Performance of Unidimensional Transient Elastography in Staging Non-Alcoholic Steatohepatitis

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Abstract

Background/aims: Transient elastography (TE) is a noninvasive method for predicting liver fibrosis, mainly validated in patients with viral hepatitis. Information is still limited concerning its performance in non-alcoholic steatohepatitis (NASH) patients. We aimed to assess the value of TE in the prediction of fibrosis stage in NASH as well as the factors determining the discordance between the TE-predicted and the biopsy-proven fibrosis stage in these patients. **Methods.** Liver biopsy and TE were performed on 72 consecutive NASH patients. Fibrosis, lobular inflammation, ballooning and steatosis were evaluated (Brunt system). **Results:** Liver stiffness (LS) values ranged from 2.80 to 16.90 kPa. In the univariate analysis, LS was correlated with fibrosis ($r=0.661$; $p<0.0001$), steatosis ($r=0.435$; $p<0.0001$), ballooning ($r=0.385$; $p=0.001$) and lobular inflammation ($r=0.364$; $p=0.002$). In multivariate analysis, only fibrosis significantly correlated with LS ($p<0.0001$). The median (and range) LS values (kPa) according to the fibrosis stages were: 4.90 (2.80-7.30) for F0; 6.15 (4.80-12.50) for F1; 6.90 (3.30-16.90) for F2 and 14.00 (10.70-14.10) for F3, with significant difference between stages, except for F1-F2 ($p=0.249$). Cut off values were calculated for predicting each fibrosis stage: 5.3kPa (AUROC=0.879) for F1; 6.8kPa (AUROC=0.789) for F2; and 10.4kPa (AUROC=0.978) for F3. Patients with false-positive results had a significantly higher ALT level than those with concordant results ($p=0.039$). **Conclusion**: In NASH patients, TE allows a reliable assessment and prediction of liver fibrosis, especially in advanced stages. Steatosis, ballooning and inflammation do not influence liver stiffness.

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Key words

Non-alcoholic steatohepatitis – fibrosis – noninvasive – transient elastography – Fibroscan – liver biopsy – liver stiffness.

Introduction

The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing in the context of the general epidemics of obesity and diabetes mellitus, which has become a public health issue [1]. The clinical importance of this condition is not only due to its high prevalence in the general population (13–23%), but also to the wide spectrum of risk factors, the indisputable link to the metabolic syndrome, and, of utmost importance, the potential evolution towards cirrhosis and hepatocarcinoma [2]. NAFLD is recognized as a clinical and pathological entity evolving from simple steatosis towards steatohepatitis, advanced fibrosis, liver failure and, in some cases, hepatocarcinoma.

The diagnosis of NAFLD was traditionally based on the histopathological changes of the liver, evaluated by needle liver biopsy (LB). Unfortunately, this is an invasive method, with potential adverse effects and great inter and intraobserver variability [3-5]. Therefore, rapid, noninvasive fibrosis assessment methods are being currently researched for non-alcoholic steatohepatitis (NASH) patients.

A new technique in the assessment of fibrosis in patients with diffuse liver diseases is unidimensional transient elastography - TE (Fibroscan®). This method has already been validated in different diseases: chronic viral C hepatitis [6-9], chronic B viral hepatitis [10, 11], HCV-HIV coinfection [12, 13], liver transplant recipients [14-17], cholestatic conditions [18], hemochromatosis [19], etc.

To our knowledge, there have been few studies published in extenso investigating the contribution of TE in the assessment of fibrosis in NASH patients [20-22]. With this in mind we set out to assess the TE performance in NASH patients, as well as the factors determining the discordance between the TE-predicted and the biopsy-proven fibrosis stage in these patients.

Patients and methods

Patients

In this study, we prospectively enrolled 72 NASH patients who had been examined in the 3rd Medical Clinic, Cluj-Napoca between May 2007 and September 2009. All of these patients subsequently had undergone a liver biopsy in order to establish the grade and stage of their condition. We excluded patients with other acute or chronic liver diseases (viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, hemochromatosis, Wilson's disease), with a history of alcohol consumption (≥ 30 g/day in men and ≥ 20 g/day in women), on hepatotoxic therapies that might induce steatosis as well as those with less than 6 portal spaces on liver biopsy.

