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Diagnostic Accuracy of Controlled Attenuation Parameter Measured by Transient Elastography for the Non-invasive Assessment of Liver Steatosis: a Prospective Study

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ABSTRACT

Background & Aims: A novel non-invasive tool based on the evaluation of ultrasound attenuation using transient elastography (TE) has been developed, called controlled attenuation parameter (CAP). We aim to establish the histopathological parameters that significantly influence CAP, the cutoff values and their performance in predicting each steatosis grade on a group of biopsied patients with chronic liver diseases (CLD) from Romania.

Methods. We prospectively analyzed 201 consecutive CLD patients who underwent CAP measurements using TE. Steatosis, liver fibrosis and necroinflammatory activity were staged and graded during the pathological analysis of bioptic specimens. Univariate and multivariate regression analyses were applied to identify the variables correlated with CAP values. The diagnostic performance of CAP for steatosis prediction was assessed using an AUC analysis.

Results. Among the histopathological factors correlating with CAP, the multivariate analysis found steatosis as the only factor independently influencing CAP values ($p<0.001$). Maximal diagnostic accuracy (DA) was obtained for the prediction of ≥34-66% (S2) fatty load and of 67-100% (S3) fatty load (82.06%, respectively 81.59%) while, for the prediction of ≥11-33% (S1) fatty load, DA reached only 76.11%. The negative predictive value for the exclusion of ≥S2 and S3 was 93.5% and 98.7%, respectively. AUCs calculated between each two steatosis grades were: 0.772 (S0 vs S1), 0.874 (S0 vs S2), 0.904 (S0 vs S3), 0.659 (S1vs S2), 0.777 (S1 vs S3), and 0.665 (S2 vs S3).

Conclusion. Steatosis is the only histopathological factor independently influencing CAP. Maximal DA could be obtained for the prediction of ≥S2 and S3 (82.06% and 81.59%), while for the prediction of S1, the accuracy reached only 76.11%.

Key words: steatosis – noninvasive – Fibroscan – vibration-controlled transient elastography – controlled attenuation parameter (CAP).

Abbreviations: AUC: area under the ROC curve; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CAP: controlled attenuation parameter; CLD: chronic liver disease; DA: diagnostic accuracy; dB/m - decibel per meter; GGT: gamma-glutamyl-transpeptidase; IQR: interquartile range; LB: liver biopsy; LR: likelihood ratio; LS: liver stiffness; NAFLD: non-alcoholic fatty liver disease; ROC: receiver operating characteristic; SR: success rate; TE: transient elastography.

INTRODUCTION

Steatosis is a frequent histological finding in patients with chronic liver diseases (CLD) [1, 2]. It may progress towards fibrosis, cirrhosis, and hepatocellular carcinoma [3], it reduces the likelihood of sustained virological response in HCV patients [4] and, in patients undergoing liver resection, it independently increases the risk of postoperative complications and death [5]. In the setting of liver transplantation, the presence of macrovesicular steatosis in the graft increases the 1-year risk of graft failure [6]. Finally, steatosis is the primary lesion in non-alcoholic fatty liver disease (NAFLD), the main cause of CLD in western countries [7]. Therefore, an accurate method to detect and quantify steatosis would be extremely useful and it has been the subject of much research in the latter years.

The current „gold standard" for evaluating steatosis is liver biopsy (LB), but it is invasive and may result in severe complications [8]. Furthermore, LB has potential sampling errors and cannot be readily repeated for adequate patient follow-up [9].

The non-invasive diagnosis of steatosis (especially blood tests and imaging techniques) has been extensively developed in the last decade, but it is not yet recommended for use in clinical practice [10]. Blood tests have been implemented for binary diagnosis or quantification of steatosis, but their diagnostic accuracy remains to be validated [11]. Computed tomography detects extensive steatosis (>30% of all hepatocytes affected), but a more refined quantification is impossible [12]; furthermore, this technique is irradiating [13]. MRI, especially proton MRI spectroscopy, is the most reliable method for quantification of fatty liver disease, with 82-97.4% sensitivity and 76.1-95.3% specificity [13], but the examination and the image-processing procedure tend to be complex [12]. In addition, it is too expensive for regular use and is therefore used mainly in clinical trials [12, 14, 15]. Ultrasonography is considered the imaging technique of choice for steatosis screening given its low cost, safety and wide availability [16], but it is operator-dependent [14] and it detects steatosis only if it involves at least 20% of all hepatocytes [17]. The method cannot establish with certainty the degree of fatty infiltration and cannot accurately discriminate steatosis from fibrosis, since both result in increased liver echogenity [18]. The ultrasonographic hepatorenal index was used in an attempt to grade steatosis [19], but it is cumbersome and time-consuming and requires specialized software.

