Analysis of Histopathological Changes that Influence Liver Stiffness in Chronic Hepatitis C. Results from a Cohort of 324 Patients

Monica Lupşor[1], Radu Badea[1], Horia Stefănescu[2], Mircea Grigorescu[2], Zeno Sparchez[2], Alexandru Serban[3], Horatiu Branda[1], Sorana Iancu[4], Anca Maniu[1]

1) Department of Ultrasonography; 2) Department of Hepatology; 3) Department of Pathology, 3rd Medical Clinic, University of Medicine and Pharmacy, Cluj-Napoca; 4) Athena Institute, VU University Amsterdam, Amsterdam, The Netherlands

Abstract

Aim. The current study aims to assess the role of the histological parameters in liver biopsy for explaining the variance of liver stiffness, as well as the performance of transient elastography in quantifying liver fibrosis in patients with chronic hepatitis C. **Methods**. 324 consecutive CHC patients were prospectively included in this study. All of them had positive HCV-RNA in serum and had underwent percutaneous liver biopsy for grading and staging the diseases (METAVIR scoring system). All were referred to liver stiffness measurement 1 day prior to biopsy. **Results**. Liver stiffness values were strongly correlated with fibrosis ($r=0.759$, $p<0.0005$). They also correlated with steatosis ($r=0.255$, $p<0.0005$), necroinflammatory activity ($r=0.378$, $p<0.0005$) and hepatic iron deposition ($r=0.143$, $p=0.03$). The univariate regression analysis demonstrated that fibrosis ($R^2=0.610$, $p<0.0005$), activity ($R^2=0.145$, $p<0.0005$) and steatosis ($R^2=0.037$, $p=0.002$) were correlated with liver stiffness. In multiple regression analysis, all three variables independently influenced liver stiffness: fibrosis ($p<0.0005$), activity ($p=0.039$) and steatosis ($p=0.025$). Together they explained 62.4% of the variance of the liver stiffness. The areas under ROC curve for the diagnosis of fibrosis $F\geq1$, $F\geq2$, $F\geq3$, and $F=4$ were 0.936, 0.862, 0.910 and 0.938, for the cut-off values of 4.9 kPa, 7.4 kPa, 9.1 kPa and 11.85 kPa respectively. **Conclusions**. Transient elastography is a useful method for chronic hepatitis C assessment. Fibrosis is the main predictor of liver stiffness, but activity and steatosis also influence liver stiffness.

Key-words

Chronic hepatitis C – noninvasive – fibrosis – transient elastography – liver stiffness.

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Address for correspondence: Dr. Monica Lupşor
Ultrasonography Dept., 3rd Medical Clinic, 19 – 21 Croitorilor Street, Cluj-Napoca, Romania
mmlupsor@yahoo.com

Abbrevations

CHC, chronic hepatitis C; LB, liver biopsy; LS, liver stiffness; LSM, liver stiffness measurement; LSP, liver stiffness prediction; TE, transient elastography.

Introduction

The prognosis and management of chronic liver diseases largely depends on the amount and progression of liver fibrosis. In patients with chronic hepatitis C (CHC), the stage of liver fibrosis is the most important predictor of disease progression and determines the need for antiviral therapy [1]. Until recently, liver biopsy (LB) was the only method to evaluate liver fibrosis and it has traditionally been considered as the gold standard [2]. However, LB is an invasive and painful procedure, often with poor patient compliance, also carrying a significant, although small risk of life-threatening complications [3].

An obvious trend in clinical practice observed in the latter years consists of finding a correct method for liver fibrosis evaluation in a noninvasive way, both by biochemical tests as well as imaging methods, as an alternative to LB. Virtually, all serological tests developed to date have not entirely met clinician expectations, as they insufficiently predict the stage of liver fibrosis on an individual level [4]. The imaging methods have the advantage of being noninvasive and, at the same time, they allow a complete evaluation of the entire organ, with a precise assessment of the disease severity when fibrosis does not affect the liver uniformly. Unfortunately, these methods have been limited for the tracing of cirrhosis and its complications. The "classical" imaging methods (ultrasonography, computerized tomography, and magnetic resonance imaging) relied on the identification of the anatomical changes of the liver during the natural evolution of the cirrhosis (e.g. the decrease in size of the right lobe and also the increase in size of the left and caudate lobe). These approaches have a high specificity but also a limited sensitivity while the significant morphological changes are present only in the advanced stages of the disease and, therefore, cannot be used to evaluate the initial stages

of fibrosis [5].

The last technological discovery in the evaluation of liver fibrosis is the Fibroscan® (Echosens, Paris, France), a specially adapted ultrasound device using the principle of the one-dimension transient elastography (TE) for the assessment of liver stiffness (LS). Transient elastography gained popularity as a user-friendly noninvasive technique for measuring LS [6], and has been shown to accurately predict liver fibrosis in a variety of clinical conditions including CHC [6-13]. In patients with CHC, it has proven to be highly reproducible approaching 0.98 inter- and intra-observer agreement rates [14].

