The performance of M and XL probes of FibroScan for the diagnosis of steatosis and fibrosis on a Brazilian nonalcoholic fatty liver disease cohort

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Objectives Recently, controlled attenuation parameter (CAP) was incorporated for XL probe. However, its performance through M and XL probes has been scarcely evaluated in nonalcoholic fatty liver disease (NAFLD). The performance of probes regarding transient elastography by Fibroscan is still under debate.

Aim Compare the performance of CAP and transient elastography in NAFLD patients obtained through XL with M probes using histological analysis as gold standard.

Methods NAFLD patients underwent liver biopsy and FibroScan/CAP with M and XL probes the same day. C-statistic evaluated CAP performance in the identification of moderate/severe (≥33%) and severe (≥66%) steatosis by both probes and transient elastography performance for identification of significant fibrosis (≥F2).

Results Eighty-one patients (74% female; age 54.2 ± 9.9 years; BMI 32.8 ± 5.2/ BMI ≥ 25 92.6%; 96% metabolic syndrome; 60% diabetes mellitus) were included. Mean CAP with M and XL probes was 314 ± 39 and 325 ± 47 dB/m, respectively. The areas under receiver operating characteristic curves (AUROCs) of the M and XL probes for steatosis detection ≥33% were 0.75 (0.64–0.84) and 0.76 (0.65–0.84) (P = 0.95) and for steatosis ≥66% 0.83 (0.73–0.90) and 0.82 (0.71–0.89) (P = 0.73), respectively, with similar performances for both degrees of steatosis. Regarding transient elastography, AUROCs of M and XL probes for ≥F2 were 0.82 (0.71–0.93) and 0.80 (0.69–0.92) (P = 0.66).

Conclusion Performance of M and XL probes is similar for the diagnosis of moderate and severe steatosis and significant fibrosis even on a overweight population with NAFLD. Eur J Gastroenterol Hepatol 2020: 231–238

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Western countries [1,2]. Patients with NAFLD are at an increased risk of more aggressive liver disease, nonalcoholic steatohepatitis (NASH) and at higher risk of death from cirrhosis, hepatocellular carcinoma (HCC) [3] and cardiovascular disease [4,5]. It should be noted that NAFLD is becoming a major cause of HCC in the United States and was associated with shorter survival time compared with other predisposing etiologies [6]. Although not necessary for the diagnosis of NASH [7], the presence of fibrosis is an important feature because most studies indicate that the fibrosis stage influences overall and liver-related mortality regardless of the presence or severity of other histological features [8–10].

Therefore, an accurate estimation of the degree of liver fibrosis is crucial for prognostication and clinical decision making [11]. Transient elastography (FibroScan, Echosens, Paris, France) has emerged as a popular noninvasive test of liver fibrosis with excellent results reported for the diagnosis of cirrhosis and advanced fibrosis in NAFLD/NASH [12–14]. Despite its usefulness in the management of liver disease, transient elastography also has limitations [15,16]. Studies have shown that obesity is the most important reason for failed and unreliable measurements [15,16]. However, NAFLD is strongly linked to obesity, with a prevalence of around 80% in obese individuals compared with only 16% in individuals with normal BMI and without metabolic risk factors [1,17]. Hence, an XL probe was developed to evaluate patients with an increased skin-liver length, a frequent finding in obese patients [18,19].

Recently, controlled attenuation parameter (CAP), a noninvasive tool for quantifying steatosis has been developed on the Fibroscan [20]. Steatosis quantification by CAP analyzes the ultrasound attenuation at the center frequency of the Fibroscan probe and is acquired at the same time of liver stiffness measurements (LSMs), with values ranging from 100 to 400 dB/m [20]. This method has several advantages: easy to perform, accurate and can identify even patients with low grades of steatosis [21–23].

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The evaluation of steatosis by CAP was initially available only for the M probe and lately was also incorporated for the XL probe [24]. Nonetheless, the performance of transient elastography and CAP through the XL probe has scarcely been evaluated, especially in a Western population exclusively with NAFLD [19,25–27]. Thereby, the aim of this study was to compare the diagnostic performance of transient elastography and CAP using M and XL probes in a population of overweight patients with histological diagnosis of NAFLD.

Methods

Study design and patients

This was a cross-sectional study with prospective inclusion conducted at the outpatient unit of Hepatology Service of the Hospital Universitário Clementino Fraga Filho of the Universidade Federal do Rio de Janeiro (Federal University of Rio de Janeiro) in Brazil. Individuals with 18 or more years of age and NAFLD diagnosis by ultrasonography whose clinicians requested a liver biopsy were included in this study.

HIV, hepatitis C virus and hepatitis B virus infected patients were excluded as well as those with other etiologies for chronic liver diseases. Patients using hepatotoxic drugs or therapy that could cause hepatic steatosis were excluded. Individuals with daily alcohol intake greater than: 20 g for women and 30 g for men were also excluded. In addition, subjects with a contraindication for liver biopsy were also excluded. The local Ethics Committee approved the study and all patients signed an informed consent form (ICF).

Study procedures

Individuals included in the study were submitted to clinical and laboratorial evaluation, liver stiffness and CAP measurements using FibroScan (by the same operator) and liver biopsy at the same day.

