

Controlled attenuation parameter for the assessment of liver steatosis in comparison with liver histology: a single-centre real life experience

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Summary

BACKGROUND AND AIMS: Accurate diagnosis and staging of non-alcoholic fatty liver disease are essential for the management of this disorder. Controlled attenuation parameter (CAP) has been suggested as a new noninvasive measurement made during transient elastography to assess liver steatosis. The aim of this study was to evaluate CAP as a diagnostic tool for identifying the presence and degree of hepatic steatosis in consecutive patients in an outpatient liver unit of a tertiary centre.

METHODS: Between March 2015 and August 2016, all patients who underwent liver biopsy underwent liver stiffness measurement with simultaneous CAP determination using the FibroScan[®] M or XL probe. Steatosis, inflammatory activity and fibrosis were assessed using the histological SAF scoring system. In addition, fibrosis was scored according to the METAVIR system, and body mass index (BMI) and the underlying liver disease were also recorded.

RESULTS: 224 patients were included in the analysis; 146 (65.2%) were male. Steatosis grades were distributed as follows: S0 n = 85 (37.9%), S1 n = 82 (36.6%), S2 n = 33 (14.7%), S3 n = 24 (10.7%). Mean BMI was 26.8 kg/m², for the S0 group 24.9 kg/m², S1 26.5 kg/m², S2 27.3 kg/m² and S3 32.5 kg/m². The CAP differed significantly between steatosis groups S0 to S3. The area under receiver operating characteristics curve for S0 vs S1–S3 was 0.78, for S0/1 vs S2/3 0.83 and for S0–2 vs S3 0.82. Calculated cut-off values were 258.5 dB/m for S0 vs S1–3, 282.5 dB/m for S0/1 vs S2/3 and 307.5 dB/m for S0–2 vs S3.

CONCLUSIONS: CAP values are strongly associated with the degree of steatosis irrespective of the underlying liver disease. Integrating CAP measurements in the standard work-up may identify patients with NAFLD.

Keywords: CAP, controlled attenuation parameter, steatosis, liver, NASH, NAFLD, FibroScan, elastography, fatty liver, SAF

Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease in industrialised countries with a prevalence of up to 35% worldwide and 25% in Europe [1–4]. NAFLD includes steatosis (isolated fat accumulation in hepatocytes) and steatohepatitis (steatosis with liver-cell injury/death and accumulation of inflammatory cells). Patients with NAFLD have a significantly increased risk of developing hepatic fibrosis or cirrhosis and therefore increased morbidity and mortality due to complications of liver cirrhosis or hepatocellular carcinoma. In addition, NAFLD has a strong association with metabolic syndrome and is also associated with an increased risk of cardiovascular disease [5]. Both cardiovascular morbidity and complications of cirrhosis are associated with a severe impairment of quality of life and represent an increasing clinical and economic burden [6–8]. Furthermore, NAFLD is also observed with increasing frequency concomitant to other liver diseases such as hepatitis B and C, autoimmune hepatitis, hemochromatosis, primary biliary cholangitis or alpha1-antitrypsin deficiency [9]. As a concomitant disease, NAFLD can accelerate the progression of the underlying disease and promote the development of hepatocellular carcinoma [10–12]. Therefore, early detection and

ABBREVIATIONS

AUROC	area under receiver operating characteristics curve
BMI	body mass index
CAP	controlled attenuated pressure
EASL	European Association for Study of the Liver
LSM	liver stiffness measurement
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NPV	negative predictive value
PPV	positive predictive value
SAF	steatosis activity fibrosis
T2DM	type 2 diabetes mellitus

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treatment of NAFLD, both as separate disease and as co-factor with other liver diseases, is crucial.