The current study aims to evaluate the performance of TE in quantifying liver fibrosis in a cohort of consecutive patients with CHC and to assess its role in the variance of LS determined by other histological parameters observed in LB.

Patients and methods

Patients

Three hundred twenty-four consecutive patients with chronic HCV infection examined at the 3rd Medical Clinic, Cluj-Napoca, Romania, between May 2007 and March 2008 were prospectively included in this study. All of them had positive serum HCV-RNA and underwent percutaneous LB for grading and staging the diseases. All patients were referred to liver stiffness measurement (LSM) 1 day prior to LB. Besides the epidemiological data, the following biological parameters were determined for all patients on the same day as LSM: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transpeptidase (GGT), total bilirubin, alkaline phosphatase, platelet count, prothrombin time [international normalized ratio (INR)], fasting blood glucose, fasting serum cholesterol and triglycerides.

Exclusion criteria were: the presence of ascites at clinical or ultrasound examination (ascites is a physical limitation to the technique because elastic waves do not propagate through liquids) [8], co-infection with HBV and/or human immunodeficiency virus, active infectious diseases other than HCV, and pregnancy.

The study was approved by the local Ethical Committee of the University of Medicine and Pharmacy Cluj-Napoca. The nature of the study was explained to the patients. Each provided written informed consent before the beginning of the study, in accordance with the principles of the Declaration of Helsinki (revision of Edinburgh, 2000).

Transient elastography

Transient elastography was performed using FibroScan® device, which consists of a 5-MHz ultrasound transducer probe mounted on the axis of a vibrator. The vibrator generates a completely painless vibration (frequency 50 Hz and amplitude 2 mm) which is similar to a „flick", generating an elastic share wave that propagates through the skin and the subcutaneous tissue to the liver. The velocity of the wave is directly related to tissue stiffness.

During the acquisition, patients were lying in a dorsal decubitus position, with the right arm in maximum abduction. The Fibroscan's transducer, covered with a drop of coupling gel, was placed perpendicularly in the intercostal space. Under control TM and A-mode, the operator choses a liver portion within the right lobe of at least 6 cm thick, free of any large vascular structure or the gallbladder. When the target area has been located, the operator presses the probe button to commence the measurements. The median value of 10 successful acquisitions, expressed in kilopascals (kPa), is kept as a representative of the LSM. As previously described [6, 7, 15] and as suggested by the provider of the instrumentation, we considered as representative measurements 10 successful acquisitions with a success rate of at least 60% (the ratio of the number of successful measurements to the total number of acquisitions) and with an interquartile range (IQR) lower than 30% of the median value. IQR reflects the variability of the validated measures.

The operators were ML and HS, who had previously performed at least 100 determinations in patients with chronic liver diseases.

Histological study

A LB was performed by using the TruCut technique with a 1.8 mm (14G) diameter automatic needle device - Biopty Gun (Bard GMBH, Karlsruhe, Germany). Liver biopsy specimens were fixed in formalin and embedded in paraffin. Unaware of the clinical data, the slides were evaluated by a single expert pathologist (AS). Only biopsy specimens with more than 6 intact portal tracts were eligible for evaluation [16]. Liver fibrosis and necroinflammatory activity were evaluated semiquantitatively according to the METAVIR scoring system [16, 17].

Fibrosis was staged on a 0-4 scale as follows: F0 – no fibrosis; F1 – portal fibrosis without septa; F2 - portal fibrosis and few septa; F3 – numerous septa without cirrhosis; F4 – cirrhosis. Necroinflammatory activity was graded as follows: A0 – none; A1 – mild; A2 – moderate; A3 – severe. Steatosis was categorized by visual assessment as: 0 - none; 1 – steatosis in <33% of hepatocytes; 2 - steatosis in 33% to 66% of hepatocytes; and 3 - steatosis in > 66% of hepatocytes

Hepatic iron deposition was identified through Perls Prussian blue staining of biopsy specimens and classified as absent or present.

Statistical analysis

Statistical analysis was performed using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). Elastography data were expressed as median values. The distribution of LS values on the various classes of the four histological parameters were visually inspected through box plots. Differences in mean values were tested by one-way analysis of variance (ANOVA) and the Kruskal-Wallis test; relationships between the parameters were characterized using the Spearman correlation coefficients.