Demographic, clinical and laboratorial variables

Demographic (gender, age), anthropometric (BMI, weight, abdominal circumference), clinical (diagnosis of diabetes mellitus, presence of systemic arterial hypertension, hypercholesterolemia, hypertriglyceridemia) and laboratorial [alanine aminotransferase test (ALT), aspartate aminotransferase test, gammaglutamil transferase, total cholesterol and its fractions and triglycerides, blood glucose – including fasting glucose and glycosylated hemoglobin, platelet count) variables were registered.

Liver stiffness and controlled attenuation parameter measures

Liver stiffness (LSM) and CAP measurements were performed by the same experienced operator using Fibroscan 502 touch, whose technique was previously described [28,29]. The 3.5-MHz M and the 2.5-MHz XL probes were used for all patients in the same measurement point. The final liver stiffness result was expressed in kilopascals (kPa) and corresponded to the median value of 10 measurements performed between 25 and 65 mm depth or 35 and 75 mm depth, to M and XL probes, respectively. Only results with 10 valid shots, interquartile range/median liver stiffness ratio <30% and success rate >60% were included in the analysis. CAP was registered when there was a valid associated LSM using the same signals as the one used to measure liver stiffness [20]. Both liver stiffness and CAP were obtained simultaneously and in the same volume of liver parenchyma. The final CAP value was the median of individual CAP values and was expressed in dB/m.

Liver histopathology

Percutaneous liver biopsy was performed under ultrasound guided using 16-gauge diameter needles. Liver specimens shorter than 15 mm were excluded. An experienced physician obtained the fragments according to standard procedures. An independent pathologist, blinded to the study data, evaluated liver biopsy specimens. The NASH clinical research network Scoring system was applied to define fibrosis staging, the diagnosis of steatohepatitis and steatosis grading [30].

Statistical analysis

Clinical and laboratory data as well as liver stiffness and CAP final values and histopathology diagnosis were recorded in case report forms and entered in the SPSS 21.0 software (IBM Corp, Armonk, New York). All variable values were analyzed as continuous variables or were categorized when appropriate. Means, medians and other summary variables were calculated. Boxplots and graphs were constructed. Univariate analyses were performed using Chi square or Fisher exact test for categorical variables, and Student's *t* test or Mann–Whitney test for continuous variables as appropriate. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. The main analysis of this study was the evaluation of diagnostic performance of the two probes. Receiver operating characteristic curves were constructed to assess the overall accuracy of LSMs and to identify optimal cutoffs. The optimal cutoffs of moderate/severe, severe steatosis and significant fibrosis were chosen at the highest Youden's index based on cases with 10 valid measurements. The areas under receiver operating characteristic curves (AUROC) were compared by the method of DeLong *et al*. [31] using MedCalc18.6 (MedCalc Software, Ostend, Belgium). All reported *P* values are two sided. A *P* value of less than 0.05 was considered statistically significant.

Results

Study population

Eighty-five patients have agreed to participate in the study and have signed the ICF. All patients were submitted on the same day, to laboratorial evaluation, transient elastography through FibroScan® with M and XL probes and liver biopsy. Four patients were excluded due to unreliable measurements obtained with M probe (criteria's previously described).

Demographic and clinical characteristics of the 81 patients included in the study are shown in Table 1. The majority of patients were female (74%) with a mean age of 54.2 ± 9.9 years. The mean BMI was 32.8 ± 5.2 Kg/m^2. Only

7.4% of patients had a BMI under 25 and 69% of patients were obese. Ninety-six percent of subjects had metabolic syndrome. Only 24.6% of patients had elevated ALT. The distribution of fibrosis according to the histological analysis was: F0 ($n=32.1\%$), F1 ($n=49.3\%$), F2 ($n=6.2\%$), F3 ($n=6.2\%$) and F4 ($n=6.2\%$). Seventy-two percent had the diagnosis of NASH in histological analysis, and the distribution of the different grades of steatosis in the histological analysis was: S0 ($n=0\%$); S1 ($n=27.2\%$); S2 ($n=49.3\%$) and S3 ($n=23.5\%$). The characteristics of the patients according to the degree of hepatic steatosis observed in liver biopsy and the comparative analysis between the different degrees of steatosis and the clinical and laboratory variables are presented in Table 2. The majority of patients presented moderate steatosis (49.3%).

Table 1. Patients' clinical, demographic and laboratorial characteristics

Variables	*N*=81
Female gender (%)	74.0
White skin self-declared (%)	62.9
Age (years)	54.2±9.9
Weight (kg)	84.9±16.3
BMI (kg/m^2)	32.8±5.2
BMI<25 (kg/m^2) (%)	7.4
BMI≥25 (kg/m^2) (%)	92.6
Abdominal circumference (cm)	109.1±10.6
Diabetes (%)	60.0
SAH (%)	80.0
Metabolic syndrome (%)	96.1
Hypertrygliceridemia (%)	65.3
Hypercholesterolemia (%)	68.4
Glucose (mg/dL)	117±34
Glycosylated hemoglobin (%)	6.8±1.2
Cholesterol (mg/dL)	187±40
HDL (mg/dL)	42±11
LDL (mg/dL)	110±38
Triglycerides (mg/dL)	159±87
Uric acid (mg/dL)	5.4±1.5
ALT (U/L)	41 (16–179)
AST (U/L)	33 (28–108)
GGT (U/L)	50 (20–435)
Elevated ALT (%)	24.6
Elevated AST (%)	18.5
Elevated GGT (%)	40.9
Platelet count (×10^3)	247±62

Values are mean (SD) for normally distributed data or proportion for categorical data.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gammaglutamiltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SAH, systemic arterial hypertension.