To overcome these limitations, a novel non-invasive tool based on the evaluation of ultrasound attenuation using the Fibroscan® device (Echosens, Paris, France) has been developed, using a novel proprietary algorithm called controlled attenuation parameter (CAP) [20]. This parameter is an estimate of the total ultrasonic attenuation (go-and-return path) at the central frequency of the regular or M probe of the Fibroscan® (3.5 MHz) and is expressed in decibel per meter (dB/m). CAP is evaluated using the same radio-frequency data and the same region of interest as the region used to assess liver stiffness (LS) [21].

Since the development of this method, some studies have been performed on patients with various diffuse liver diseases [20, 22-30]. Among histopathological parameters, these studies analyzed mainly the influence of steatosis and fibrosis on CAP and, in some studies, also that of necroinflammatory activity. To our knowledge, only one study, performed on NASH patients, included the influence of lobular inflammation and ballooning on CAP, apart from that of steatosis and fibrosis [31]. Until the present day, no study has yet analysed the concomitant influence of all histopathological parameters (steatosis, fibrosis, necroinflammatory activity, ballooning) which independently influence the CAP value and its performance in predicting each steatosis grade on biopsied patients with CLD from Romania.

The purpose of our study was to establish all the histopathological parameters that significantly influence CAP (steatosis, fibrosis, necroinflammatory activity, ballooning–quantified according to liver disease etiology), the CAP cutoff values and their performance in predicting each steatosis grade on a group of prospectively followed biopsied patients with CLD from Romania.

PATIENTS AND METHODS

Patients

Two hundred and one consecutive patients with different diffuse CLDs (viral hepatitis C, viral hepatitis B, non-alcoholic steatohepatitis, primary biliary cirrhosis, autoimmune hepatitis) examined in our department between January 2012 and June 2014 were prospectively included in this study. All of them underwent percutaneous liver biopsy for disease grading and staging. All patients were referred for CAP measurement, one day prior to liver biopsy.

Apart from the epidemiological data, the following biological parameters were determined for all patients on the same day as the CAP measurements: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transpeptidase (GGT), total bilirubin, alkaline phosphatase, platelet count, fasting serum glucose, cholesterol and triglycerides.

The exclusion criteria were: the evidence of ascites at physical or ultrasound examination (ascites is a physical limitation of the technique because elastic waves do not propagate through liquids) [32], other conditions associated with severe cholestasis or right heart failure, proven to influence the LS value [33, 34], pregnancy, malignancy or other terminal disease, and a LB unsuitable for steatosis grading (when the LB specimen contained <6 portal tracts).

The study was approved by the local Ethical Committee of our University (no. 464/2011 and 98/2014). The nature of the study was explained to the patients. Informed signed consent was obtained from each patient before the beginning of the study, in accordance to the principles of the Declaration of Helsinki (Edinburgh revision 2000).

CAP measurements using transient elastography

CAP has been designed to measure the liver ultrasonic attenuation (go and return path) at 3.5 MHz using signals acquired by the Fibroscan. CAP uses a sophisticated guidance process based on Vibration-Controlled Transient Elastography. The principles of CAP measurement have been described elsewhere [20].

All CAP measurements were performed using the Fibroscan® device by an experienced operator (MLP), who had long-term experience in TE measurements as well as in the ultrasonographic examination of the abdomen and had performed more than 8,000 TE measurements. The measurements were made after an overnight fast using the 3.5 MHz M probe, on which the CAP measurement software was installed [35].

During the acquisition, patients were positioned in a dorsal decubitus position, with the right arm in maximum abduction. The Fibroscan transducer, covered with a drop of coupling gel, was placed perpendicularly on the intercostal space. Under TM and A-mode control, the operator chose a liver zone within the right lobe, free from any large vascular structure or the gallbladder. Then, the operator pressed the probe button to commence the measurement. The final CAP value

considered for analysis was the median of 10 individual CAP values, regardless of the success rate (SR), and was expressed in dB/m.