The predictive value of the histological parameters on the LSM was investigated through uni- and multivariate standard

regression. Evaluation of the assumptions for regression analysis led to the transformation of LS on a logarithmic scale to reduce skewness, the number of outliers and to improve the normality, linearity and homoscedasticity of residuals. Variables that showed a significant effect ($p<0.05$) on the log transformed LS results in univariate analysis were included in multivariate regression analysis, in order to evaluate their simultaneous influence on the log transformed LS results. We computed the model's R-squared, the standardized and unstandardized weights, partial correlation coefficients and the p-values for all the variables included in the analysis. Only correlations with a p-value under 0.05 were considered significant. The predictive model was then tested on an additional sample. For each of these patients, a new parameter was calculated (liver stiffness prediction, LSP), using the equation yielded by the multivariate regression analysis. The agreement between LSP and LSM was first inspected visually, through the Bland-Altman plot. This method reports on the relationship between the differences and the averages of the two measurements and allows for the identification of any systematic biases and outliers [18].

Further, the agreement between LSM and LSP was quantified using the two-way mixed intraclass correlation coefficient (ICC) [19]. The ICC quantifies the agreement between two measurements as poor (ICC=0.00-0.20), fair to good (ICC=0.40-0.75) or excellent (ICC>0.75) [20].

The diagnostic performance of LSM was assessed using sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), accuracy, likelihood ratios (LR) and receiver operating characteristic (ROC) curves. The ROC curve is a plot of sensitivity versus 1-specificity for all possible cut-off values. The most commonly used index of accuracy is the area under the ROC curve (AUROC), with values close to 1.0 indicating higher diagnostic accuracy. Optimal cut-off values for LS were chosen to maximize the sum of sensitivity and specificity, and positive and negative predictive values were computed for these cut-off values. We also calculated the adjusted AUROC, taking into account the prevalence of each fibrosis stage, using DANA method, as described by Poynard et al. [21].

Results

Baseline characteristics of patients

Clinical and biochemical characteristics of the study patients are summarized in Table I. The median length of the LB samples was 11.38 mm, and the mean number of portal spaces was 11.6. The fibrosis stage distribution in our patients was as follows: F0 –13 (4%), F1 – 101 (31.2%), F2 –104 (32.1 %), F3 – 37 (11.4%), F4 – 69 (21.3%).

Liver stiffness

Failure of LSM appeared in 6 patients (1.69%), all of them having a BMI>35 kg/m^2. According to this, 6 patients were excluded, so that only 318 patients (115 men, 202 women; mean age 48.41 ± 10.88 years) were fully examined and analyzed in respect to the study protocol.

Table I. Baseline characteristic of the patients

Characteristics of patients	Mean ± SD or number (%)
Sex (male)	115 (35.5%)
Age (years)	48.41 ± 10.88
BMI (kg/m^2)	26.49 ± 4.71
AST (U/l)	60.45 ± 41.90
ALT (U/l)	89.04 ± 58.03
GGT (U/l)	83.11 ± 87.465
Total bilirubin (mg/dl)	0.89 ± 1.49
Alkaline phosphatase (U/l)	230.54 ± 117.01
Glucose (mg/dl)	105.31 ± 28.56
Cholesterol (mg/dl)	189.96 ± 42.59
Triglycerides (mg/dl)	118.03 ± 64.54
Platelet count (10^9/L)	199.94 ± 70.77
INR	1.03 ± 0.18

Abbreviation: body mass index (BMI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transpeptidase (GGT), international normalized ratio (INR)

Liver stiffness values ranged from 2.90 to 75 kPa (median 12.10 kPa). Figure 1 shows the box-plots of LSM value variability according to different stages of fibrosis, activity grades, steatosis grade and iron deposition.

Correlation between liver stiffness and different histopathological parameters

Liver stiffness values were significantly correlated with all histological parameters evaluated; we found correlations with hepatic iron deposition ($r=0.143$, $p=0.03$), steatosis ($r=0.255$, $p<0.0005$), activity ($r=0.378$, $p<0.0005$) and a strong correlation with fibrosis ($r=0.759$, $p<0.0005$).

The univariate regression analysis showed that fibrosis ($R^2=0.610$, $p<0.0005$), activity ($R^2=0.145$, $p<0.0005$) and steatosis ($R^2=0.037$, $p=0.002$) were correlated with log-transformed LSM. In multiple regression analysis, all three variables independently influenced the log-transformed LSM: fibrosis ($p<0.0005$), activity ($p=0.039$) and steatosis ($p=0.025$) (Table II).

The fibrosis, activity and steatosis together explained 62.4% of the variance of the LS ($R^2=0.624$, $p<0.0005$); the remainder of 37.6% was not explained by these relationships. The three significant parameters uniquely explained 45.95% of the amount of R^2, with fibrosis making the most unique contribution; the difference of 16.25% (62.4%-45.95%) was accounted for by the joint contribution of the three parameters.

The prediction model computed from this study can be expressed as follows:

Liver stiffness (log-transformed) = 0.493 + 0.180*fibrosis stage + 0.034*steatosis + 0.033*activity grade.