Analysis of the controlled attenuation parameter performance with the M and XL probes in the diagnosis of hepatic steatosis

The mean CAP with the M probe was 314±39 and 325±47 dB/m with the XL probe (Table 3) ($P<0.01$). CAP, through the M and XL probes, presented progressively higher values in patients with mild, moderate and severe steatosis (Table 3 and Fig. 1).

For the analysis of the performance between the M and XL probes, the diagnoses of moderate/severe (S2/S3) and severe (S3) steatosis were considered because there were no patients in the sample without steatosis to evaluate the performance of CAP for the diagnosis of mild steatosis.

The performances of the M and XL probes for the detection of moderate/severe (S1 vs. S2S3) and severe steatosis (S1S2 vs. S3) were similar (Fig. 2a and b). The AUROC of CAP for diagnosis of moderate/severe steatosis for the M and XL probes was 0.75 (0.64–0.84) and 0.76 (0.65–0.84), respectively ($P=0.95$) (Fig. 2a). When

Table 2. Patients' clinical, demographic and laboratorialcharacteristics according to steatosis grade in liver biopsy (S1, S2 and S3) (*n*=81)

Variables	S1 (*n*=22; 27.2%)	SS2 (*n*=40; 49.3%)	S3 (*n*=19; 23.5 %)	*P* value
Female gender (%)	68.2	75.0	78.9	0.72
White skin self-declared (%)	50.0	70.0	63.2	0.29
Age (Years)	53.8±7.0	57.2±8.5	46.8±13.7	0.02[a]
Weight (kg)	85.2±17.5	83.8±15.9	89.4±17.9	0.28
BMI (kg/m^2)	33.1±5.6	32.1±4.5	36.6±5.2	0.025[b]
Abdominal circumference (cm)	108.7±11.2	108.4±10.4	113.1±11.8	0.032[c]
Diabetes (%)	54.5	61.5	63.1	0.82
SAH (%)	76.2	90.0	63.2	0.48
Metabolic syndrome (%)	90.9	97.4	100.0	0.27
Hypertrygliceridemia (%)	50.0	72.9	68.7	0.19
Hypercholesterolemia (%)	63.6	74.2	62.5	0.59
Glucose (mg/dL)	106±24	123±35	123±44	0.19
Glycosylated hemoglobin (%)	6.6±0.9	6.9±1.1	7.1±1.8	0.31
Cholesterol (mg/dL)	184±42	192±42	191±33	0.50
HDL (mg/dL)	42±12	43±10	43±12	0.97
LDL (mg/dL)	109±41	116±40	112±36	0.47
Triglycerides (mg/dL)	147±99	148±81	176±97	0.60
Uric Acid (mg/dL)	5.4±0.9	5.7±2.0	4.9±1.3	0.35
ALT (U/L)	38 (24–58)	41 (19–179)	72 (20–130)	0.036[d]
AST (U/L)	23 (14–68)	25 (13–58)	33 (14–77)	0.13
GGT (U/L)	72 (25–276)	48 (29–177)	92 (20–435)	0.10
Elevated ALT (%)	5.2	21.8	57.1	0.003
Elevated AST (%)	10.5	15.6	35.7	0.15
Elevated GGT (%)	35.0	28.1	78.5	0.005
Platelet Count (× 10^3)	237±55	252±68	257±77	0.46

Values are mean (SD) for normally distributed data or proportion for categorical data.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gammaglutamiltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SAH, systemic arterial hypertension.
[a]S2 vs. S3, $P=0.035$.
[b]S1 vs. S3, $P=0.047$; S2 vs. S3, $P=0.040$.
[c] S1 vs. S3, $P=0.038$.
[d]S1 vs. S3, $P=0.036$; S2 vs. S3, $P=0.048$.

Table 3. Elastography and controlled attenuation parameter results with M and XL probes according to steatosis grade (S1, S2 and S3)

Variables	*N* = 81	S1 (*n* = 22)	S2(*n* = 40)	S3 (*n* = 19)	*P* value
M probe					
Elastography (kPa)	8.0 (3.3–28.8)	6.3 (4.4–14.4)	7.1 (3.3–25.7)	10.9 (4.7–28.4)	0.72
IQR elastography (%)	14 ± 5	15 ± 6	14 ± 4	15 ± 7	0.13
CAP (dB/m)	314 ± 39	283 ± 30	317 ± 36	384 ± 28	<0.001
IQR CAP	8 ± 5	10 ± 7	8 ± 4	7 ± 3	0.33
XL probe					
Elastography (kPa)	6.6 (3.3–28.4)	6.1 (3.3–12.0)	6.1 (3.7–21.8)	8.2 (4.3–28.4)	0.15
IQR elastography (%)	13 ± 5	15 ± 5	13 ± 4	14 ± 7	0.93
CAP (dB/m)	325 ± 47	289 ± 43	334 ± 38	357 ± 30	< 0.001
IQR CAP	12 ± 6	13 ± 5	14 ± 7	10 ± 6	0.28

Values are mean (SD) for normally distributed data, median (minimun-maximun) for asymmetrically distributed data.
CAP, controlled attenuation parameter; IQR, interquartile range.