Liver biopsy is the gold standard for both the diagnosis of NAFLD and the differentiation between isolated steatosis and non-alcoholic steatohepatitis (NASH). However, liver biopsy is an invasive procedure, involves a certain risk of complications, is costly and bears the risk of sampling errors [13, 14]. Therefore, a non-invasive diagnostic approach is preferred. The current guidelines from the European Association for Study of the Liver (EASL) mention ultrasonography, serum biomarkers and magnetic resonance spectroscopy (¹H-MRS) as non-invasive tests for NAFLD [15]. However, ultrasonography and serum biomarker tests are relatively reliable in predicting steatosis, but not in its classification. In comparison, ¹H-MRS is the optimal examination for grading the steatosis and avoids sampling errors [16, 17]. However, ¹H-MRS is expensive and only available at selected centres.

Measurement of controlled attenuation parameter (CAP) is a novel tool for the non-invasive quantitative and qualitative assessment of liver steatosis. CAP is integrated in the elastography tool FibroScan[®], which is widely used to assess liver fibrosis in chronic viral hepatitis [18, 19]. CAP measures ultrasonic attenuation (in dB/m) at a frequency of 3.5 MHz (on a go-and-return path). Values range from 100 to 400 dB/m [20–22]. To date, CAP has been studied in various patient cohorts, mainly in Europe and Asia and in patients with metabolic syndrome and hepatitis B [23–27].

The aim of this study was to evaluate CAP as a diagnostic tool for identifying the presence and the degree of hepatic steatosis in consecutive patients in an outpatient liver unit of a tertiary centre.

Patients and methods

Study population

This retrospective study was conducted at the Cantonal Hospital St Gallen, a tertiary referral centre in Switzerland. Consecutive patients who underwent liver biopsy between March 2015 and August 2016 were evaluated for inclusion in the study. Every patient considered for liver biopsy without a focal lesion and aged over 18 years was eligible for inclusion. The following clinical parameters were recorded: age, gender, body mass index (BMI) and liver diseases. Data analysis was in accordance with the ethical guidelines of the Helsinki Declaration 1975 and the study was approved by the Ethics Committee Ostschweiz (EKOS), St Gallen (Project ID 2018-02092).

Transient elastography and CAP measurement

Transient elastography and CAP measurements were performed with the FibroScan[®] Touch 502 device using M or XL probes (Echosens, Paris, France). Details on CAP measurements are described elsewhere [28, 29]. The result is the median of ≥ 10 valid measurements. The measurements were taken at a depth of between 25 and 65 mm, measured on the go-and-return path at 3.5 MHz. The measurements (and the biopsy) were made on the right lateral side (intercostal space) in the liver segment VI or VII. If CAP measurement and biopsy were performed on the same day, CAP measurement was always performed before biopsy. If CAP measurement and biopsy were not performed on the

same day, the maximum allowed interval was 7 days. Only valid measurements were included, based on the device's internal algorithm. Reliability was defined as (1) ≥ 10 valid measurements, (2) interquartile range / median (IQR/med) $< 30\%$ in elastography and (3) success rate $> 60\%$ [30]. The physicians performing the elastography (and biopsy) had knowledge of the available clinical information.

Liver biopsy and histology

The liver biopsy was always performed by two physicians together (SB, IB or DS). The biopsies were always taken under sonographic control from the right lateral side (segment VI or VII) at the same place as the elastography (if performed before). All biopsies were made with BioPince[®] Full Core Biopsy needles with a diameter of 18G and a cylinder length of 33 mm. Liver biopsy specimens were formalin-fixed and paraffin-embedded. Serial sections were stained with haematoxylin and eosin, and chromotrope aniline blue using standard methods. Sections were evaluated by two experienced liver pathologists (WJ and JN), who were blinded to CAP and elastography results. Liver steatosis was categorised as S0, S1, S2 or S3 based on the percentage of hepatocytes with large or medium-sized fat droplets, using the SAF (steatosis, activity, fibrosis) scoring system: S0 $< 5\%$; S1 5–33%, mild; S2 34–66%, moderate; S3 $> 66\%$, marked [31–32]. In addition, liver sections were evaluated for hepatocyte ballooning, lobular inflammation and fibrosis using the SAF scoring system. The degree of fibrosis was also scored using the METAVIR system [33].