CAP was computed by the Fibroscan equipment software in an area located between 25 and 65 mm from the skin and in the same region the biopsy specimen was taken from, in order to grade and stage the disease.

Histological study

Liver biopsy was performed using the TruCut technique with a 1.8 mm (14G) diameter automatic needle device – Biopsy Gun (Bard GMBH, Karlsruhe, Germany). The specimens were fixed in formalin and embedded in paraffin. Only biopsy specimens with more than 6 intact portal tracts were eligible for evaluation [36, 37].

Liver fibrosis stage and necroinflammatory activity grade were evaluated according to the Metavir scoring system in all patients, except those with NASH, evaluated according to the Brunt system [37, 38].

Fibrosis was staged on a 0-4 scale: F0–no fibrosis; F1–portal fibrosis without septa; F2-portal fibrosis and few septa; F3–numerous septa without cirrhosis; F4–cirrhosis (Metavir score) or F0–no fibrosis, F1–zone 3 perisinusoidal fibrosis, F2–as above with portal fibrosis, F3–as above with bridging fibrosis and F4–cirrhosis (Brunt score in NASH). Necroinflammatory activity was graded as: A0–none; A1–mild; A2–moderate; A3–severe. 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.

In all patients, steatosis was estimated by visual assessment as a percentage of hepatocytes with fatty accumulation; to be able to pool all patients, steatosis was converted into the following grading system: S0: steatosis in less than 10% of hepatocytes, S1: 11%-33%, S2: 34%-66% and S3: 67%-100% of hepatocytes, a system which was also used in other studies [12, 20, 22, 24, 26, 27]. The histological type of steatosis was specified, as pure macrovesicular, mixed (i.e. macrovesicular and microvesicular) or pure microvesicular [39].

For the purpose of the present study, all LBs were analyzed by the same experienced liver pathologist who was blinded to the results of CAP.

Statistical analysis

The statistical analysis was performed using the SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA) and MedCalc software version 12.4.0 (Mariakerke, Belgium). Categorical variables were presented as numbers and percentages and were compared using the $\chi 2$ test. The continuous variables were expressed as mean value, standard deviation, median and range. Box plots were used to estimate the CAP distributions between each steatosis grade. The differences between two continuous variables were analyzed with the Mann-Whitney test and the differences between more than two independent groups were tested with the Kruskal-Wallis test. The relationships between CAP and different histological parameters were characterized using the Spearman correlation coefficients. Variables reaching statistical significance ($p<0.05$) in univariate analysis were included into a multivariate logistic regression analysis in order to identify those that were independently associated with a certain factor. The diagnostic performance of CAP for steatosis prediction was assessed using sensitivity (Se), specificity (Sp), positive (PPV) and negative (NPV) predictive values, likelihood ratios (LR), accuracy (DA) and receiver operating characteristic (ROC) curves. The optimal CAP cut-off values were defined by maximizing the sum of sensitivity and specificity. To determine the ability of CAP to differentiate between individual steatosis grades, we also calculated AUCs between two steatosis grades only (e.g. S0 vs. S1, excluding patients with S2–S3). AUC values were interpreted as follows: 0.90-1.0 = excellent; 0.80-0.90 = good; 0.70-0.80 = fair; <0.70 = poor.

RESULTS

Baseline characteristics of patients

Of the 201 patients in the study, 118 (58.7%) had been diagnosed with HCV hepatitis, 48 (23.88%) with HBV hepatitis, 24 (23.88%) with NASH and 11 (5.47%) with other diffuse CLD (primary biliary cirrhosis, autoimmune hepatitis). The majority of patients were female (61.2%), with a median age of 51 years. The median size of the liver biopsy specimens was 15 (12-20) mm, with a median of 14 (10-22) portal spaces. The clinical, biochemical and histopathological characteristics of the patients are presented in Table I. No patient had a history of alcohol consumption (i.e. >30 g/day in men and >20 g/day in women).