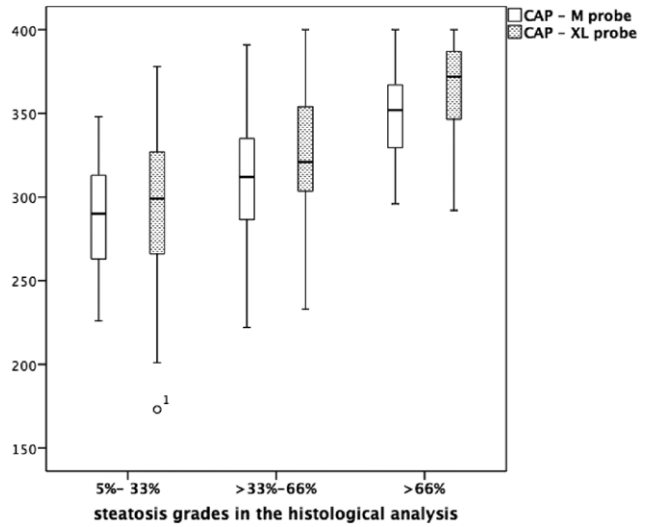


Fig. 1. CAP with M and XL probes according to steatosis grades in histological analysis. CAP, controlled attenuation parameter.

comparing CAP performance for the diagnosis of severe steatosis (S1S2 vs. S3), AUROC of 0.83 (0.73–0.90) and of 0.82 (0.71–0.89) for the M and XL probes, respectively, showed similar performance ($P = 0.73$) (Fig. 2b). In stratified analysis by gender, there was no statistically significant difference in performance for the diagnosis of moderate/severe and severe hepatic steatosis with M and XL probes. Cutoffs for M and XL probes for the diagnosis of moderate/severe steatosis were 298 and 291 dB/m, respectively. For the evaluation of severe steatosis, the best cutoffs for M and XL probes were 322 and 348 dB/m, respectively. The cutoff points found presented similar performances of sensitivity, specificity, positive and negative predictive values (Table 4).

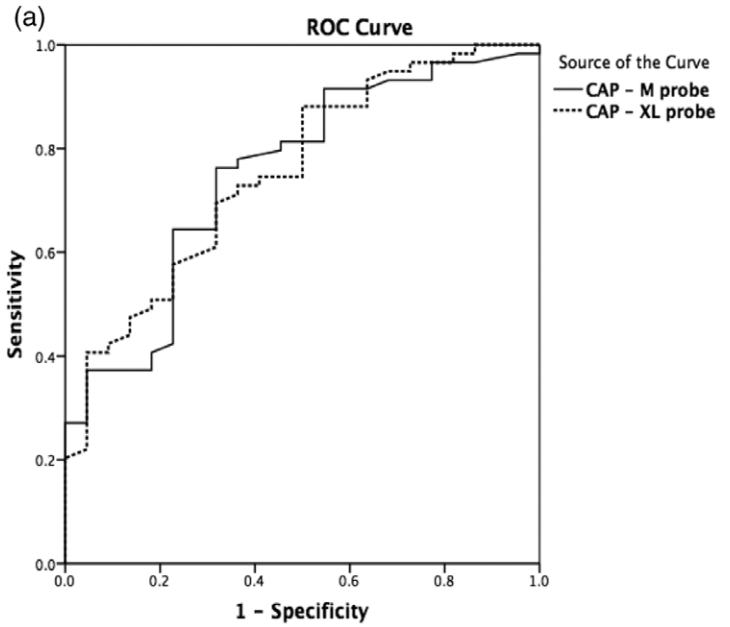
Analysis of the transient elastography performance with M and XL probes for diagnosis of significant fibrosis

The median of the liver stiffness with the M probe was 8.0 kPa (3.3–28.8 kPa) and 6.6 kPa (3.3–28.4 kPa) with the XL probe ($P < 0.001$) (Table 3). The performance of both probes for the diagnosis of significant fibrosis ($F \geq 2$) is shown in Fig. 3. The AUROC for the M probe of 0.82 (0.71–0.93) and for the XL probe of 0.80 (0.69–0.92) ($P = 0.66$).

We identified the transient elastography value of 8.2 kPa as the best cutoff point for discriminating significant fibrosis (F2 – F4) for both M and XL probes. Descriptions of sensitivity, specificity, positive predictive value, negative predictive values of cutoff points were presented in Table 5.

