

Transient elastography in non-alcoholic fatty liver disease

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a relevant issue in public health owing to its epidemiological burden. It represents the most common chronic liver disease in the general population and is expected to increase in future as a result of an ageing population. Liver biopsy is still considered the "gold standard" for distinguishing the broad range of NAFLD. However, liver biopsy is often not recommended in the NAFLD patients, because of its cost, the potential risk of bleeding and the absence of consensus regarding the histopathological criteria that firmly differentiate between the NAFLD entities. Due to the remarkable increase in the prevalence of NAFLD and the concomitant efforts in developing novel therapies, a non-invasive, simple and reproducible technique is needed in the clinical practice. Transient elastography is a non-invasive technique for liver stiffness measurement (LSM) as a function of the extent of hepatic fibrosis. This review focuses on practical issues in the use of LSM in the NAFLD patients and suggests areas for further research and development.

Key words. Liver. Stiffness. Elastic wave. Steatosis. Obesity.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents the most common chronic liver disease in the general population and is expected to increase in future as a result of an ageing population, the improving control of other major causes of the chronic liver disease and the epidemics of obesity and diabetes.^{1,2}

Liver steatosis consists of an accumulation of fat in the liver cells and it is not a harmful disease itself. It is an aggravating factor in certain pathological situations of varying degrees of gravity and becomes dangerous when it is associated with inflammation or fibrosis. However, it is reversible, through diet and exercise. In fact, NAFLD may present a spectrum ranging from asymptomatic steatosis with elevated or normal aminotransferases to

steatohepatitis [non-alcoholic steatohepatitis (NASH)], all the way to cirrhosis with the complications of liver function to hepatocellular carcinoma. NAFLD occurs in 60-95% patients with obesity, in 28-55% patients with type 2 diabetes mellitus (T2DM) and in 27-92% patients with dyslipidemia. Insulin resistance with compensatory hyperinsulinemia is a common denominator of obesity, T2DM and dyslipidemia, and it can play a pathogenetic role in NAFLD. Accordingly, it has been reported that the insulin resistance is a single laboratory finding most closely associated with NAFLD in a great number of patients, irrespective of the body mass index (BMI), fat distribution or glucose tolerance. Therefore, NAFLD has recently been proposed as an additional feature of the metabolic syndrome.³

Liver biopsy (LB) is still considered the gold standard for distinguishing between the broad range of NAFLD. However, LB is often not recommended in the NAFLD patients because of its cost, the potential risk of bleeding, and the absence of consensus regarding the histopathological criteria that firmly define NASH and differentiate between NAFLD entities.⁴ Due to the remarkable increase in the prevalence of NAFLD and the concomitant efforts in developing the new therapies for fatty liver, reproducible and simple non-invasive techniques are needed.⁵

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Currently, many techniques exist to detect steatosis, although ultrasound (US) is perhaps the easiest and most common approach in the clinical practice and for epidemiological studies. In particular, US is the least expensive modality for detecting the changes associated with NAFLD. However, echography-based US techniques become sensitive at moderately high levels of steatosis (33% or more) and suffer from low intra- and inter-operator repeatability.⁶

In the last years, a new technique called vibration-controlled transient elastography ([VCTE], Fibroscan®, Echosens, Paris, France) has been proposed for liver stiffness measurement (LSM). It is a technique using elastic waves of low-audio-frequency (50 Hz), whose propagation velocity depends on tissue elasticity, indicative of liver stiffness, considered to be one of the direct consequences of the fibrotic evolution of chronic liver disease.⁷

A few meta-analyses have suggested liver stiffness measurement (LSM) by VCTE to be a reliable tool to detect advanced liver fibrosis and early liver cirrhosis.^{8,9} In the NAFLD patients, various degrees of hepatic steatosis may attenuate the elastic shear wave, but does not change its underlying speed which is the parameter used for the stiffness measurement. Clearly positive correlation between LSM and the severity of liver fibrosis in the patients with NAFLD has been reported in the literature. Recently, a new measurement technique, the controlled attenuation parameter (CAP®, EchoSens, Paris, France), has been introduced which provides good accuracy even with the low levels of steatosis in the liver. Owing to its sensitivity at early phases of steatosis, CAP could provide a means to monitor the progression or regression of steatosis. A combination of the measurement by VCTE and CAP could provide the means to follow the NAFLD patients, during the different phases of the disease history.