Statistical analysis

Descriptive statistics are reported for all included patients, for patients stratified according to their steatosis category and for the subgroup with a diagnosis of NAFLD. For measurements on a continuous scale the mean and standard deviation and for nominal characteristics the number of observations and percentages were reported. Continuous measures were assumed to follow a normal distribution, except for the liver stiffness and steatosis grade (in %). Parametric test statistics were used to compare the null hypothesis of no differences between patient groups. For comparisons between two patient groups, a p-value based on a two-sided t-test was reported. In the case of more than two groups, the reported p-values were based on an F-test. The liver stiffness and steatosis grade (in %) were compared by a nonparametric Mann-Whitney U-test and a Kruskal-Wallis test. Characteristics on a nominal scale were compared by Fisher's exact test.

The performance of the CAP parameter to predict the steatosis category was examined by the area under the curve (AUC) obtained from the receiver operating characteristics (ROC). A 95% confidence interval (CI) for the AUC of each patient group was provided and for the cut-off point, which is related to the maximum Youden index (defined as sensitivity + specificity – 1), the specificity, sensitivity, negative predictive value (NPV), positive predictive value (PPV), the accuracy and the positive as well as the negative likelihood ratio (LR). For all these measures a 95% CI was reported, if available. The ROC was carried out for groups with different steatosis categories: first the S0 patients were compared against all other and then higher steatosis grade categories were reassigned con-

tinually to the S0 category. In a second analysis all steatosis grade categories were compared group wise in a separate ROC. All analyses were made with R version 3.2.3 (2015-12-10) and for the ROC analyses the R-package pROC version 1.8 was used [34, 35].

The influence of possible confounders, which could have an impact on the association between CAP and steatosis, was established by a multiple linear regression model, which includes the CAP measurements as outcome. The included, possible confounding factors, were the patient's age, sex, BMI, liver stiffness and the steatosis grade in percent and if a patient suffered from NASH or NAFLD. The reference category refers to female patients with no NASH.

Results

Patient characteristics

From March 2015 to August 2016 a total 290 patients were screened and 224 were included in the analysis (fig. 1). Thirty-nine patients were not included because of unreliable CAP measurements. In 7 patients fewer than 10 measurements were valid, in 11 the success rate was $\leq 60\%$ and in 21 LSM IQR/med was $\geq 30\%$. In 27 cases the interval from CAP measurement to liver biopsy was more than 7 days. The majority (65%) of the 224 patients were male. Median age was 49 years (IQR 39–60). Median BMI was 25.6 kg/m² (IQR 23.5–29.4, range 17.5–51.8 kg/m²). A total of 114 patients had a BMI ≥ 25 kg/m². BMI values were not available for 21 patients. The following liver diseases were diagnosed: hepatitis C (n = 61), hepatitis B (n = 38),

NAFLD/NASH (n = 52), ASH (n = 6); autoimmune hepatitis (n = 20), primary biliary cholangitis (n = 8), sarcoidosis n = 4, drug induced liver injury (n = 12), others (n = 25). Patient characteristics are summarised in table 1.