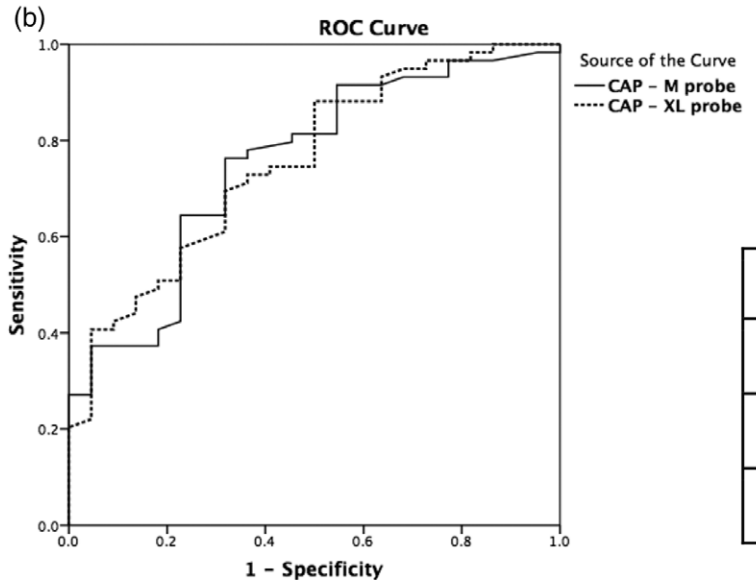
Discussion

This study evaluates the performance of transient elastography and CAP through the XL probe, comparing with the M probe, in a predominantly overweight population exclusively compound of patients with fatty liver disease and it has two important findings. First, CAP presented good accuracy with M and XL probes for the diagnosis of moderate/severe and severe steatosis in patients with NAFLD. Second, it demonstrates, similarly, that even in patients with high abdominal circumference and obesity, the accuracy of transient elastography with both probes for the diagnosis of significant fibrosis was good. CAP was recently incorporated in the XL probe. Only two previous studies show the comparison between CAP values obtained with both probes [26,27]. The first study addressing this issue was the study by de Lédinghen *et al.* [26]. In this study, 236 patients with different etiologies of liver diseases (20.8% NAFLD) were evaluated and the results obtained with both probes were compared. The M and XL probe showed similar performance for the diagnosis of mild ($P = 0.82$), moderate ($P = 0.63$) and severe ($P = 0.64$) steatosis. They also proposed the use of similar cutoff points for both probes. However, this study evaluated a heterogeneous population, with various etiologies for liver diseases. Only a fifth of the patients presented NAFLD. These patients are the ones of greater interest because they are in essence those with hepatic steatosis and metabolic syndrome. In our study, we evaluated only patients with NAFLD, a homogeneous population. Although we could not analyze the CAP performance for detecting mild steatosis, because in our sample we did not have patients without steatosis, the analysis for the diagnosis of moderate/severe and severe steatosis also presented similar accuracy. The performance of the M and XL probes for moderate/severe steatosis were similar (AUROC 0.75 M vs. 0.75 XL, $P = 0.95$) as well as for severe steatosis (AUROC 0.83 M vs. 0.82 XL, $P = 0.73$). The performance for the diagnosis of moderate/severe steatosis was regular with both probes. This may be explained by the difficult diagnosis of this intermediate grade of steatosis by noninvasive methods. For the diagnosis of severe steatosis, the performance of CAP in the present study was satisfactory, although slightly lower than that found in the previously mentioned article [26]. Of note, we may also consider the anthropometric profile



$P = 0.95$

Moderate/severe Steatosis (≥ 33%)	
Probe	AUROC (IC)
M	0.75 (0.64-0.84)
XL	0.76 (0.65–0.84)



$P = 0.73$

Severe Steatosis (≥ 66%)	
Probe	AUROC (IC)
M	0.83 (0.73-0.90)
XL	0.82 (0.71–0.89)

Fig. 2. (a) Performance of M and XL probes for the diagnosis of moderate/severe steatosis. (b) Performance of M and XL probes for the diagnosis of severe steatosis. AUROC, areas under receiver operating characteristic curve; CAP, controlled attenuation parameter.

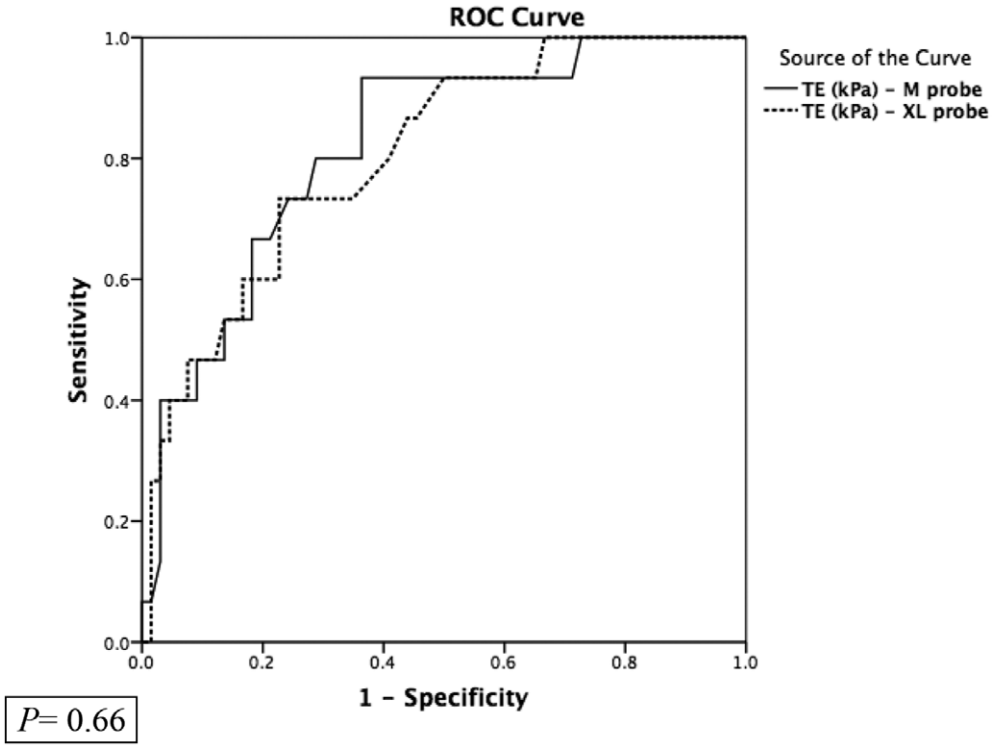
Table 4. Performance of M and XL probes according to steatosis grade (moderate/severe and severe)