We tried to validate this prediction model in a new group of 42 patients, where LSP was calculated, according to the regression equation. The fibrosis stage distribution in this new sample of patients was as follows: F0 –2 (4.8%), F1 – 16 (38.1%), F2 –17 (40.5 %), F3 – 6 (14.3%), F4 – 1

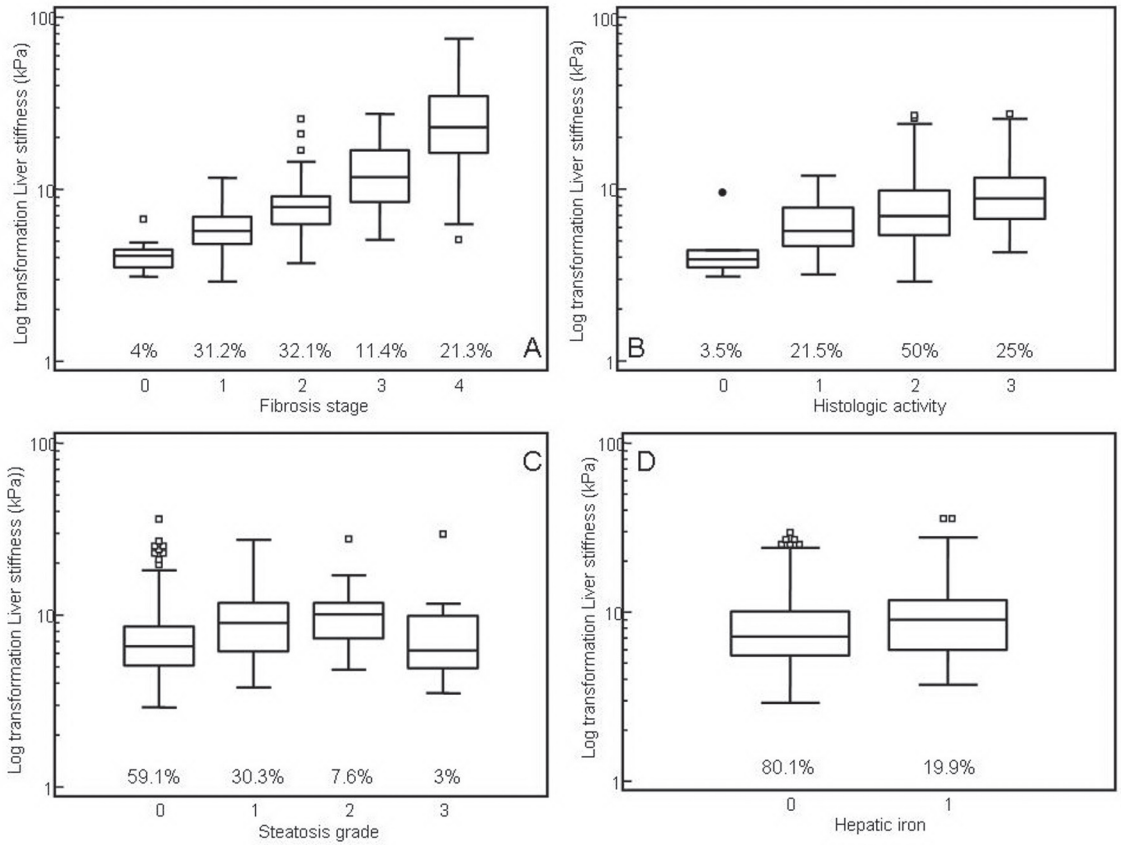

Fig 1. Box plots of liver stiffness values and patients' distribution (%) for each fibrosis stage (A), activity grade (B), steatosis grade (C) and iron deposition [present – 1, or absent – 0] (D). Because of the wide range of LSM values for F4, the vertical axis is in logarithmic scale. The top of the bottom of the boxes are the first and third quartiles, respectively. The length of the box thus represents the interquartile range within which 50% of the values were located. The line through the middle of each box represents the median. The error shows the minimum and maximum values (range).

Table II. Standard multiple regression of fibrosis, steatosis and activity grade on the log-transformed liver stiffness measurements

Variables	Fibrosis (Metavir)	Activity (Metavir)	Steatosis	B	β	Unique contribution (%)[1]
Fibrosis	1.00	0.395	0.137	0.180**	0.733	44.49
Steatosis	0.137	0.023	1.00	0.034	0.090	0.79
Activity	0.395	1.00	0.023	0.033	0.089	0.67

*p<0.05, **p<0.01; Intercept = 0.493; R2 = 62.4%, R = 0.79; Unique variability = 45.95%, Shared variability =16.45%

[1]Squared partial correlation coefficients

(2.4%). The mean value of LSM in sample group was 8.064 kPa (values ranged between 3.7-16.8 kPa), compared to the mean LSP value of 8.54 kPa (values ranged between 3.1-23.9 kPa). Figure 2 shows the plotted data of LSP against LSM, with the plots scattered along the line Y=X (LSP=LSM). Further investigation of the plot of the difference between LSM and LSP against their mean (the Bland-Altman plot) showed no systematic over or under-estimation between the two methods of measurement (mean difference -0.5). Only 3 patients scored outside the limits of agreement. This agreement was quantified as good: ICC = 0.718 (0.478-0.848, p<0.0005).