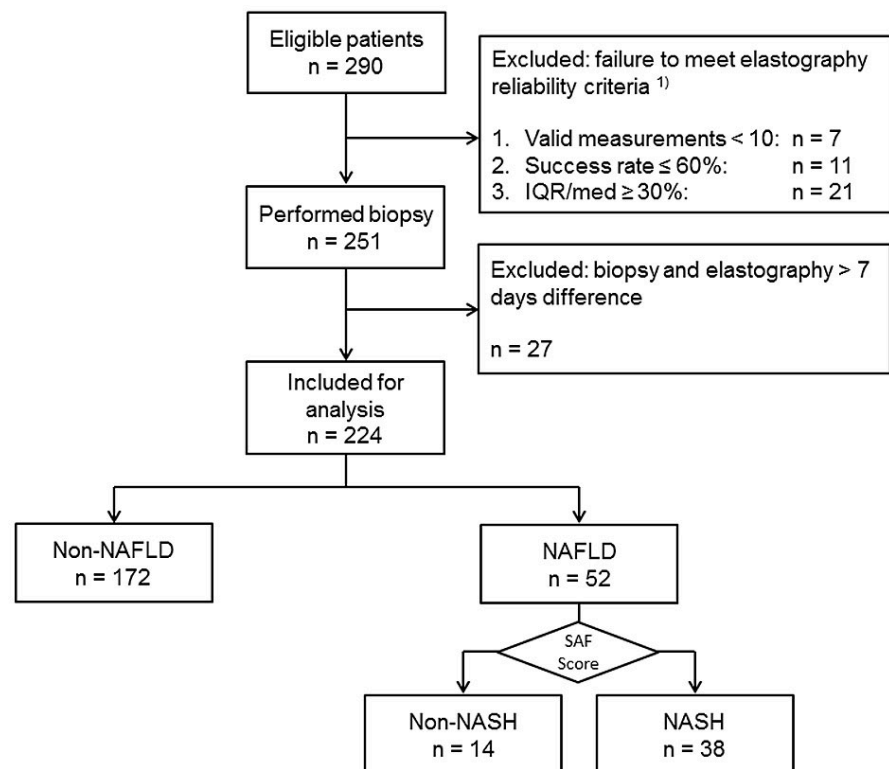
Histopathological findings

All biopsy samples were evaluated as representative. Steatosis was observed in 139/224 (62%) liver biopsies. Steatosis categories had the following distribution: S0 n = 85 (38%), S1 n = 82 (37%), S2 n = 33 (15%), and S3 n = 24 (11%).

CAP measurements

The range of CAP values was 109–400 dB/m (median 257, IQR 219–307). The mean number of measurements taken per patient was 13.3 (range 10–21) and the mean number of valid measurements per patient was 12.4 (range 10–20). For patients with S0, median CAP was 227 dB/m (IQR 199–249), for S1 265 dB/m (IQR 228–295), for S2 307 dB/m (IQR 283–338) and for S3 335 dB/m (IQR 319–358) (fig. 2). Patients with steatosis (S1–S3) had significantly higher CAP values than patients without steatosis (S0) (p < 0.0001). Patients with no and low steatosis (S0, S1) had significantly lower CAP than patients with moderate or high steatosis (S2, S3) (p < 0.0001). Patients with no to moderate steatosis (S0–S2) had significantly lower CAP than patients with high steatosis (S3) (p < 0.0001). CAP values were also compared between steatosis categories, which revealed significant differences between the S0/S1

Figure 1: STARD flow diagram. ¹⁾ A total of 39 patients were excluded because of unreliable measurements. Among them 21 (54%) suffered from non-alcoholic fatty liver disease (NAFLD). IQR/med = interquartile range / median; NASH = non-alcoholic steatohepatitis



($p < 0.0001$), S1/S2 ($p = 0.00012$), and S2/S3 ($p = 0.03$) categories. The distributions of CAP values for each steatosis group are presented in supplementary figure S3 (appendix 1).

CAP values of patients with NAFLD ($n = 52$) were significantly higher than CAP values of patients with other liver diseases ($n = 172$): median CAP 324.5 dB/m (IQR 297–352.25) vs 239.5 dB/m (IQR 214.75–280); $p < 0.001$ (fig. 3). Among the patients with NAFLD, CAP values were comparable between patients with isolated steatosis

or steatohepatitis. CAP values for patients with NAFLD are summarised separately in table 2.

The M or XL probe was chosen according to the device's internal algorithm. The XL probe was used on 20% of the patients. For patients with NAFLD, the XL probe was used in 69%, for non-NAFLD patients in 14%; for BMI ≥ 25 kg/m² the XL probe was used in 32% and for BMI < 25 kg/m² in 7%.

