remains difficult to explain the reason why TE results were significantly and independently influenced by liver cell necroinflammatory activity in both HBV and HCV, whereas, in patients with chronic hepatitis C, TE results were influenced by moderate to severe steatosis only. Necroinflammatory activity might influence TE assessment through increased portal pressure and/or tissue oedema accompanying liver cell necrosis and swelling [3,5,7]. Indeed, a relationship between TE and hepatic necroinflammatory activity was shown in patients with acute viral hepatitis [4] as well as in patients with chronic hepatitis B experiencing an ALT flare [3], which is synonymous for the necroinflammatory changes of the hepatic parenchyma.

In a previous study, the confounding effect of liver cell necroinflammation on TE assessment of F in HCV patients [6] resulted in an increase of TE results by 1.7 to 2.4 kPa, moving from A1 to A2/A3, and from A0 to A2/A3, respectively, within each F stage. This could partially explain why a diagnostic strategy based on the application of serum tests as Fibrotest or Actitest and TE could successfully predict disease severity in viral hepatitis patients since a test like Actitest includes parameters that sense virus-related necroinflammatory activity of the liver [25].

Steatosis might cause TE to overestimate liver fibrosis in HCV patients due to its ability to affect the evolutionary course of hepatitis C [26] by increasing both hepatic fibrogenesis and liver cell inflammation. This was not clearly highlighted by previous studies [6–8] that enrolled too a few patients with moderate to severe steatosis, compared to our study, which enrolled 27–43% of patients with significant steatosis in each etiologic stratum, thus being powered to assess the influence of steatosis on TE.

We previously reported that TE reproducibility was significantly attenuated in patients with moderate to severe steatosis, supporting the view that an interaction of fat in liver cells with low-frequency vibrations of TE could virtually affect the signal

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**Table 5. Univariate analysis in 453 HCV patients.** Patients' characteristics in discordant (overestimation) vs. concordant results for the diagnosis of significant fibrosis (F ⩾2).

| Patients characteristics | | Overestimation vs concordance Univariate | | Underestimation vs concordance Univariate | |
|---|---|---|---|---|---|
| | | OR (CI) | *p* value | OR (CI) | *p* value |
| Males | | 1 | 0.041 | 1 | 0.334 |
| Female | | 0.50 (0.26-0.97) | | 1.34 (0.74-2.44) | |
| Age | <40 years | 1 | | 1 | |
| | 41-60 | 1.31 (0.54-3.16) | 0.834 | 3.18 (0.93-10.82) | 0.107 |
| | >60 | 1.22 (0.47-3.18) | | 3.87 (1.1-13.58) | |
| BMI | ≤25 kg/m$^2$ | 1 | 0.077 | 1 | 0.108 |
| | >25 kg/m$^2$ | 1.75 (0.94-3.24) | | 0.58 (0.3-1.13) | |
| AST | ≤2 ULN | 1 | 0.727 | 1 | 0.085 |
| | >2 ULN | 0.88 (0.43-1.8) | | 0.48 (0.21-1.11) | |
| AST | ≤2 ULN | 1 | 0.123 | 1 | 0.156 |
| | >2 ULN | 1.62 (0.88-2.98) | | 0.63 (0.33-1.19) | |
| GGT | ≤2 ULN | 1 | 0.068 | 1 | 0.203 |
| | >2 ULN | 0.37 (0.13-1.07) | | 0.56 (0.23-1.37) | |
| Necroinflammation | 0-1 | 1 | 0.569 | 1 | 0.171 |
| | 2-3 | 0.83 (0.45-1.56) | | 1.52 (0.84-2.76) | |
| Steatosis | 0-1 | 1 | 0.849 | 1 | 0.700 |
| | 2-3 | 0.94 (0.5-1.78) | | 0.88 (0.47-1.67) | |
| IQR | <20% | 1 | 0.171 | 1 | 0.558 |
| | >20% | 1.53 (0.83-2.82) | | 1.20 (0.65-2.19) | |
| Liver biopsy length | ≤20 mm | 1 | 0.671 | 1 | 0.446 |
| | >20 mm | 0.86 (0.42-1.74) | | 1.35 (0.63-2.89) | |
| Perisinusoidal fibrosis | 0-1 | 1 | 0.047 | 1 | 0.351 |
| | 2-3 | 2.25 (1.01-4.99) | | 1.43 (0.68-3) | |

to noise ratio [5]. This interpretation, however, is largely debated. In a study of 324 HCV patients, hepatic steatosis was found to influence liver stiffness together with hepatic fibrosis and necroinflammation [8], a finding, however, that was not replicated by others [1,6].