This review focuses on practical issues in the use of LSM in NAFLD patients and suggests areas for further research and development.

Elastography assessment of NAFLD

The development of non-invasive methods to evaluate liver disease stages is a clinical and research priority. NAFLD is strongly associated with metabolic syndrome and obesity. NASH may progress to cirrhosis and hepatocellular carcinoma. The prognosis depends heavily on histological severity. Several questions concerning the management of the patients with NAFLD and, in particular, how to diagnose NAFLD and its type, how to select the patient

candidates for the treatment and how to treat these patients have been debated in the literature.

The limitations of LB have stimulated the search for non-invasive approaches for the assessment of steatosis and liver fibrosis in the patients with NAFLD. A variety of new methods, including serum markers, imaging techniques such as US, computed tomography, magnetic resonance imaging, and more recently LSM, have been proposed. In particular, LSM has shown excellent results for the diagnosis of severe fibrosis and cirrhosis and moderate results for the diagnosis of significant fibrosis in the patients with NAFLD/NASH.

A prospective study aimed to assess the ability of VCTE to identify histological parameters, including steatosis, in asymptomatic healthy individuals such as the potential liver donors, and to compare these findings with the results in the patients with liver disease.¹⁰ Forty-seven patients with abnormal liver function and/or hepatitis symptoms and eighty living related potential liver donors were consecutively enrolled. LB and LSM were performed in each subject. Histological parameters were evaluated according to Metavir score by a single pathologist. In the liver disease patients, stiffness was significantly correlated with fibrosis stage ($P < 0.001$), and the optimal stiffness cut-off values for $F \geq 2$, $F \geq 3$, and $F = 4$ were 7.35, 8.85, and 15.1 kPa respectively. In the potential liver donors, stiffness was not correlated with fibrosis ($P = 0.851$). In the latter group, the area under the receiver-operating characteristics curve (AUROC) was 0.70 (95%, confidence interval [CI], 0.58-0.81), and the optimal stiffness cut-off value was 4.00 kPa for $F \geq 2$, which was lower than that in the liver disease patients. Steatosis was not correlated with stiffness ($P = 0.463$) in the potential liver donors. The authors concluded that VCTE preset limited the value for detecting steatosis in the asymptomatic healthy subjects.

Nobili, *et al.* assessed the value of VCTE in a cohort of the paediatric patients with NASH.¹¹ LSM was performed in fifty consecutive biopsy-proven NASH patients, age 13.6 ± 2.44 years. The AUROC for the prediction of different degrees of liver fibrosis on the basis of Brunt score were 0.977, 0.992, and 1, for respectively any significant and advanced fibrosis (Table 1). The calculation of multilevel likelihood ratios showed that the VCTE values were < 5 , < 7 , and < 9 kPa, suggesting the presence of no fibrosis, significant fibrosis, and advanced fibrosis, respectively. The VCTE values between 5 and 7 kPa predicted a fibrosis stage of 1, but with some degree of uncertainty. The VCTE va-

lues between 7 and 9 kPa predicted fibrosis stages 1 or 2, but could not discriminate between these two stages. The VCTE values of at least 9 kPa were associated with the presence of advanced fibrosis. In summary this study reported the reliability of VCTE to predict a normal liver or the presence of significant fibrosis and advanced fibrosis in these special populations.

Yoneda, *et al.* investigated the usefulness of LSM in the assessment of liver fibrosis in the NAFLD patients.¹² VCTE was performed for LSM in 97 NAFLD patients. The authors also investigated the relationship between LSM and the serum levels of hyaluronic acid and type IV collagen 7s domain. Liver stiffness was well correlated with the different stages of Metavir score ($P < 0.0001$). The AUROC were (Table 1):

- 0.927 for \geq F1.
- 0.865 for \geq F2.
- 0.904 for \geq F3.
- 0.991 for \geq F4.