In our patients, CAP values varied between 124-377 dB/m (median 241). No complications occurred during or after examination. The success rate of CAP measurements varied between 9 and 100% (median 90%). In 9.5% of cases, the success rate (SR) was <60%, but 10 valid measurements were nevertheless recorded. These patients had a higher body mass index (BMI) than the patients with SR ≥60% [29.76 (23.95-42.86) kg/m^2 versus 25.78 (16.41-36.41) kg/m^2, $p<0.0001$], while the rest of the parameters (age, sex, glucose, AST, ALT, alkaline phosphatase, GGT, total bilirubin, cholesterol and triglycerides) did not differ significantly. The mean value of the interquartile range (IQR) of CAP measurements was 0.15 (0-0.30).

Correlation between CAP and histopathological parameters

CAP correlated significantly only with steatosis ($r=0.568$, $p<0.0001$) and the steatosis type ($r=0.235$, $p=0.02$). No correlation was found between CAP and fibrosis ($r=0.019$, $p=0.8$ according to the Metavir scoring system, $r=0.158$, $p=0.5$ according to the Brunt scoring system in NASH patients, respectively), activity ($r=0.003$, $p=0.9$), ballooning ($r=0.408$, $p=0.09$) or lobular inflammation ($r=0.034$, $p=0.9$). Among the histopathological factors correlating with CAP, the multivariate analysis showed that only steatosis influences independently CAP in CLD patients ($p<0.0001$), but not the steatosis type ($p=0.0889$).

CAP performance in the assessment of steatosis in chronic liver diseases

The distribution of CAP values for each steatosis grade is represented in Fig. 1. The median (range) CAP values (dB/m)

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

Patients' characteristics	Value	
	Mean	Median (range)
*Sex (female)	123 (61.2%)	
Age (years)	49.61±10.98	51 (20-74)
BMI (kg/m^2)	26.44±4.55	26.44 (16.41-42.86)
AST (U/L)	52.79±55.12	37 (12-401)
ALT (U/L)	62.15±88.46	42 (4-725)
GGT (U/L)	68.71±74.83	42 (14-499)
†Total bilirubin (mg/dL)	0.74±0.40	0.62 (0.11-2.71)
Alkaline phosphatase (U/L)	210.50±121.74	176.5 (90-1021)
Glucose (mg/dL)	99.30±25.13	93.1 (59-294)
Cholesterol (mg/dL)	190.87±58.01	178 (79-493)
Triglycerides (mg/dL)	125.49±61.70	109.5 (33-368)
Platelet count (10^9/L)	233.46±61.70	226 (21.8-566)
CAP (dB/m)	243.86±55.56	241 (124-377)
*Fibrosis stage (Metavir)		
F0	17 (9.60%)	
F1	60 (33.89%)	
F2	49 (27.68%)	
F3	32 (18.07%)	
F4	19 (10.73%)	
*Necroinflammatory activity (Metavir):		
A0	7 (4.3%)	
A1	47 (12.8 %)	
A2	97 (52.1 %)	
A3	52 (30.8 %)	
*Steatosis:		
S0 (≤10%)	110 (54.7 %)	
S1 (11-33%)	58 (28.6 %)	
S2 (34-65%)	21 (10.3 %)	
S3 (≥66%)	12 (5.9 %)	
*Fibrosis stage (Brunt):		
F0	4 (16.66%)	
F1	12 (50%)	
F2	6 (25%)	
F3	2 (8.33%)	
*Ballooning (Brunt):		
B0	4 (16.66%)	
B1	11 (45.83%)	
B2	8 (33.33%)	
B3	1 (4.16%)	
*Lobular inflammation (Brunt):		
LI 0	2 (8.33%)	
LI 1	14 (58.33%)	
LI 2	5 (20.83%)	
LI 3	3 (12.5%)	

Note. Unless otherwise indicated, data are mean ±standard deviation and median, with ranges in parentheses. *Data are numbers of patients, with percentages in parentheses. †To convert to Systéme International units (micromoles per liter), multiply by 17.1. CAP: Controlled Attenuation Parameter, BMI: body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl-transpeptidase

according to the steatosis grades were: 212 (124-359) for S0; 266 (153-353) for S1; 304 (215-359) for S2 and 321 (218-377) for S3. The differences were statistically significant between all the steatosis grades, except S2 vs S3 (Fig. 1).

Figure 2 shows the ROC curves according to three different steatosis grade thresholds: AUCs were 0.813 for ≥S1, 0.822 for ≥S2 and 0.838 for S3. Table II shows the optimal cut-off values as well as the corresponding sensibility, specificity, positive and negative predictive values. Maximal DA could be obtained for the prediction of ≥S2 and S3 (82.06% and 81.59%, respectively), while for the prediction of grades ≥S1, the accuracy reached only 76.11%.