Biological parameters were determined on a blood sample taken 12 hours after overnight fasting: alanin aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (APh), total cholesterol, triglycerides, total bilirubin, basal glycemia and platelets.

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, who provided written informed consent before the beginning of the study, in accordance with the the Declaration of Helsinki (Edinburgh revision, 2000).

Transient elastography

It was performed one day before the liver biopsy, using a FibroScan® device (Echosens, Paris, France), which consists of a 5-MHz ultrasound transducer probe mounted on the axis of a vibrator. The vibrator generates a completely painless vibration (with a frequency of 50 Hz and an amplitude of 2 mm) which generates an elastic share wave propagating through the skin and the subcutaneous tissue to the liver. The velocity of the wave is directly related to the tissue stiffness [23].

The acquisition was performed with patients lying in a dorsal decubitus position, with the right arm in maximum abduction. The Fibroscan transducer was placed perpendicularly to the intercostal space, in an area free of any large vascular structure. The median value of 10 successful acquisitions, expressed in kilopascals (kPa), was kept as a representative of the liver stiffness (LS).

Histopathological study

Liver biopsy was performed by the TruCut technique with a 1.8 mm (14G) diameter automatic needle device - Biopty Gun (Bard GMBH, Karlsruhe, Germany). The LB specimens were stained with haematoxylin-eosin, reticulin and Masson trichrome. Only biopsy specimens with more than 6 intact portal tracts were considered as eligible for evaluation. The slides were evaluated according to the Brunt criteria by a single expert pathologist blinded to the clinical data [24].

Fibrosis was scored as follows: 0 – no fibrosis; 1 – zone 3 perisinusoidal fibrosis; 2 – as above with portal fibrosis; 3 – as above with bridging fibrosis; and 4 – cirrhosis. Steatosis in the liver specimens was arbitrary scored by the percentage of hepatocytes containing fat deposits as: 0: <5%; 1: 5-33%; 2: 34-66% and 3: >66%. Lobular inflammation was graded on a 4-point scale on a 200 x field as: 0: no foci; 1: <2 foci; 2: 2-4 foci; and 3: >4 foci. Hepatocyte ballooning was graded as: 0: none; 1: occasional ballooned hepatocytes (mainly zone 3); 2: obvious zone 3 ballooning degeneration; 3: widespread ballooning.

Statistical analysis

Statistical analysis was performed using the SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA).

Continuous variables were presented as median values and range. Data was compared using the Mann-Whitney U and the $\chi 2$ test for continuous and categorial variables, respectively. Differences between more than two independent groups were tested by the Kruskal-Wallis test. Relationships between LS and different histological parametres were characterized using the Spearman correlation coefficients.

Variables that showed a significant effect ($p<0.05$) on LS in univariate analysis were included in a multivariate regression analysis.

The diagnostic performance of LS 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 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.

Subsequently the patients were divided into three groups on the basis of the agreement between LS measurement and liver biopsy: A – concordant results; B – false positive and C – false negative results. The factors that could influence the occurrence of false results were analysed using non-parametric tests.

Results

Characteristics of the study group

Most patients were male (70.8%), with a median age of 42 years. The median size of the LB was 11 (6-20) mm, with a median of 11 (7-22) portal spaces. The clinical, biochemical and histopathological characteristics are presented in Table I.