In other etiologies, like nonalcoholic fatty liver disease, liver stiffness was found to correlate with hepatic fibrosis but not with steatosis [27], thus further strengthening the importance of viral etiology in the discrepancies we observed between TE and LB in patients with chronic hepatitis. The fact that in healthy subjects, the presence of metabolic syndrome was associated with higher values of liver stiffness [28], and that in non-diabetic patients with genotype 1 chronic hepatitis C insulin resistance was associated with increased liver stiffness independently of hepatic fibrosis [29], further indicates that the role of steatosis on liver stiffness is unclear and could be multifactorial. From a physical point of view, increased liver stiffness leading to TE values may result from the intracellular accumulation of substances other than fat, including amyloid [30].

In our study, the viral etiology of hepatitis did somehow influence some discordance between TE and LB assessments of fibrosis; necroinflammatory activity of the liver was the only confounder in HBV whereas in HCV TE assessment of fibrosis was independently overestimated in patients with moderate to severe necroinflammatory activity and steatosis. The fact that this finding of TE overestimation by steatosis in HCV patients was not reported by others, once again points to differences in patient selection among studies, particularly as far as the prevalence of patients with moderate/severe steatosis is concerned. This could also partially account for our inability to identify steatosis as a TE confounder in HBV patients, who compared to HCV patients, were less frequently found to have moderate to severe liver steatosis (27% vs. 43%).

We were puzzled by the identification of perisinusoidal fibrosis as a factor causing TE overestimation of F ⩾2, even though this histological predictor was not confirmed in the multivariate model. It is intriguing that co-morbidities like alcohol intake or metabolic syndrome may occur in up to 20% of patients with chronic viral hepatitis in association with perisinusoidal fibrosis [31]. Perisinusoidal fibrosis escapes assessment by Metavir (and Ishak), thus making the interpretation of this histological confounder even more difficult. Though one third of our patients were overweight, neither BMI nor histologic steatosis significantly correlated with perisinusoidal fibrosis. We previously reported an association between perisinusoidal fibrosis in patients with recurrent hepatitis C after liver transplantation [7] and liver stiffness in the univariate analysis, which again was not confirmed in the multivariate model as an independent predictor of TE. Based on these findings, we speculate that in some patients, the assessment of perisinusoidal fibrosis could

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**Table 6. Univariate and multivariate analysis in 453 HCV patients.** Patients' characteristics in discordant (overestimation) vs. concordant results for the diagnosis of cirrhosis (F = 4).

| Patients characteristics | | Overestimation vs concordance Statistical analysis | | | |
|---|---|---|---|---|---|
| | | Univariate | | Multivariate # | |
| | | OR (CI) | *p* value | OR (CI) | *p* value |
| Males | | 1 | 0.058 | - | - |
| Female | | 0.53 (0.27-1.02) | | - | |
| Age | <40 years | 1 | | 1 | |
| | 41-60 | 1.56 (0.51-4.74) | 0.020 | 1.41 (0.44-4.53) | 0.138 |
| | >60 | 3.38 (1.12-10.2) | | 2.54 (0.79-8.13) | |
| BMI | ≤25 kg/m$^2$ | 1 | 0.124 | - | - |
| | >25 kg/m$^2$ | 1.65 (0.87-3.12) | | - | |
| AST | ≤2 ULN | 1 | <0.001 | 1 | 0.922 |
| | >2 ULN | 3.14 (1.67-5.9) | | 1.04 (0.48-2.23) | |
| AST | ≤2 ULN | 1 | 0.092 | - | - |
| | >2 ULN | 1.70 (0.92-3.16) | | - | |
| GGT | ≤2 ULN | 1 | <0.001 | | 0.009 |
| | >2 ULN | 5.08 (2.66-9.69) | | 2.74 (1.29-5.81) | |
| Necroinflammation | 0-1 | 1 | <0.001 | 1 | 0.001 |
| | 2-3 | 6.26 (2.94-13.35) | | 3.84 (1.69-8.75) | |
| Steatosis | 0-1 | 1 | <0.001 | 1 | 0.032 |
| | 2-3 | 3.52 (1.86-6.66) | | 2.13 (1.07-4.24) | |
| IQR | <20% | 1 | 0.719 | - | - |
| | >20% | 1.12 (0.6-2.09) | | - | |
| Liver biopsy length | ≤20 mm | 1 | 0.723 | - | - |
| | >20 mm | 0.87 (0.41-1.85) | | - | |
| Perisinusoidal fibrosis | 0-1 | 1 | 0.131 | - | - |
| | 2-3 | 1.86 (0.83-4.16) | | - | |