A deeper view on the multiple influences of histopathological changes on LS was carried out by analyzing subgroups of patients with the same fibrosis stage on liver biopsy and identical activity grade, and the same degree of steatosis, respectively.

The 52 patients with F2A2 were divided according to the absence (0-5% fatty load – 21 patients) or presence (> 6% fatty load – 31 patients) of steatosis. The mean values of the two subgroups were 7.28 kPa and 8.18 kPa respectively, showing no significant difference (p=0.21).

The 61 patients with F2 and no steatosis were divided according to the severity of necroinflammatory activity: A1 – 7 patients, A2 – 30 patients, A3 – 24 patients. Mean values of LS in the three subgroups are shown in Table III. On the whole, there was a significant difference among the mean values of LS according to activity (p=0.004), but the only significant difference was found between the A2 and A3 subgroups (p=0.034).

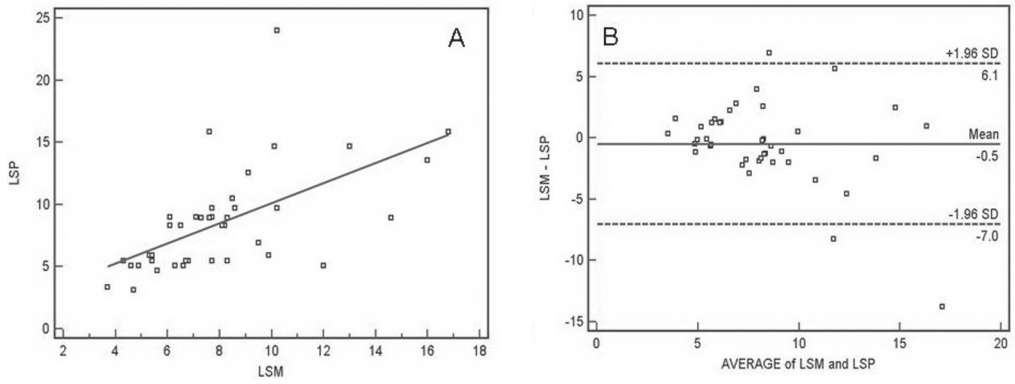

Fig 2. Comparison of LSM and LSP as measurements of liver stiffness, with the LSM=LSP line (A). Bland-Altman plot. (the solid line represents the mean of the difference of ratings; the dashed lines define the limits of agreement: mean of the difference ± 2 SD) (B)

According to our data and to those already published, liver fibrosis is the most important predictor of liver stiffness, therefore we analyzed how the fibrosis stage influences the LS value.

The mean values of LS for each fibrosis stage were 4.13 kPa (for F0), 6.12 kPa (for F1), 8.34 kPa (for F2), 12.66 kPa (for F3) and 27.28 kPa (for F4). The significance of differences between the groups were as follows: F1 vs F2

Table III. Variability of liver stiffness according to activity in the absence of steatosis in F2 subgroup of patients (N=61)

Liver stiffness		A1 (N=7)	A2 (N=30)	P* value (A1vs A2)	A3 (N=24)	P* value (A2 vs A3)	P* value (overall)
Mean (kPa)		6.4000	7.2833	0.818	9.7250	0.034	0.004
95% CI	Lower bound	4.3182	6.3562		7.7677		
	Upper bound	8.4818	8.2105		11.6823		