Variables	Cutoff (dB/m)	Probes	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive predictive value (%)	Negative predictive value (%)
Steatosis grade						
Moderate/severe ($n = 59$)	298	M	76.2 (63.4–86.4)	68.1 (45.1–86.1)	86.5 (77.4–92.3)	51.7 (38.4–64.7)
	291	XL	88.1 (77.1–95.1)	50.0 (28.2–71.8)	82.5 (75.4–87.8)	61.1 (41.1–77.9)
Severe ($n = 19$)	322	M	89.4 (66.9–98.7)	72.5 (59.8–83.1)	50.0 (39.3–60.6)	95.7 (85.7–98.8)
	348	XL	73.6 (48.8–90.9)	79.0 (66.8–88.3)	51.8 (38.2–65.1)	90.7 (82.0–95.4)

CI, confidence interval.

of the population included in this study compounded of predominantly overweight/obese patients that might have contributed to a lower performance of both probes in the evaluation of moderate/severe steatosis, although still good for the evaluation of severe steatosis.

In 2018, Chan *et al*. [27] published a study with 180 patients, most of them with NAFLD (86.7%). The study applied the CAP cutoff points proposed by the meta-analysis of Karlas *et al*. [32] to the CAP measurements obtained with the XL probe. They concluded that these cutoffs could be used successfully in the analyzed population. Nevertheless, it is also a cohort with several etiologies, and principally, not submitted to transient elastography and liver biopsy on the same day. Thirty percent of the sample have been submitted to transient elastography with more than one month of difference, and of



F ≥ 2	
Probe	AUROC (IC)
M	0.82 (0.71-0.93)
XL	0.80 (0.69–0.92)

Fig. 3. Performance of M and XL probes for the diagnosis of significant fibrosis. AUROC, areas under receiver operating characteristic curve.

Table 5. Performance of M and XL probes for significant fibrosis ($F \geq 2$)

Variables	Cutoff (kPa)	Probes	AUROC	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive predictive value (%)	Negative predictive value (%)
Fibrosis stage							
Significant fibrosis ($F \geq 2$) $N = 15$	8.2	M	0.78 (0.68–0.86)	93.3 (68.1–99.8)	63.6 (50.9–75.1)	36.8 (29.2–45.2)	97.6 (86.2–99.6)
	8.2	XL	0.75 (0.64–0.84)	73.3 (44.9–92.2)	77.2 (65.3–86.7)	42.3 (29.9–55.7)	92.7 (84.5–96.7)

AUROC, areas under receiver operating characteristic curve; CI, confidence interval.

these subjects almost 20% had more than three months between the liver biopsy and transient elastography. NAFLD patients may change anthropometric characteristics quickly because they gain and lose weight sometimes on a short interval, leading to a rapid change in the pattern and, consequently, results of transient elastography and even liver biopsy. Thus, smaller intervals between transient elastography and histological evaluation, as we presented in this study, propitious more accurate results [33,34].

Another issue addressed in our study is the comparative performance of M and XL probes for the diagnosis of significant liver fibrosis in an exclusively NAFLD population. Few studies analyzed transient elastography results with XL probe compared with those found in liver biopsy [19,25,35,36]. In 2012, Wong *et al.* [25] demonstrated in a French-Chinese bicentric study that the XL probe had lower cutoff points than those previously identified with the M probe. A cutoff value of 8.2 kPa with the XL probe had 90% of specificity to rule in F2 disease in that study

and the cutoff point for XL probe for the settlement of significant liver fibrosis was 6.2 kPa. The cutoff point found in our study, for both probes, for the diagnosis of $F \geq 2$ was also 8.2 kPa. The sensibility was superior with both probes in comparison with Wong's results (M probe – 93%/XL probe – 73% vs. 57%). However, the specificity was lower in our study (M probe – 63%/XL probe – 77%). We believe that the definition of $F \geq 2$ cutoff points an important target. Patients with significant fibrosis are eligible to therapy (different to those with mild disease) and have greater risk to progressive liver disease [37]. Both probes presented similar performance for the diagnosis of $F \geq 2$, even in a predominant obese population. The fact that we can use the same cutoff for both probes simplifies daily practice. Another interesting point in the study of Wong *et al*. [25] is the fact that a large number of patients had failed transient elastography measurements with M probe (10%) besides only 2% with XL probe. These results unbalanced the intention-to-diagnosis analysis with the M probe, with AUROCs showing approximately 0.50 in the different fibrosis stages. Although in the present study we did not perform the intention-to-diagnosis analysis, we only lost 4% of our patients evaluated with the M probe. The AUROC for the diagnosis of $\underline{F} \geq 2$ was similar with the M and XL probes, even though in our population the median BMI was higher compared with Wong study (32.8 vs. 28.9 Kg/m^2).