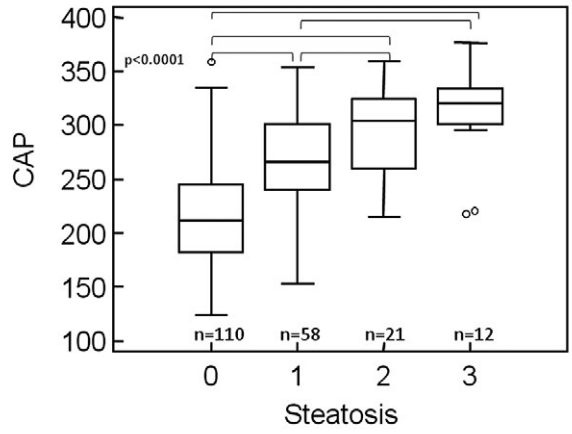

Fig. 1. Box plots of Controlled Attenuation Parameter values for each steatosis grade. The top and the bottom of the boxes are the first and third quartiles, respectively. The length of the box represents therefore the interquartile range including 50% of the values. The line through the middle of each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

Table II. CAP cut-off values for the diagnosis of steatosis grades ≥S1, ≥ S2 and ≥S3

	≥ S1 (S0 vs S123)	≥ S2 (S01vs S23)	≥ S3 (S012 vs S3)
CAP cutoff value (dB/m)	260	285	294
Se (95% CI) (%)	64.84 (54.1-74.6)	69.70 (51.3-84.4)	83.33 (51.6-97.9)
Sp (95% CI) (%)	87.27 (79.6-92.9)	85.12 (78.8-90.1)	82.54 (76.4-87.7)
+LR (95% CI)	5.09 (3.1-8.5)	4.68 (3.1-7.2)	4.77 (3.2-7.1)
-LR (95% CI)	0.40 (0.3-0.5)	0.36 (0.2-0.6)	0.20 (0.06-0.7)
PPV (95% CI) (%)	80.8 (69.9-89.1)	47.9 (33.3-62.8)	23.3 (11.8-38.6)
NPV (95% CI) (%)	75 (66.5-82.3)	93.5 (88.3-96.8)	98.7 (95.5-99.8)
AUC (95% CI)	0.813 (0.75-0.86)	0.822 (0.76-0.87)	0.838 (0.78-0.88)
DA (%)	76.11	82.08	81.59

Note: Se=sensitivity, Sp=specificity, PPV=positive predictive values, NPV=negative predictive values, LR=likelihood ratios, DA=diagnostic accuracy, AUC= area under the receiver operating characteristic curves

AUCs calculated between each two steatosis grades were: 0.772 (for S0 vs S1), 0.874 (S0 vs S2), 0.904 (S0 vs S3), 0.659 (S1 vs S2), 0.777 (S1 vs S3), and 0.665 (S2 vs S3), respectively.

DISCUSSION

This study confirms, on a series of Romanian patients, the preliminary results of previous studies [20, 22-31], namely that, among all histopathological parameters assessed during various diffuse liver diseases, CAP is independently influenced only by the amount of steatosis, not by fibrosis, necroinflammatory activity, ballooning or lobular inflammation (quantified according to liver disease etiology). The CAP value increased alongside the increase in the steatosis degree. Despite some overlap in adjacent steatosis grades, the overall differences between any two steatosis grades were statistically significant, except S2 vs S3, finding which is also confirmed by other authors [23-25, 40, 41]. Moreover, this situation is also