Table 1: Patient characteristics.

	Total	Steatosis score				p-value	
		S0	S1	S2	S3		
Patients, n (%)	224	85 (37.9)	82 (36.6)	33 (14.7)	24 (10.7)		
Male, n (%)	146 (65.2)	49 (57.6)	55 (67.1)	25 (75.8)	17 (70.8)	0.239	
Age, median (IQR)	49 (39–60)	44 (36–56)	53 (42–64)	54 (46–61)	42 (30–56)	0.003	
BMI kg/m ² median (IQR)*	25.6 (23.5–29.4)	24.8 (22.6–27.5)	25.6 (23.8–28.9)	26.6 (23.7–30.1)	31.7 (27.5–35.4)	<0.0001	
Diagnosis, n (%)	HBV hepatitis	38 (17)	18 (21)	16 (20)	3 (9)	1 (4)	0.754
	HCV hepatitis	61 (27)	25 (29)	25 (30)	9 (27)	2 (8)	
	NAFLD/NASH	52 (23)	0	15 (18)	18 (55)	19 (79)	
	ASH	6 (3)	1 (1)	3 (4)	0	2 (8)	
	Autoimmune hepatitis	20 (9)	16 (19)	3 (4)	1 (3)	0	
	Drug-induced liver injury	12 (5)	4 (5)	7 (9)	1 (3)	0	
	Primary biliary cholangitis	8 (4)	5 (6)	2 (2)	1 (3)	0	
	Sarcoidosis	4 (2)	3 (4)	1 (1)	0	0	
Others	23 (10)	13 (15)	10 (12)	0	0		
Fibrosis score METAVIR, n (%)†	F0	45 (20.1)	22 (25.9)	11 (13.4)	5 (15.2)	7 (29.2)	0.754
	F1	78 (34.8)	28 (32.9)	28 (34.1)	13 (39.4)	9 (37.5)	
	F2	48 (21.4)	19 (22.4)	19 (23.2)	6 (18.2)	4 (16.7)	
	F3	21 (9.4)	7 (8.2)	10 (12.2)	3 (9.1)	1 (4.2)	
F4	31 (13.8)	8 (9.4)	14 (17.3)	6 (18.2)	3 (12.5)		
Liver stiffness (kPa), median (IQR)	7.8 (5.4–13.1)	6.4 (5.0–12.2)	8.8 (6.0–14.1)	7.7 (6.2–11.6)	8.2 (5.3–13.1)	0.223	
CAP (dB/m), median (IQR)	257 (219–307)	227 (199–249)	265 (228–295)	307 (283–338)	336 (319–358)	<0.001	

CAP = controlled attenuation parameter; BMI = body mass index; HBV = hepatitis B virus; HCV = hepatitis C virus; IQR = interquartile range; NAFLD = non-alcoholic fatty liver disease; NASH = non-alcoholic steatohepatitis * $n = 203$, 21 values not available; † $n = 223$, 1 value not available

Table 2: Characteristics of patients with non-alcoholic fatty liver disease.

	Total	Steatosis	Steatohepatitis	p-value
Patients, n (%)	52	14 (27)	38 (73)	
Age (years), median (IQR)	49 (39–62)	48 (40–54)	51 (39–62)	0.41
Male, n (%)	36 (69.2)	9 (64.3)	27 (71.1)	0.738
BMI (kg/m ²), median (IQR)	29.7 (25.9–32.9)	30.7 (25.4–32.6)	28.9 (26.1–32.9)	0.82
BMI ≥ 25 kg/m ² , n (%)	42 (80.7)	10 (71.4)	32 (84.2)	
SAF steatosis, n (%)	1	11 (21)	5 (13)	
	2	19 (37)	15 (39)	
	3	22 (42)	18 (47)	
SAF ballooning, n (%)	0	5 (10)	0	
	1	30 (58)	21 (55)	
	2	17 (32)	17 (45)	
SAF lobular inflammation, n (%)	0	13 (25)	0	
	1	36 (69)	35 (92)	
	2	3 (6)	3 (8)	
SAF fibrosis, n (%)	0	12 (23)	8 (21)	
	1	10 (19)	6 (16)	
	2	13 (25)	11 (29)	
	3	10 (19)	9 (24)	
	4	7 (13)	4 (11)	
Steatosis (%), median (IQR)	50.0 (40.0–70.0)	45.0 (30.0–57.5)	50 (40.0–70.0)	0.133
Liver stiffness (kPa), median (IQR)	7.8 (3.4–11.7)	8.4 (5.5–10.3)	7.7 (5.4–12.3)	0.951
CAP (dB/m), median (IQR)	325 (297–353)	310 (284–327)	331 (305–358)	0.441