Only the fibrosis stage was strictly correlated with LSM by multiple regression analysis. Liver stiffness was also strongly correlated with the serum levels of type IV collagen 7s domain ($r = 0.525$,

$P < 0.0001$) and hyaluronic acid ($r = 0.457$, $P < 0.0001$). These data showed the significant correlation between LSM and the fibrosis stage in the NAFLD patients, as confirmed by the results of LB.

Lupsor, *et al.* assessed VCTE performance in the NASH patients, as well as the factors determining the discordance between the VCTE-predicted and the fibrosis stage evaluated by the Brunt system.¹³ LB and VCTE were performed on 72 consecutive NASH patients. LSM ranged from 2.80 to 16.90 kPa. In the univariate analysis, LSM was correlated with fibrosis ($P < 0.0001$), steatosis ($P < 0.0001$), ballooning ($P = 0.001$) and lobular inflammation ($P = 0.002$). In multivariate analysis, only fibrosis significantly correlated with LSM ($P < 0.0001$). The median LSM values according to the fibrosis stages were:

- 4.90 kPa (range: 2.80-7.30) for F0.
- 6.15 kPa (4.80-12.50) for F1.
- 6.90 kPa (3.30-16.90) for F2, and
- 14.00 kPa (10.70-14.10) for F3, with significant difference between stages, except for F1-F2 ($P = 0.249$).

The cut-off values were calculated for predicting each fibrosis stage (Table 1):

Table 1. Analysis of VCTE cut-off for the diagnosis of NAFLD in adult patients. AUROC: area under receiver operative curve. PPV: positive predictive value. NPV: negative predictive value. Histological evaluation by *Metavir and †Brunt score.

Lead author	Yoneda (N = 97)*	Nobili (N = 50) †	Lupsor (N = 72) †	Wong (N = 246) †	Mori (N = 36) †
• F \geq 2					
AUROC	0.86	0.99	0.78	0.84	-
Optimal cut-off (kPa)	6.6	7.4	6.8	7.0	8.3
Sensitivity (%)	88	100	100	79	-
Specificity (%)	74	92	97	76	-
PPV (%)	79	80	71	70	-
NPV (%)	85	100	100	84	-
• F \geq 3					
AUROC	0.90	1.0	0.978	0.93	-
Optimal cut-off (kPa)	9.8	10.2	10.4	8.7	14.7
Sensitivity (%)	85	100	100	84	-
Specificity (%)	81	100	97	83	-
PPV (%)	64	100	71	59	-
NPV (%)	93	100	100	95	-
• F \geq 4					
AUROC	0.99	-	-	0.95	-
Optimal cut-off (kPa)	17.5	-	-	10.3	19.6
Sensitivity (%)	100	-	-	92	-
Specificity (%)	97	-	-	88	-
PPV (%)	75	-	-	46	-
NPV (%)	100	-	-	99	-

- 5.3kPa (AUROC = 0.879) for F1.
- 6.8kPa (AUROC = 0.789) for F2, and
- 10.4kPa (AUROC = 0.978) for F3.

The patients with false positive results had a significantly higher alanine aminotransferase (ALT) level than those with concordant results ($P = 0.039$). These data show that steatosis degree, ballooning and inflammation do not influence LSM.