#Multiple logistic regression model: only variables statistically significant in univariate analysis.

help interpreting discordant results between TE and LB, including HCV patients with known co-morbidities like alcohol abuse or metabolic syndrome. However, the predictive value of perisinusoidal fibrosis in HCV patients needs to be further evaluated, whereas the role of perisinusoidal fibrosis in HBV could not be evaluated in our studies owing to the limited number of patients with chronic hepatitis B enrolled.

Interestingly, we found <19 BMI to be the only predictor of fibrosis underestimation by TE, although the significance of this association disappeared when data were stratified by other BMI cut offs.

Overall, the discrepancies we observed between TE and LB results reinforce the clinical usefulness of LB, which allows for the identification and dissection of the role of each of these confounders in patients with chronic viral hepatitis. The robustness of our results was validated by the rates of failures (8%), which were comparable to those reported in previous studies [1,2,5,11–12], whereas they were definitively lower than those reported by a large prospective, multicentre study in France [24], rating a 18% of uninterpretable liver stiffness measurements. Since obesity was by far the main factor associated with both failure and unreliable TE examinations, in all studies, a lower rate of obese patients in our study could account for the differences in the rates of unsuccessful results reported between studies, even though data on waist circumference that appeared to be a relevant factor in the French study were not available in our study.

The robustness of our study was also validated after patient stratification by the predictor cut-offs of TE that were reported by others for HBV [21,22] and HCV patients [2], showing substantially unchanged correlations. Importantly, the determinants of discordances identified using the TE cut-off of other studies were similar to those generated in our series, even though studies differed in terms of prevalence of patients with significant fibrosis and cirrhosis, thereby potentially accounting for variation in the overall performances of TE in fibrosis stages. Recently, Lucidarme et al. [32], in an analysis of the determinants of discordance between TE and LB, found that the most relevant independent factor of fibrosis overestimation was the ratio between interquartile range on the median value of TE (IQR/M), in particular the value of 0.21 being the stronger discriminant TE cut off. In our patients, this cut-off showed no predictive power for discordance between TE and LB, despite differences in the definition of discordance in Lucidarme's and our study that could account for the discrepancy between studies.

We acknowledge that the use of LB as a "gold standard" was a potential limitation of our study, since this procedure may yield false-negative results in up to 30% of cases, leading to an underestimation of the accuracy of the surrogate test under investigation, where a false-negative result of LB was perceived

as a false-positive result of TE [33]. Even if our criteria for adequate LB should have minimized this risk, we concur that LB is an "imperfect" reference standard to assess the diagnostic performances of any non-invasive test under investigation [34], and acknowledge that only outcome studies evaluating the prognosis of patients with discrepant results could define the actual advantage of one test over the other.

In conclusion, host and hepatitis-related factors other than hepatic fibrosis may influence the assessment of disease severity in patients with chronic viral hepatitis. Of note, false TE results more commonly occur among patients with moderate to severe histological features of necroinflammation or steatosis that can only be diagnosed with confidence by histology. Therefore, a baseline diagnostic LB remains a prerequisite for reliable staging and the follow-up of single patients with chronic viral hepatitis, since it enables the recognition of important TE confounders like liver cell necroinflammation and steatosis that can inappropriately aggravate the prognosis of hepatitis as established on the ground of TE.

### Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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