In a German study with 50 patients with NAFLD [19], the diagnostic performance for the diagnosis of $F \geq 2$ for the M and the XL probes has also shown similar performance for both probes ($P = 0.68$) as we found in our study, with AUROCs of 0.80 and 0.82, respectively, for each probe. Therefore, despite the recommendation to use XL probe in patients with BMI above 30 Kg/m^2, we observed that, concerning fibrosis, the use of M probe even for obese patients when XL probe is not available would not impact on the accuracy of the results. In that study, the mean elastography results for M and XL probes were 8.4 kPa with M vs. 6.9 kPa with XL, very similar to what we found, 8.0 kPa with M probe and of 6.6 kPa with XL probe, even considering that in our study patients had a higher BMI compared to those included in the study by Friedrich-Rust *et al*. [19].

In our study, as previously mentioned, only four patients failed to perform transient elastography with M probe and were excluded. All the subjects obtained valid exams with XL probe. In the literature, four studies have reported the failure rates associated with the use of the M and the XL probes [25,36,38,39]. In a meta-analysis published in 2018 [40], the failure rate for LSM with the M probe was 10.1%. Comparing XL with the M probe, the first one had a lower risk of failure rate, as we found in our study.

This study has some limitations. Although it is a population with a high prevalence of steatosis, the prevalence of significant fibrosis or higher was low, this makes the definition of cutoff points for advanced liver fibrosis more fragile. In order to overcome this limitation, we chose to evaluate only the cutoff for significant fibrosis, which is usually linked to a progressive disease, needs intervention and a rigorous follow-up [37]. The absence of a group without steatosis is also a limitation because we could not perform the CAP analysis for detecting mild steatosis. However, the initial diagnosis of steatosis in the present study was performed by ultrasonography which sensitivity to mild degrees of steatosis is smaller compared to moderate and severe liver steatosis. Additionally, it is possible that the diagnosis of mild steatosis would not have a significant prognostic impact in NAFLD patients because in general patients with mild steatosis are considered as having a benign disease. In conclusion, this study showed that even on a population of predominantly overweight/obese patients with NAFLD both M and XL probes have shown good performances both for the diagnosis of moderate/severe and severe steatosis and also for the diagnosis of significant fibrosis.

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Conflicts of interest

There are no conflicts of interest.

References

1 Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, *et al*. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; 140:124–131.

2 Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; 64:73–84.

3 Ioannou GN, Green P, Lowy E, Mun EJ, Berry K. Differences in hepatocellular carcinoma risk, predictors and trends over time according to etiology of cirrhosis. *Plos One* 2018; 13:e0204412.

4 Volzke H, Robinson DM, Kleine V, Deutscher R, Hoffmann W, Ludemann J, *et al*. Hepatic steatosis is associated with an increased risk of carotid atherosclerosis. *World J Gastroenterol* 2005; 11:1848–1853.

5 Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, *et al*. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007; 30:1212–1218.

6 Younossi ZM, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, *et al*. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015; 62:1723–1730. doi: 10.1002/hep.28123.

7 Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. *Semin Liver Dis* 2001; 21:3–16.

8 Younossi ZM, Stepanova M, Rafiq N, Makhlouf H, Younoszai Z, Agrawal R, Goodman Z. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. *Hepatology* 2011; 53:1874–1882.

9 Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, *et al*. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2015; 149:389–397. e10.

10 Ekstedt M, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, Hultcrantz R. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015; 61:1547–1554.

11 de Franchis R, Dell'Era A. Non-invasive diagnosis of cirrhosis and the natural history of its complications. *Best Pract Res Clin Gastroenterol* 2007; 21:3–18.

12 Wong GL, Wong VW, Choi PC, Chan AW, Chum RH, Chan HK, *et al*. Assessment of fibrosis by transient elastography compared with liver