In the NAFLD patients, various degrees of hepatic steatosis may attenuate the elastic shear wave, possibly leading again to an underestimation of liver damage. Wong, *et al.* aimed to evaluate the accuracy of VCTE in the diagnosis of fibrosis and cirrhosis in the patients with NAFLD and to study the factors associated with discordance between LSM and histological data.¹⁴ Two hundred forty-six consecutive patients from two ethnic groups had successful LSM and satisfactory liver biopsy specimens. The AUROC of VCTE for F3 or higher and F4 disease was 0.93 and 0.95, respectively, and was significantly higher than that of other biochemical scores for fibrosis prediction. AUROC ranged from 0.62 to 0.81, $P < 0.05$ for all comparisons. At a cut-off value of 7.9 kPa, the sensitivity, specificity and positive and negative predictive values for F3 or greater disease were 91, 75, 52 and 97%, respectively (Table 1). LSM was not affected by hepatic steatosis, necro-inflammation, or BMI. Discordance of at least two stages between VCTE and histology was observed in 33 (13.4%) patients. By multivariate analysis, LB length less than 20 mm and F0-F2 disease were associated with discordance. Unsatisfactory LB specimens rather than VCTE accounted for most cases of discordance. However, when the diagnostic characteristics were compared with the inclusion of the subjects who had unsuccessful LSM, the sensitivity and specificity values were not dissimilar from the clinical/biochemical models.

A prospective study assessed the accuracy and the efficacy of VCTE for the detection of fibrosis in the patients with the liver disease of different aetiology (chronic hepatitis B - CHB, C- CHC and NAFLD) and evaluated the effect of steatosis on LSM.¹⁵ VCTE was performed in 219 consecutive patients with the chronic liver disease (35% CHC, 32% CHB, and 33% NAFLD) within 6 months of the liver biopsy. LSM was related to the fibrosis stage in each group:

- CHC: $P = 0.596$, $P < 0.001$.
- CHB: $P = 0.418$, $P < 0.001$.
- NAFLD: $P = 0.573$, $P < 0.001$.

But the correlation was less strong in the CHB and NAFLD than in CHC patients. The median LSM values were:

- 7 kPa (3.2-26) in the HCV patients ($n = 77$).
- 7.6 kPa (3.7-30.7) in the HBV patients ($n = 70$).
- 6.6 kPa (3.0-44.3) in the NAFLD patients ($n = 72$) compared to 4.35 kPa (range 2.6-7) in the controls ($n = 40$), ($P < 0.001$).

The median values of LSM according to the fibrosis stage evaluated by Metavir were in the 72 NAFLD patients:

- F0: 5.3 kPa (3.0-9.7).
- F1: 6.15 kPa (3.2-12.1).
- F2: 7.75 kPa (4.3-13.9).
- F3: 6.5 kPa (4.3-10.3).
- F4: 11.9 kPa (7.9-44.3), ($P = 0.001$).

VCTE underestimated the stage of fibrosis in 75% of patients with F3 and steatosis $> 33\%$. At the multiple logistic regression analysis, in the CHC and CHB patients, LSM was the only predictive variable of severe fibrosis/cirrhosis (odds ratio [OR] = 1.42, $P = 0.003$ and OR = 1.354, $P = 0.003$, respectively), while in the NAFLD subjects the BMI and transaminase levels (OR = 1.433, $P = 0.002$ and OR = 1.053, $P = 0.020$, respectively), but not LSM, were independently related with advanced fibrosis and cirrhosis. This study confirmed that VCTE could be considered a valid support to detect fibrosis in chronic liver disease related to HCV, but it should be interpreted cautiously in the CHB and NAFLD patients.

In a recent study, Mori, *et al.* confirmed the relationship between LSM and the Brunt score ($P = 0.000149$) in 36 NAFLD biopsy (Table 1).¹⁶ In addition, the authors showed that liver stiffness significantly correlated with the stage of fibrosis determined by the Sirius red-positive fibrotic area ($r = 0.390$, $P = 0.0184$) and α -smooth muscle actin-positive area ($r = 0.333$, $P = 0.0471$), respectively, the markers of collagen deposit and myofibroblasts presence.

Elastography assessment of NAFLD in obese patients

LSM can be influenced by metabolic syndrome even in the absence of biological features in NAFLD. It would be important to identify the rates and factors associated with unreliable LSM or LSM

failure, as the results may provide guidance to referral physicians regarding the optimal candidates for LSM. The knowledge would also assist clinicians to counsel patients before arranging LSM. The measurement is normally difficult for the obese patients because of the thick subcutaneous and perihepatic fat.