* according to Kruskal-Wallis test

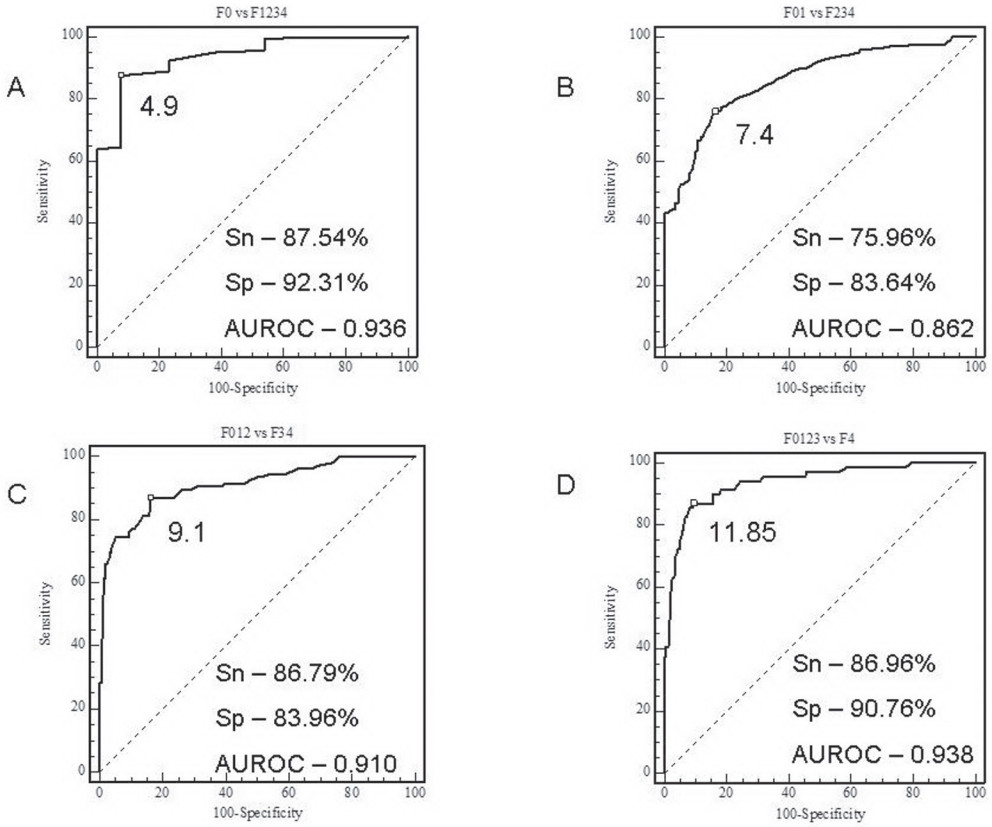

Fig 3. ROC curves for LSM for different fibrosis thresholds: A: F0 vs F1-F4 (≥F1); B: F0-F1 vs F2-F4 (≥F2); C: F0-F2 vs F3-F4 (≥F3); D: F0-F3 vs F4 (F=4).

Table IV. Liver stiffness cut-off values for the diagnosis of METAVIR scores ≥ F1, ≥ F2, ≥F3, F4

	≥ F1 F0vsF1234	≥ F2 F01vsF234	≥ F3 F012vsF34	F4 F0123vsF4
Liver stiffness cutoff value (kPa)	4.9	7.4	9.1	11.85
Se (%)	87.54	75.96	86.79	86.96
95% CI	83.3 - 91.0	69.6 - 81.6	78.8 - 92.6	76.7 - 93.8
Sp (%)	92.31	83.64	83.96	90.76
95% CI	63.9 - 98.7	75.4 - 90.0	78.3 - 88.6	86.5 - 94.1
+ LR	11.38	4.64	5.41	9.41
- LR	0.13	0.29	0.16	0.14
PPV	99.63	89.77	73.02	72.29
NPV	24.00	64.79	92.71	96.17
AUROC	0.936	0.862	0.910	0.938
SE	0.019	0.020	0.020	0.021
95% CI	0.903 to 0.960	0.819 to 0.898	0.873 to 0.939	0.906 to 0.962
Significance level p (Area = 0.5)	0.0001	0.0001	0.0001	0.0001
DA	87.74	78.62	84.91	89.94

Se sensitivity, Sp specificity, PPV positive predictive value, NPV negative predictive value CI confidence interval, +LR Positive likelihood ratio, -LR Negative likelihood ratio, AUROC area under ROC curve, SE standard error, DA diagnosis accuracy

(p=0.328), F3 (p=0.001), and F4 (p< 0.0005); F2 vs F3 (p=0.054) and F4 (p<0.0005); F3 vs F4 (p<0.0005).

The most discriminate cut-off values were determined from the distribution of stiffness values according to fibrosis stage. Figure 3 shows the ROC curves according to four different fibrosis stage thresholds: F0 versus F1, F2, F3 and F4 patients (F≥1), F0 and F1 versus F2, F3 and F4 patients (F≥2), F0, F1, F2 versus F3 and F4 patients (F≥3), and F0, F1, F2 and F3 versus F4 patients (F=4). The areas under the ROC curves (95% CI) were 0.93 (0.90 – 0.96) for F≥1, 0.86 (0.81-0.89) for F≥2, 0.91 (0.87-0.93) for F≥3, 0.93 (0.90-0.96) for F=4. Table IV shows the optimal cut-off values as well as corresponding sensibility, specificity, positive and negative predictive values.

Taking into account the prevalence of each fibrosis stage, the adjusted AUROC values using the DANA method were as follows: 0.964 (for F≥1), 0.920 (for F≥2), 0.938 (for F≥3) and 0.953 (for F4). These values did not significantly differ from the observed AUROCs, except F≥2 (p=0.315, p=0.040, p=0.322 and p=0.613 respectively).