Analysis of liver stiffness

Liver stiffness kept within the 2.80-16.90 kPa interval, with a median value of 6.10 (2.80-16.90) kPa. The mean rate of success was 84.22%. No valid measurement was obtained for 3 of the patients, while in another 4 the rate of success did not reach the required 60%. All these patients had a BMI over 30 kg/m^2. The patients with a success rate < 60% had a BMI larger than those with a success rate of > 60%, but the difference was not statistically significant ($p=0.110$). No significant differences were recorded among

Table I. Clinical, biochemical and histopathological characteristics of the study group

Characteristics		Median (and range), number (%)
Gender males / females		51 (70.8%) / 21 (29.2%)
Age (years)		42 (20-69)
BMI (kg/m2)		28.71 (20.96-41.53)
AST (U/l)		36.00 (7-197)
ALT (U/l)		80.00 (15-343)
GGT (U/l)		55.00 (18-366)
Total bilirubin (mg/dl)		0.63 (0.30-2.34)
Alkaline phosphatase (U/l)		192.50 (127-360)
Glycemia (mg/dl)		99.50 (66-266)
Cholesterol (mg/dl)		227.50 (98-779)
Triglycerides (mg/dl)		197.00 (39-2987)
Platelets (10^9/L)		235.00 (158-364)
Liver stiffness (kPa)		6.10 (2.80-16.90)
IQR		0.80 (0.10-3.90)
Success rate (%)		100 (0-100)
Steatosis (S)	1	28 (38.9%)
	2	24 (33.3%)
	3	20 (27.8%)
Ballooning (B)	0	6 (8.3%)
	1	28 (38.9%)
	2	36 (50%)
	3	2 (2.8%)
Lobular inflammation (LI)	0	1 (1.4%)
	1	30 (41.7%)
	2	25 (34.7%)
	3	16 (22.2%)
Fibrosis (F)	0	25 (34.7%)
	1	29 (40.3%)
	2	13 (18.1%)
	3	5 (6.9%)
	4	-

the other parameters in the two groups, but the group was too small to allow a rigorous analysis of the factors leading to low success rates (Table II).

Correlation between liver stiffness and various histological parameters

Liver stiffness correlated with fibrosis (rs=0.661, p<0.0001) hepatocyte ballooning (rs = 0.385, p=0.001), lobular inflammation (rs=0.364, p=0.002) and steatosis (rs = 0.435, p<0.0001). No correlation was found between LS and steatosis type (rs= - 0.072, p=0.557).

Among the factors correlating with LS, multivariate analysis found fibrosis as the only factor influencing independently LS in NASH patients (p<0.001) (Table III).

The median (range) LS values (kPa) according to the fibrosis stages were: 4.90 (2.80-7.30) for F0; 6.15 (4.80-12.50) for F1; 6.90 (3.30-16.90) for F2 and 14.00 (10.70-14.10) for F3.

Table II. Univariate analysis of the parameters in relation with the success rate

	Success rate <60%	Success rate ≥ 60%	P*
No. patients	7 (9.7%)	65 (90.3%)	
Gender: males	3 (42.9%)	48 (73.8%)	0.183
females	4 (57.1%)	17 (26.2%)	
Age (years)	50 (24 – 53)	41 (20 – 69)	0.342
BMI (kg/m^2)	30.85 (27.34–37.37)	28.40 (20.96-41.53)	0.110
AST (U/l)	36.00 (13-152)	39.25 (7-197)	0.953
ALT (U/l)	80.00 (15-343)	92.00 (15-257)	0.695
GGT (U/l)	76.00 (35-366)	54.00 (18-339)	0.238
Total bilirubin (mg/dl)	0.60 (0.43-0.89)	0.635 (0.30-2.34)	0.409
Alkaline phosphatase (U/l)	206 (187-303)	192.50 (127-360)	0.283
Glycemia (mg/dl)	102 (83-187)	99.50 (66-266)	0.631
Cholesterol (mg/dl)	211 (167-335)	229 (98-779)	0.945
Triglycerides (mg/dl)	195 (112-454)	203 (39-2987)	0.976
Platelets (10^9/L)	223 (158-281)	239 (158-354)	0.145
Fibrosis: F 0,1	6 (85.7%)	48 (73.8%)	0.672
F 2,3	1 (14.3%)	17 (26.2%)	
Steatosis: S 1	4 (57.1%)	24 (36.9%)	0.419
S 2,3	3 (42.9%)	41 (63.1%)	
Ballooning:		30 (46.2%)	0.700
B 0,1	4 (57.1%)		
B 2,3	3 (42.9%)	35 (53.8%)	
Lobular inflammation:			0.132
LI 0,1	5 (71.4%)	26 (40%)	
LI 2,3	2 (28.6%)	39 (60%)	

*Mann-Whitney U and $\chi 2$ tests

Table III. Multivariate analysis of factors independently influencing the liver stiffness in patients with non-alcoholic steatohepatitis.