Studies have shown that BMI > 28 is an independent risk factor for a LSM failure.¹⁷ A prospective pilot study evaluated the new XL-probe in 99 obese patients (mean BMI 40.5 kg/m²).¹⁸ LSM was successful in 45% of the cases with M-probe, versus 76% of the cases with XL-probe (P < 0.001). 59% of those who could not be measured (< 10 valid measurements) using M-probe could successfully be measured using XL-probe. Both probes were successfully measured in 44 patients. LSM was correlated with the platelet count, prothrombin time, gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), fasting glucose, AST/platelet ratio index, Forns (combines age, GGT, cholesterol, and platelet count) and FIB-4 (combines age, AST, platelet count, and ALT) scores. XL-probe provided a higher rate of LSM than M-probe in the patients with an increased BMI and showed the promising results for the evaluation of liver fibrosis.

Wong, *et al.* have recently investigated the rates of unreliable LSM and LSM failure in the patients suffering from chronic liver diseases using the same procedure.¹⁹ They also aimed to evaluate the factors including BMI and central obesity associated with unreliable LSM and LSM failure. Among 3205 Chinese patients with LSM, 370 (12%) with liver steatosis, 371 (11.6%) and 88 (2.7%) had unreliable LSM and LSM failure, respectively. The rates started to increase when BMI \geq 28.0 kg/m². Comparing the patients with BMI \geq 28.0-29.9 kg/m² with those with BMI \geq 30.0 kg/m² the rates of unreliable LSM (16.4 vs. 18.9%; P = 0.62) and LSM failure (11.8 vs. 17.8%; P = 0.16) were similar. BMI \geq 28.0 kg/m² was the most important factor associated with unreliable LSM (OR = 2.9, 95% CI = 2.1-3.9, P < 0.0001) and LSM failure (OR = 10.1, 95% CI = 6.4-14.2, P < 0.0001). Central obesity, defined as a waist circumference > 80 cm in women and > 90 cm in men, was another independent risk factor of unreliable LSM (OR 1.3, 95% CI = 1.0-1.6, P = 0.04) and LSM failure (OR = 5.8, 95% CI = 2.9-11.5, P < 0.0001).

Finally, Petta, *et al.* evaluated the reliability of LSM in 169 NAFLD patients and the role of BMI.²⁰ Twenty-three patients (14%) failed to obtain 10 valid LSM acquisitions due to the higher BMI (P <

0.001), and showed a not-significantly different prevalence of significant (F2-F4) and severe (F3-F4) fibrosis (P = 0.20), evaluated by Kleiner score. A LSM value > 7.25 kPa was the best cut-off for predicting significant fibrosis at biopsy (AUC 0.794); however, this cut-off still failed to rule out F2-F4 fibrosis in 31% (false-negative rate) or rule in F3-F4 in 29% (false-positive rate). Similarly, a LSM value > 8.75 kPa was the best cut-off for severe fibrosis (F3-F4) (AUC 0.870) with a rate of false-negatives 24% and of false-positives 2%. The authors found that BMI values reduced the performance of LSM in evaluating the stage of fibrosis.

CAP approach to liver steatosis

Fat affects US propagation. US is the most common liver imaging technique used to detect the presence of steatosis. In B-mode images, steatosis appears as an increased parenchymal echogenicity caused by the reflectivity induced by fatty accumulation. Many studies have reported averaged specificity and sensitivity of this technique as between 60 and 95%. However, US can only detect steatosis from around 30% of fatty infiltration. Furthermore, it is, therefore, impossible to distinguish between increased echogenicity caused by an extensive fibrosis from increased echogenicity caused by fatty infiltration. However, a quantitative attenuation parameter can overcome this limitation.