Discussion

Transient elastography is a novel and promising clinical method for the rapid and noninvasive assessment of CHC patients. Transient elastography is currently subjected to extensive validation in cross-sectional and longitudinal studies, both for diagnosis and monitoring the response to antiviral treatments, and needs to be confirmed in the other chronic liver diseases, where specific consensus cut-off is still to be developed [22]. A major scope in this process of validation concerns the identification of potential factors leading to an over- and under-estimation of the fibrotic transformation of liver tissue assessed by this technique. In this context, the necroinflammatory activity and the grade of steatosis could represent important confounding factors in patients with chronic hepatitis C.

In most studies, LS was significantly correlated only to fibrosis, not steatosis or necroinflammatory activity [6, 8]. However, none of the patients in these studies had massive steatosis. On the other hand, the study by Fraquelli et al strongly suggested that factors other than fibrosis might have impact on the results of LSM [14]. Pinzani et al suggested that the extent of the necroinflammatory activity influences LSM, with a steady increase of LSM in parallel with the degree of histological activity [23]. The influence of the extent of necroinflammatory activity is also strongly suggested by a report showing that significant variations in LS occur during a flare in ALT levels in patients with chronic viral hepatitis [24] and is confirmed by results obtained in patients with acute viral hepatitis [25].

In this context, we prospectively assessed the role of the histopathological parameters seen in LB in explaining the variance of LS, as well as the performance of TE in quantifying liver fibrosis in patients with CHC.

We used LB as the reference standard because it is recommended for pre-treatment assessment of CHC in the latest consensus statements [26]. However, its reproducibility is poor, owing to heterogeneity in liver fibrosis and sample size, and also to inter and intra-observator variability. Ragev et al reported a difference of at least 1 fibrosis stage between the right and left lobes in 33% of 124 patients [27], and Siddique et al showed in 45% of patients a difference of at least 1 fibrosis stage between 2 specimens (at least 15 mm long) obtained at the same puncture site [28].

First, LS in patients with HCV was found to be highly correlated with the degree of fibrosis assessed by LB, but we found also a weak correlation with hepatic iron deposition and steatosis and a correlation with activity.

In multiple regression analysis, fibrosis, activity and steatosis independently influenced LSM. Iron deposition does not seem to influence the LS in CHC patients. This finding might be due to the fact that iron deposition is mainly intracellular, while the stiffness is mainly determined by the extracellular matrix. Further studies are needed to elucidate this matter.

Fibrosis, activity and steatosis together explained 62.4% of the variance of the LS. The three significant parameters uniquely explained 45.95% of the amount of LS, with fibrosis making the most unique contribution (44.49%); the difference of 16.25% (62.4%-45.95%) was accounted for by the joint contribution of the three parameters. The size and the direction of the relationships suggest that higher values of LSM are obtained for patients with advanced fibrosis, increased necroinflammatory activity and increased steatosis. Between these three, however, the stage of fibrosis is the single most important predictor, as suggested by the squared partial correlation.

We computed a prediction model which demonstrated the relationship between LS and fibrosis, necroinflammatory activity and steatosis. To our knowledge, this is the first evidence that quantifies the influence of different morphological changes in LSM in CHC patients. As expected, fibrosis is the main predictor, but activity and steatosis cannot be neglected, and might explain the LS variability within the same fibrosis stage. It should be mentioned that we did not observe in our group high ALT values which were demonstrated to influence LS, suggesting that the LS variability may be only due to changes observed on LB [25].

It is advisable that any model obtained by a research to be validated on another group of patients, with similar characteristics. Because our study was a prospective one, and the primary data analysis was already made on 324 patients, we managed to validate the equation on a smaller group (42 patients, prospectively included) and with almost the same distribution of fibrosis stages, except from the F4 (2.4%). We obtained a good agreement between LSM and LSP. However, the prediction model that we managed to compute needs further validation, both internal and external, on larger series of patients.

When we analyzed the influence of steatosis on LS in the subgroup of patients with the same grade and stage of the liver disease (A2F2) we did not find any significant influence ($p=0.21$). This might be due to the fact that in this subgroup there were not any patients with severe fatty load (>66%). Our hypothesis is that only severe steatosis significantly influences LS. This aspect needs further studies to clarify the role of pure steatosis, without variations of fibrosis, on LS in CHC patients.

Furthermore, we found that, in patients with the same fibrosis stage, after eliminating the influence of steatosis, LS increases alongside the necroinflammatory activity grade, but this particularity is significant only in those patients with severe activity (A3). This finding supports a recent report demonstrating a correlation between LS and high transaminase values, as markers of liver necroinflammation. [23]. However, our data suggest that the variance of LS within the interval validated for each fibrosis stage might be due to the activity grade in the absence of steatosis. Further studies are necessary to elucidate this hypothesis.