Parameter	Regression coefficient	Standard error	P value	P-value for model significance
Fibrosis	1.715	0.316	<0.0001	<0.001
Ballooning	0.132	0.423	0.312	
Lobular inflammation	0.481	0.360	0.187	
Steatosis	0.253	0.360	0.485	

The differences between the groups were as follows: F0 vs F1 (p<0.0001); F1 vs F2 (p=0.249); F2 vs F3 (p=0.004). There was an apparent overlap of the LS values, especially for the F1-F2 patients (Fig. 1).

TE performance in the assessment of fibrosis in NASH patients, according to the Brunt score

The discriminant cut-off values were determined from the distribution of LS values according to the fibrosis stage.

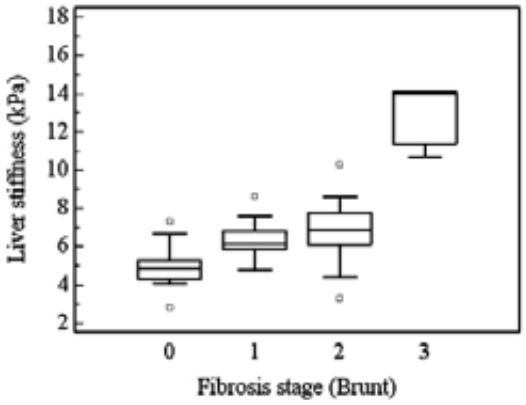

Fig 1. Box plots of liver stiffness values for each fibrosis stage. The top of the bottom of the boxes are the first and third quartiles, respectively. The length of the box represents therefore the interquartile range including 50% of the values. The line through the middle of each box represents the median. The error shows the minimum and maximum values (range).

Table IV. TE performance in quantifying fibrosis in NASH patients

	F≥1 F0vsF123	F≥2 F01vsF23	F3 F012vsF3
Cutoff value of liver stiffness (kPa)	5.3	6.8	10.4
Se (%)	93.48	66.67	100
95% CI	82.1-98.6	41-86.8	48-100
Sp (%)	78.26	84.31	96.87
95% CI	56.3-92.5	71.4-93.0	89.1-99.5
+LR	4.30	4.25	32.00
-LR	0.08	0.40	0
PPV	89.6	60.0	71.4
NPV	85.7	87.8	100
AUROC	0.879	0.789	0.978
SE	0.040	0.069	0.046
95% CI	0.779-0.945	0.674-0.878	0.910-0.997
P (Area=0.5)	0.0001	0.0001	0.0001
DA (%)	86.95	75.36	95.65

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

Fig. 2 shows the ROC curves according to three different fibrosis stage thresholds. The areas under the ROC curves were 0.879 for F≥ 1, 0.789 for F≥ 2, and 0.978 for F≥ 3. Table IV shows the optimal cut-off values as well as the corresponding sensibility, specificity, positive and negative predictive values. Maximal diagnostic accuracy could be obtained only for the prediction of severe fibrosis (95.65%), while, for the prediction of ≥ F2 stages the accuracy reached only 75.36%.

Analysis of the concordance between the biopsy-proven and the TE fibrosis stage

The discordances in the staging of fibrosis between the two methods (LB and TE) were further analysed in order to identify the responsible factors. Based on this model, 85% of F0 patients (according to LB), 65.4% of F1 patients, 40% of those with F2 and 62.5% of those with F3 were correctly classified using TE. The largest discordance was found in patients with F2 biopsy-proven fibrosis (Table V).