Recently a new parameter has been developed to detect and quantify the degree of liver steatosis. This parameter is based on the US properties of the radiofrequency back propagated signals acquired by VCTE. It is called CAP because it is devised to target the liver specifically.²¹ This coefficient is an estimate of the total US (go-and-return path) at 3.5 MHz and is expressed in decibel per meter (dB.m⁻¹). CAP is evaluated using the same radio-frequency data and in the same region of interest used for LSM and is only appraised if the acquisition is valid. CAP is, therefore, VCTE guided, ensuring the operator obtains an ultrasonic attenuation value of the liver only. Therefore, CAP can be assessed by an operator who does not have any ultrasound imaging skills. Furthermore, CAP is designed to be an immediate, reproducible operator and machine-independent. The performance of CAP was evaluated by Sasso, *et al.* on 115 patients, taking the histological grade of steatosis as reference.²¹ Liver conditions were stratified in CHC (42), CHB (17), alcoholic liver disease (39) and NAFLD (17). The mean BMI was 25 \pm 4 kg/m². CAP was significantly correlated

to steatosis ($P = 10^{-16}$). AUROC was equal to 0.91 and 0.95 for the detection of more than 10 and 33% of steatosis, respectively. These data showed that CAP can efficiently separate several steatosis grades. CAP appears to be a promising diagnostic tool for non-invasive assessment and quantification of steatosis, enhancing the spectrum of the non-invasive method for the exploration and follow-up of the patients with fatty liver. However, the validation of this novel indicator is ongoing in a large multi-aetiology cohort study.

CONCLUSION

LB will still be a part of the clinical practice in the coming years, but the progress in medicine will challenge previously entrenched assumptions and will change our current approach to liver diseases in future. VCTE is a simple, non-invasive and inexpensive technique to assess LS, in particular, in the patients with CHC.²² In other highly prevalent diseases, such as NAFLD, there is still room for improvement.^{23,24}

A new statistical approach is needed in order to obtain reliable and clinically useful information from LSM.²⁵ In fact, most of the published studies on the NAFLD patients reported the cut-off values selected by binary measures, like sensitivity and specificity. This seems insufficiently informative to discriminate the optimal cut-off value in a wide range of values, especially in the context of liver steatosis. However, the data have shown that the degree of hepatic steatosis does not appear to affect liver stiffness, which is an important consideration with the adoption of transient elastography as a tool for fibrosis assessment in NAFLD. In addition, the recent studies on patients with chronic viral hepatitis have reported that the necro-inflammatory hepatic activity or transaminase levels contribute significantly to hepatic stiffness, interfering with the LSM.¹¹⁻¹⁵ To the contrary, in the NAFLD patients, this relation is not reported, probably due to the lower inflammatory grade observed in NAFLD than in viral hepatitis.¹⁹ In this setting, a high BMI represents the major determinant of diagnostic errors in predicting significant and severe fibrosis.

A new parameter, named CAP, has been developed to process the raw ultrasonic signals stored in the VCTE examination file. Performance of CAP has to be validated into specific etiologic groups. In addition, the influence of the steatosis topography and other histological features specific to each aetiology has to be considered. Large cohorts are required to determine

the appropriate threshold to detect and quantify steatosis. Furthermore, the development of new probes, as well as that for the obese patients, can broaden the utility of LSM in the clinical practice.

ABBREVIATIONS

- **NAFLD:** Non-alcoholic fatty liver disease.
- **NASH:** Non-alcoholic steatohepatitis.
- **T2DM:** Type 2 diabetes mellitus.
- **BMI:** Body mass index.
- **LB:** Liver biopsy.
- **US:** Ultrasound.
- **VCTE:** Vibration-controlled transient elastography.
- **LSM:** Liver stiffness measurement.
- **CAP:** Controlled attenuation parameter.
- **AUROC:** Area under the receiver-operating characteristics curve.
- **CI:** Confidence interval.
- **ALT:** Alanine aminotransferase.
- **CHB:** Chronic hepatitis B.
- **CHC:** Chronic hepatitis C.
- **GGT:** Gamma-glutamyltransferase.
- **AST:** Aspartate aminotransferase.
- **OR:** Odds ratio.
- **PPV:** Positive predictive value.
- **NPV:** Negative predictive value.

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