When we focused on the diagnosis of the fibrosis stage, the LS performances were very good. The areas under ROC curve for the diagnosis of fibrosis $F\geq1$, $F\geq2$, $F\geq3$, and $F=4$ were 0.936, 0.862, 0.910 and 0.938, respectively. These findings support the idea that liver fibrosis has the higher influence on LS values, but, as stated before, the role of necroinflammatory activity and steatosis cannot be neglected. The diagnostic performance of Fibroscan was good (Table V), similar to those previously published by other authors [7, 8].

Poynard et al demonstrated that the prevalence of liver fibrosis stages defining advanced fibrosis is a major factor of variability in assessing the diagnostic value of a fibrosis marker [21]. In our study, the adjusted AUROC according to the prevalence of each individual fibrosis stage did not significantly differ from the observed ones, except $F\geq2$.

It is also worth mentioning the good data we have obtained for diagnosing patients with $F\geq1$. This subcategory was poorly studied, and very few cut-off values for $F\geq1$ were provided, mainly because the lack of patients with documented F0 on LB.

Interestingly, for the F4 prediction, the cut-off value we obtained was lower than previously reported (11.8 kPa vs 12.5 kPa and 14.1 kPa) even though the AUROC values were very close (0.938 vs 0.91 vs 0.97) [7, 8]. In our opinion, the probability to actually have F4 in LB in a patient with LS between 12 and 14 kPa is high. Therefore, it is advisable to consider that the patient has liver cirrhosis until the contrary can be demonstrated.

It has been previously demonstrated and now it is widely recognized that the main advantage of TE compared to fibrosis biomarkers and biochemical scores is that it measures the quantitative physical parameters directly on the liver and there are no interferences from extra hepatic disorders. It represents a totally different approach and therefore could be complementary to fibrosis biomarkers and biochemical scores, in the better assessment of liver fibrosis, without the use of LB.

In relation to other diagnostic indices or predictive models based on laboratory tests, the fibrosis evaluation based on elastography (FibroScan®) has some leading advantages: it is easy to use, noninvasive, painless, does not imply anesthesia or hospitalization and thus it is easily accepted by the patients. It is rapid (acquisition speed < 1/10 sec), the acquisitions are not biased by cardiac or respiratory movements or by simultaneous diseases. The results are available immediately and independent by from the operator. Transient elastography measures LS in a volume that

Table V. Comparison of LSM diagnostic performances for different stages of fibrosis [7,8]

		Stiffness (kPa)	Sensitivity (%)	Specificity(%)	PPV (%)	NPV (%)	AUROC
F ≥ 1	Ziol (2005)	-	-	-	-	-	-
	Castera (2005)	-	-	-	-	-	-
	Present study	4.9	87.54	92.31	99.63	24.00	0.936
F ≥ 2	Ziol (2005)	8.8	56	91	88	56	0.79
	Castera (2005)	7.1	67	89	95	48	0.83
	Present study	7.4	75.96	83.64	89.77	64.79	0.862
F ≥ 3	Ziol (2005)	9.6	86	85	71	93	0.91
	Castera (2005)	9.5	73	91	87	81	0.90
	Present study	9.1	86.79	83.96	73.02	92.71	0.910
F = 4	Ziol (2005)	14.6	86	96	78	97	0.97
	Castera (2005)	12.5	87	91	77	95	0.91
	Present study	11.8	86.96	90.76	72.29	96.17	0.938

Se sensitivity, Sp specificity, PPV positive predictive value, NPV negative predictive value, AUROC area under ROC curve.

approximates a cylinder 1 cm wide and 4 cm long, between 25 and 65 mm below the skin surface. This volume is at least 100 times bigger than a biopsy sample and therefore is far more representative of the hepatic parenchyma [8].

Regarding the limitation of TE, 6 (1.69%) of the patients in our study, all overweight or obese, could not be assessed properly by this method. Stiffness measurements can be difficult to obtain in obese patients and impossible to obtain in patients having ascites [6].

This study is the first to demonstrate the diagnostic performance of TE in CHC patients in Romania. Our findings can represent valuable evidence, since the cohort of patients is very large and well documented (324 CHC patients with LB). We hope that our work can become the first step towards implementation in Romania, as well as in other European countries, of the noninvasive algorithm for the assessment of CHC patients [29].

In **conclusion**, our findings suggest that transient elastography is a useful noninvasive method for assessment of fibrosis in chronic hepatitis C patients. We have demonstrated the relationship between liver stiffness and fibrosis, necroinflammatory activity and steatosis. Fibrosis is the main predictor of liver stiffness, but activity and steatosis also influence it. The fibrosis, activity and steatosis together explained 62.4% of the variance of the liver stiffness. Further studies are needed in order to identify all the parameters that might influence liver stiffness, both histopathological and biochemical, for a better, complete and noninvasive evaluation of chronic hepatitis C patients. The use of artificial intelligence might represent a big development in this field, a big step forward towards the goal of a "virtual liver biopsy".

Conflicts of interes

None to declare.

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