Patients with false-positive results had a significantly higher ALT level than those with concordant results: 108 (27-343) vs 69 (15-257), p=0.039. No significant differences

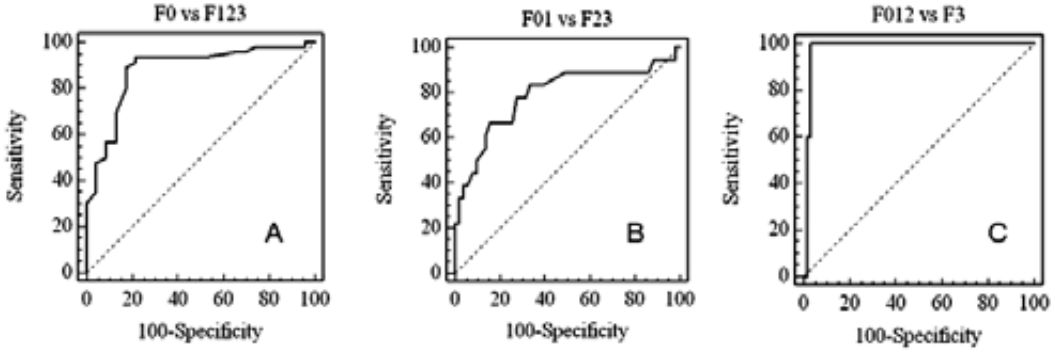

Fig 2. ROC curves for LS for different fibrosis thresholds: A: F0 vs F1-F3 (F≥1); B: F0-F1 vs F2-F3 (F≥2); C: F0-F2 vs F3 (F≥3)

Table V. Concordance between biopsy proven and TE predicted fibrosis

		Biopsy-proven fibrosis stage			
		F0 on biopsy	F1 on biopsy	F2 on biopsy	F3 on biopsy
TE predicted fibrosis stage	F0 (≤ 5.2 kPa)	17 (85%)	1 (5%)	2 (10%)	-
	F1 (5.3-6.7 kPa)	5 (19.2%)	17 (65.4%)	4 (15.4%)	-
	F2 (6.8-10.3 kPa)	1 (6.7%)	8 (53.3%)	6 (40%)	
	F3 (≥10.4 kPa)	-	2 (25%)	1 (12.5%)	5 (62.5%)

Table VI. Analysis of factors associated with the occurrence of false positive and false negative results

	Concordant results (Group A)	False positive results (Group B)	False negative results (Group C)	p	
				A vs B	A vs C
Number of patients	45 (65.2%)	17 (24.6%)	7 (10.1%)		
Gender - males	32 (71.1%)	13 (28.9%)	5 (71.4%)	0.673	0.986
- females	13 (7605)	4 (23.5%)	2 (28.6%)		
Age (years)	45 (20-69)	38 (25-56)	40 (22-58)	0.136	0.648
BMI (kg/m^2)	29.26 (22.32-41.53)	28.40 (22.86-37.72)	27.95 (20.96-33.95)	0.862	0.126
AST (U/l)	34 (7-197)	49 (14-170)	45 (15-113)	0.309	0.758
ALT (U/l)	69 (15-257)	108 (27-343)	78 (20-163)	0.039	0.727
GGT (U/l)	51 (18-339)	65 (28-141)	49.50 (29-101)	0.212	0.938
Total bilirubin (mg/dl)	0.61 (0.3-2.34)	0.72 (0.43-1.35)	0.85 (0.32-1.27)	0.165	0.504
Alkaline phosphatase (U/l)	194 (127-360)	189 (137-337)	160 (144-248)	0.581	0.264
Glycemia (mg/dl)	101.40 (66-266)	97 (83-149)	97 (89.3-119)	0.682	0.900
Cholesterol (mg/dl)	238 (98-779)	223 (114-335)	209 (98-779)	0.560	0.117
Triglycerides (mg/dl)	198 (46-2987)	210 (39-454)	154 (105-269)	0.744	0.153
Specimen size (mm)	11 (6-19)	12 (6-20)	11 (9-15)	0.592	0.829
Portal spaces number	12 (7-21)	11 (7-22)	11 (10-19)	0.752	0.752
IQR/liver stiffness	0.12 (0.01-0.45)	0.17 (0.07-0.28)	0.10 (0.05-0.15)	0.301	0.120
Success rate	100 (15-100)	83 (40-100)	100 (77-100)	0.066	0.431
Steatosis:					
S 1 n (%)	20 (44.4%)	5 (29.4%)	2 (28.6%)	0.282	0.429
S 2,3 n (%)	25 (55.6%)	12 (28.6%)	5 (71.4%)		
Ballooning:					
B 0, 1 n (%)	25 (55.6%)	5 (29.4%)	2 (28.6%)	0.090	0.241
B 2, 3 n (%)	20 (44.4%)	12 (70.6%)	5 (71.4%)		
Lobular inflammation:					
IL 0, 1 n (%)	21 (46.7%)	6 (35.3%)	2 (28.6%)	0.568	
IL 2, 3 n (%)	24 (53.3%)	11 (64.7%)	5 (71.4%)		