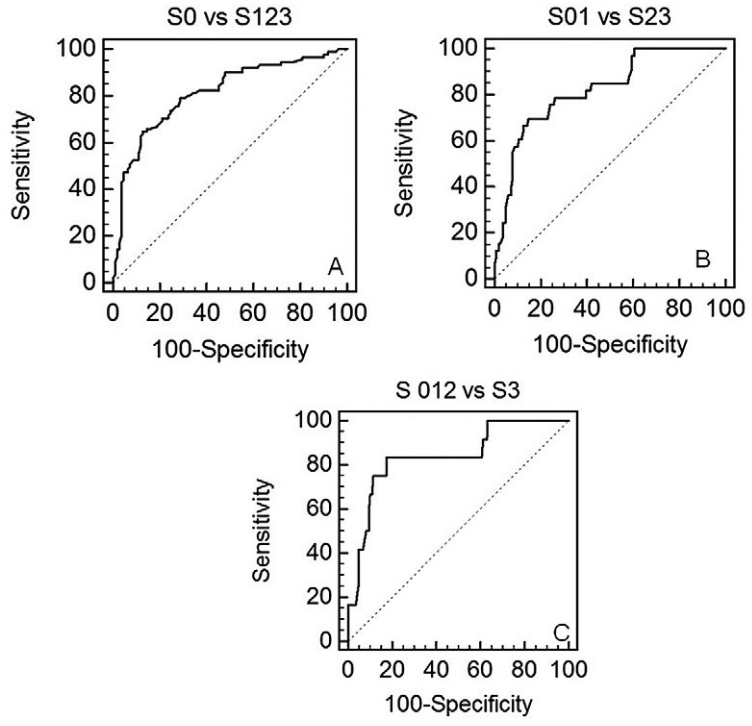

Fig. 2. The ROC curves for CAP for different steatosis thresholds: A: S0 vs S1-S3 (cut-off value 260 dB/m); B: S0-S1 vs S2-S3 (cut-off value 285 dB/m); C: S0-S2 vs S3 (cut-off value 294 dB/m).

encountered when quantifying steatosis using ^{1}H-magnetic resonance spectroscopy [41], which raises the suspicion of bias on steatosis quantification on LB for those grades.

The CAP values in our study are in range with those published so far [20-31, 40, 41]; however, there is a lack of agreement on histological quantification for early steatosis in various studies. In a meta-analysis assessing the CAP accuracy for steatosis detection [12], the median optimal CAP cut-off values were 232.5 dB/m, 255 dB/m and 290 dB/m for ≥S1, ≥S2 and S3, respectively, and the summarized sensitivity and specificity values were 0.78 (95% CI, 0.69–0.84) and 0.79 (95% CI, 0.68–0.86) for ≥S1, 0.85 (95% CI, 0.74–0.92) and 0.79 (95% CI, 0.71–0.85) for ≥S2, and 0.83 (95% CI, 0.76–0.89) and 0.79 (95% CI, 0.68–0.87) for S3. In our study, CAP sensitivity in detecting steatosis was lower than in the above cited meta-analysis (64.84%, 69.70% and 83.33% for the prediction of ≥S1, ≥S2 and S3 grades, respectively); nevertheless, the specificity was somewhat higher (87.27%, 85.12% and 82.54%, respectively), at cutoff values of 260 dB/m, 285 dB/m and 294 dB/m. We should mention that CAP cutoff values vary among studies depending on liver disease etiology, prevalence of different steatosis grades in the study group and the desired objective (maximum specificity and sensitivity, maximum accuracy, cutoff to obtain a greater specificity for a sensitivity higher than 0.90, etc.). In our study, the optimal cut-off values were defined by maximizing the sum of sensitivity and specificity.

In the present study, the positive predictive value of CAP for steatosis ≥S2 and S3 was low (47.9% and 23.3%, respectively), possibly due to the low prevalence of these steatosis grades in our group; nevertheless, the negative predictive value was high (93.5% and 98.7%, respectively), which suggests that CAP would be a useful clinical tool to help exclude, rather than confirm, the presence of moderate or severe steatosis. Performance of CAP was good, with an AUC of 0.813, 0.822 and 0.838 for the detection of ≥S1, ≥S2 and S3. Maximal diagnostic accuracy was obtained for the prediction of ≥S2 and S3 while, for the prediction of ≥S1, the accuracy reached only 76.11%.

With the exception of S1/S2 and S2/S3 comparisons, the results were fair in differentiating between S0/S1 (AUC 0.772) and S1/S3 grades (AUC 0.777), good in differentiating between S0/S2 grades (AUC 0.874) and excellent in differentiating between S0/S3 grades (AUC 0.904). The study by Sasso et al. [20] reported good results for the differentiation of adjacent grades (S0/S1, S1/S2) and excellent for the differentiation of grades further apart (S0/S2, S1/S3, S0/S3).