biopsy and morphometry in chronic liver diseases. *Clin Gastroenterol Hepatol* 2008; 6:1027–1035.
13 Yoneda M, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, *et al*. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Liver Dis* 2008; 40:371–378.
14 Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, *et al*. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; 51:454–462.
15 Castéra L, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, *et al*. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. *Hepatology* 2010; 51:828–835.
16 Wong GL, Wong VW, Chim AM, Yiu KK, Chu SH, Li MK, Chan HL. Factors associated with unreliable liver stiffness measurement and its failure with transient elastography in the Chinese population. *J Gastroenterol Hepatol* 2011; 26:300–305.
17 Bellentani S, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F, *et al*. Prevalence of and risk factors for hepatic steatosis in northern Italy. *Ann Intern Med* 2000; 132:112–117.
18 de Lédinghen V, Vergniol J, Foucher J, El-Hajbi F, Merrouche W, Rigalleau V. Feasibility of liver transient elastography with fibroscan using a new probe for obese patients. *Liver Int* 2010; 30:1043–1048.
19 Friedrich-Rust M, Hadji-Hosseini H, Kriener S, Herrmann E, Sircar I, Kau A, *et al*. Transient elastography with a new probe for obese patients for non-invasive staging of non-alcoholic steatohepatitis. *Eur Radiol* 2010; 20:2390–2396.
20 Sasso M, Beaugrand M, de Ledinghen V, Douvin C, Marcellin P, Poupon R, *et al*. Controlled attenuation parameter (CAP): a novel VCTE™ guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: preliminary study and validation in a cohort of patients with chronic liver disease from various causes. *Ultrasound Med Biol* 2010; 36:1825–1835.
21 Sasso M, Tengher-Barna I, Ziol M, Miette V, Fournier C, Sandrin L, *et al*. Novel controlled attenuation parameter for noninvasive assessment of steatosis using fibroscan(®): validation in chronic hepatitis C. *J Viral Hepat* 2012; 19:244–253.
22 de Lédinghen V, Vergniol J, Foucher J, Merrouche W, le Bail B. Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography. *Liver Int* 2012; 32:911–918.
23 Cardoso AC, Beaugrand M, de Ledinghen V, Douvin C, Poupon R, Trinchet JC, *et al*. Diagnostic performance of controlled attenuation parameter for predicting steatosis grade in chronic hepatitis B. *Ann Hepatol* 2015; 14:826–836.
24 Sasso M, Audière S, Kemgang A, Gaouar F, Corpechot C, Chazouillères O, *et al*. Liver steatosis assessed by controlled attenuation parameter (CAP) measured with the XL probe of the fibroscan: a pilot study assessing diagnostic accuracy. *Ultrasound Med Biol* 2016; 42:92–103.
25 Wong VW, Vergniol J, Wong GL, Foucher J, Chan AW, Chermak F, *et al*. Liver stiffness measurement using XL probe in patients with nonalcoholic fatty liver disease. *Am J Gastroenterol* 2012; 107:1862–1871.
26 de Lédinghen V, Hiriart JB, Vergniol J, Merrouche W, Bedossa P, Paradis V. Controlled attenuation parameter (CAP) with the XL probe of the fibroscan®: a comparative study with the M probe and liver biopsy. *Dig Dis Sci* 2017; 62:2569–2577.
27 Chan WK, Nik Mustapha NR, Mahadeva S, Wong VW, Cheng JY, Wong GL. Can the same controlled attenuation parameter cut-offs be used for M and XL probes for diagnosing hepatic steatosis? *J Gastroenterol Hepatol* 2018; 33:1787–1794.
28 Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, *et al*. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; 29:1705–1713.
29 de Lédinghen V, Vergniol J. Transient elastography (fibroscan). *Gastroenterol Clin Biol* 2008; 32:58–67.
30 Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, *et al*.; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; 41:1313–1321.
31 DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; 44:837–845.
32 Karlas T, Petroff D, Sasso M, Fan JG, Mi YQ, de Lédinghen V, *et al*. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. *J Hepatol* 2017; 66:1022–1030.
33 Cassidy S, Thoma C, Hallsworth K, Parikh J, Hollingsworth KG, Taylor R, *et al*. High intensity intermittent exercise improves cardiac structure and function and reduces liver fat in patients with type 2 diabetes: a randomised controlled trial. *Diabetologia* 2016; 59:56–66.
34 Horowitz JF, Ortega JF, Hinko A, Li M, Nelson RK, Mora-Rodriguez R. Changes in markers for cardio-metabolic disease risk after only 1-2 weeks of a high saturated fat diet in overweight adults. *Plos One* 2018; 13:e0198372.
35 Myers RP, Pomier-Layrargues G, Kirsch R, Pollett A, Duarte-Rojo A, Wong D, *et al*. Feasibility and diagnostic performance of the fibroscan XL probe for liver stiffness measurement in overweight and obese patients. *Hepatology* 2012; 55:199–208.
36 de Lédinghen V, Wong VW, Vergniol J, Wong GL, Foucher J, Chu SH, *et al*. Diagnosis of liver fibrosis and cirrhosis using liver stiffness measurement: comparison between M and XL probe of fibroscan®. *J Hepatol* 2012; 56:833–839.
37 European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; 64:1388–1402.
38 Friedrich-Rust M, Romen D, Vermehren J, Kriener S, Sadet D, Herrmann E, *et al*. Acoustic radiation force impulse-imaging and transient elastography for non-invasive assessment of liver fibrosis and steatosis in NAFLD. *Eur J Radiol* 2012; 81:e325–e331.
39 Cassinotto C, Lapuyade B, Aït-Ali A, Vergniol J, Gaye D, Foucher J, *et al*. Liver fibrosis: noninvasive assessment with acoustic radiation force impulse elastography–comparison with fibroscan M and XL probes and fibrotest in patients with chronic liver disease. *Radiology* 2013; 269:283–292.
40 Xia B, Wang F, Friedrich-Rust M, Zhou F, Zhu J, Yang H, *et al*. Feasibility and efficacy of transient elastography using the XL probe to diagnose liver fibrosis and cirrhosis: a meta-analysis. *Medicine (Baltimore)* 2018; 97:e11816.