were found among the other biochemical, anthropometric or hystological parameters. There were no significant differences among the biochemical, anthropometric, or histological parameters in the group evidencing concordant results, compared to the group of false negative results (Table VI).

Discussion

The current study represents at this moment one of the few attempts to analyse the utility of TE in an adult NASH population; other studies published in extenso focused either on the pediatric population [21], or on Japanese adults [20, 22]. As a limitation, we acknowledge the relatively small study population (72 patients), in spite of the relatively long studied period, the reason possibly being the difficulties associated with obtaining a LB in a largely uncooperative NASH group. Furthermore, in our study population we did not have F4 patients.

In NASH patients, the LS correlated moderately with fibrosis and weakly, but significantly, with hepatocyte ballooning, lobular inflammation and steatosis. Nevertheless, no correlation with the type of steatosis was found. From all of these factors, multivariate analysis evidenced fibrosis as the only factor influencing independently LS in NASH patients. Our results are in agreement with those reported by other studies [22, 25], according to which only fibrosis can independently influence LS in NASH patients. Nobili's study did not assess the possible interfering role of steatosis, necroinflammatory activity and hepatocyte ballooning because of the temporal gap between LB and TE (≤6 months) which could alter the results [21]. In our study, LB was performed one day after TE.

Given the small number of patients, these results should be regarded with reservation. Further studies are necessary, conducted on large groups of patients having undergone biopsy, aimed at proving whether LS is not influenced indeed by either steatosis or inflammation.

As far as the degree of the correlation of LS with fibrosis is concerned, it was smaller in the NASH patients (rs =0.661), in comparison to previous studies performed on HCV patients (r=0.79 and r=0.73 respectively) [9, 26]. Our observation is supported by a recent study [27], which analysed LS depending on the fibrosis area on the entire biopsy specimen assessed by quantitative digital image analysis. Interestingly, LS was highly correlated to the fibrosis amount in chronic

hepatitis before the cirrhotic stage, but not in NASH. This can be explained by a different fibrosis distribution pattern. In chronic hepatitis (viral B, C or autoimmune), fibrosis is mainly developed from portal myofibroblasts and gives rise to a dense, stellate, and regularly-distributed portal fibrosis, whereas fibrosis in NASH is mainly located in the perisinusoidal space of the centrilobular area, and in the wall of the centrilobular vein. Dense and stellate fibrosis regularly merging from all portal tracts modifies LS in a proportionate manner. In contrast, perisinusoidal fibrosis preferentially distributed in centrilobular areas does not seem to affect LS in a proportionate manner, as proven by morphometric studies [27].

Regarding the prediction of fibrosis, Nobili et al [21] reported the following cutoff and AUROC values: 5.1 KPa (0.97) for F1; 7.4 KPa (0.99) for F2 and 10.2 KPa (1.0) for F3, while Yoneda et al [22] reports quite similar data: 5.9 KPa (0.93) for F1; 6.65 KPa (0.86) for F2 and 9.8 KPa (0.9) for F3. The last study was the only one until now which reports data for F4 in this category of patients: 17.5 KPa, with AUROC 0.99.

As shown in Table IV, our data are comparable to those reported so far. Unfortunately, our study population contained no F4 patients and we could not establish a TE value predicting liver cirrhosis in NASH patients.