With regard to the examination technique, clearly established criteria for a reliable CAP measurement have not yet been defined. In the present study, we considered a CAP measurement as a representative for 10 successful acquisitions, regardless of the success rate (SR). While analyzing the differences between patients depending on SR, we noted that the only parameter that varied significantly was the BMI, higher in patients with SR <60% than in those with SR ≥60%. The present study included only patients in whom 10 valid CAP measurements were obtained, meaning we could not determine the predictive factors for total measurement failure (SR=0). Our opinion is that SR is not a relevant parameter, as long as 10 valid measurements are obtained, but that examination becomes increasingly difficult at higher BMIs (with a lower SR). Lately, however, the CAP assessment can also be performed using the XL probe, which is of great importance in obese patients [42, 43]. On the other hand, in all patients the IQR values were <0.3, and therefore we could not make any analysis on the influence of high IQR on CAP performance. Further studies, on larger groups of biopsied patients, are necessary to clearly establish the technical criteria required for a CAP measurement.

All our patients were examined after overnight fasting in order to prevent any possible changes of the CAP value due to the postprandial state. Although no proof of a possible influence of postprandial CAP measurement was yet reported, an obvious increase in LS values measured by TE was found in a recent study at 15 to 45 min after the meal onset with return to baseline pre-meal levels within 120 min in all patients [35]. Further studies are required to establish whether CAP values are significantly influenced by the patient's postprandial status.

In comparison to other modalities, CAP is a non-invasive, quantitative, and non-ionizing procedure. Furthermore, it is easy to perform and provides immediate results; it is also machine-independent and does not require corrections to be made for gain, frequency, focusing or beam diffraction and is also not subject to operator interpretation [21]. Compared to LB, CAP is less prone to sampling error as it explores a liver volume about 100 times larger [21, 22].

This study has some limitations. Firstly, the standard used in the quantification of the steatosis grade was LB, interpreted visually by one pathologist. We cannot overlook the fact that, in some cases, CAP performance may be affected by a failure in LB interpretation because of the inhomogeneous distribution of steatosis in the liver, even at a macroscopic level [9, 44] as well as to intraobserver variability, to some extent [9, 44, 45], which can lead to poor reproducibility, even when performed by expert pathologists [46]. As for interobserver variability, its analysis was not one of the aims of the study. A more accurate method of steatosis quantification is required, such as the computerized interpretation of the histologic image of the entire sample. However, for the moment, LB remains the gold standard for steatosis assessment, but it is an „imperfect gold standard". On the other hand, CAP may also be limited by sampling errors in the same way that LB is. Secondly, the structure of our series of patients does not allow us to determine the CAP performance for the prediction of each steatosis grade, depending on the etiology of liver disease. Further studies are required on large groups of biopsied patients with different etiologies of liver disease, in order to analyze CAP values and CAP performance in predicting each grade of steatosis, in correlation with the etiology of liver disease. Thirdly, the present study included only patients in whom 10 valid CAP measurements were obtained, meaning that we could not determine the predictive factors for total measurement failure (SR=0) with the M probe, on which the CAP measurement software was installed in our center at the time of the study.

The signification of CAP measurement (liver fat content, macro versus micro vesicular steatosis, etc.) and its potential confounding factors have yet to be determined for an accurate

interpretation of its results. In addition, studies taking into consideration the skin-capsule distance would be of great importance, since a distance above 25 mm may lead to overestimation of CAP.

CONCLUSIONS

Our findings suggest that CAP is a non-invasive method for the assessment of steatosis in chronic liver diseases patients with a diagnosis accuracy of 76.11-82.06% and that it is independently influenced only by the amount of steatosis. Having a negative predictive value of 93.5-98.7%, CAP could be a useful clinical tool especially in excluding significant steatosis grades. Other prospective studies conducted on larger groups of patients with different chronic liver diseases are mandatory in order to accurately establish the performance of CAP in assessing steatosis according to etiology of liver disease, the technical parameters that should be met to ensure a high-quality examination and the factors that may influence the accuracy of steatosis evaluation using this method.

Conflicts of interest: None.

Authors' contribution: M.L.-P.: study concept and design, data acquisition, statistical analysis, data interpretation and manuscript writing. D.F., A.T. and A.M.: data aquisition. D.F. and H.S.: contribution to the research. Z.S.: liver biopsy. E.B.: histological study. R.B.: study supervision. All authors read and agreed with the final version of the manuscript.

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