The patients with false positive results had an ALT level significantly higher than those with concordant results. As a matter of fact, it was proved in viral hepatitis patients that the necroinflammatory activity influences LS, increasing it in direct relationship with the histological activity [10, 28-30]. The impact of these non-fibrotic changes on LS has been proven by its progressive decrease alongside the decrease in transaminase levels [31-33]. To our knowledge, the involvement of transaminases in inducing false positive results has not yet been described in NASH patients. The ensuing practical conclusion is that, in NASH patients as well, interpretation algorithms for the LS results are imperative. These algorithms should definitely take into account liver tests, with special attention to transaminases.

In our study, the patients with false negative results did not significantly differ from those with concordant results between elastographic-predicted and biopsy-proven fibrosis. This might suggest that there may be other factors inducing the TE underrating of fibrosis.

Indeed, we cannot overlook the fact that, in some cases, the discordances may be due to failure in the LB interpretation because of inhomogeneous distribution of fibrosis in the liver, as well as to a certain amount of intraobserver variability. In HCV patients, for instance, differences of at least 1 stage were reported in 33% of the cases between the right and left lobe [4] or even 45% differences between two specimens taken from the same area [34]. In NASH patients, the inhomogeneous distribution of fibrosis seems to be more apparent than in HCV patients [35]. Some studies [5] have shown that, by performing two biopsies in the right lobe in each NASH patients, in only 74% of the cases did the fibrosis stage correspond between the two specimens, while in 41% of the cases there was a difference of at least 1 stage, and in 12% of the cases – of at least two stages. Stages F3 and F4 were diagnosed in 12% of the patients on one LB but not on the second. However, only one third of the cirrhotic patients obtained this diagnosis on both specimens, while the others had an F3 stage established on the second specimen.

In the light of these observations, in NASH, the results of validation studies for a certain noninvasive diagnostic method should be carefully interpreted, since it is compared to an imperfect "gold standard".

Transient elastography measures the stiffness of a cylindrical volume 1 cm in diameter and 4 cm in length, 25 to 45 cm from the skin. As a result, the technique can prove to be difficult in obese patients, since the transmited vibrations are reduced much by the fatty tissue. This situation is frequently encountered in NASH patients [36-38]. In our study, in 3 patients no valid measurement could be acquired, while in the other 4, the success rate did not reach 60%. The biochemical, anthopometric or histological parameters did not yield significant differences between the two groups, but the entire group was too small for a rigorous analysis of the factors generating a low success rate. It may be possible, as reported by some authors [39], that a thick chest wall is a limitative factor for the success of the measurement, rather than the BMI increase per se.

The measurement failure rate in our study (9.7%) was greater than that reported so far in NASH patients (4-6%) [21, 22]. The results of the cited studies must be nevertheless cautiously interpreted, since they were performed on small groups of Japanese and pediatric populations respectively, all having low BMIs (26.6±4.2 and 26±4.0, respectively).

Lately, there has been increased research for technical, probe-related solutions that might overcome the shortcomings of the current elastographic method in assessing obese patients. Recently, a new, especially constructed probe has become available, having the central transducer frequency of 2.5 MHz (as opposed to the 5 MHz of the usual transducer) and assessing the LS at a 35-75 mm distance from the skin level (as opposed to the previous 25-45 mm). With the aid of this new probe, valid measurements were obtained in 49% of the patients having BMI≥ 30 kg/m2, in which LS could not be assessed using the usual probe [40].

Unidimensional TE has been developed as a method for staging liver diseases. The method cannot establish the cause of the disease and cannot formulate subtle differentials (for instance, steatosis vs. NASH) [41, 42]. In addition, it cannot grade the necroinflammatory activity, but can prove to be useful in assessing the progression of NASH towards cirrhosis [43, 44].

In **conclusion**, our study has shown the existence of a good correlation between LS and fibrosis stage in NASH patients, but weaker than that previously observed in chronic viral hepatitis patients, due to a different distribution pattern of fibrosis. Unidimensional TE is therefore useful for the prediction of fibrosis in NASH patients, especially in the case of severe fibrosis, where the accuracy reaches 95.65%.

Conflicts of interest

Nothing to declare.

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