BMI = body mass index; IQR = interquartile range; NAFLD = non-alcoholic fatty liver disease; NASH = non-alcoholic steatohepatitis; SAF = steatosis, activity and fibrosis

Association between CAP and BMI

The mean CAP value of the group with BMI <25 kg/m² (n = 89) was 229 dB/m (IQR 206–267) and 280 dB/m (IQR 232–321) for the group ≥25 kg/m² (n = 114), p <0.0001 (fig. 4).

Figure 2: Controlled attenuation parameter (CAP) values according to the degree of steatosis S0 to S3.

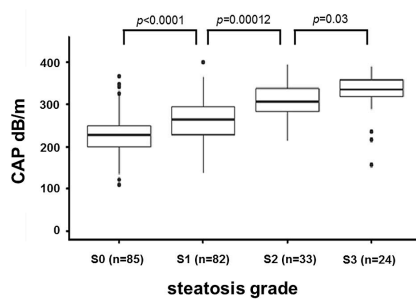


Figure 3: Comparison of controlled attenuation parameter (CAP) values in patients with non-alcoholic fatty liver disease (NAFLD) (median 324.5dB/m) vs patients without NAFLD (median 239.5dB/m), p <0.0001. NASH = non-alcoholic steatophepatitis

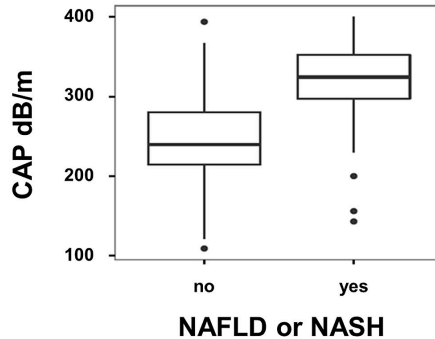
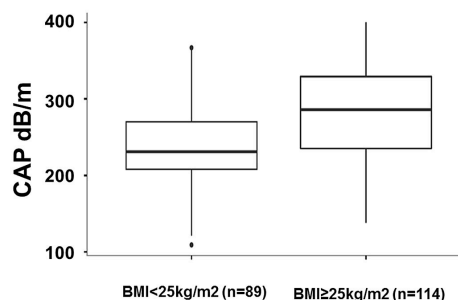


Figure 4: Comparison of controlled attenuation parameter (CAP) values in patients with body mass index (BMI) <25 kg/m² (median 229 dB/m) vs BMI ≥25kg/m² (median 280 dB/m).



Association between CAP and fibrosis

A potential association between CAP and the degree of fibrosis was analysed. A comparison of the median CAP values in the fibrosis groups F0 to F4 showed no significant difference (fig. 5).

Association between CAP and type 2 diabetes mellitus

In total, 27 (12%) patients with type 2 diabetes mellitus (T2DM) were identified. Of these 27 patients, 13 (48%) had a diagnosis of NAFLD. Median CAP was 288 dB/m (range 143–400) in the T2DM group, and 253 dB/m (109–400) in the non-T2DM group (p = 0.032). Median CAP was 323 dB/m (range 143–400) in the subgroup with T2DM and NAFLD, and 264 dB/m (range 164–341) in the subgroup T2DM without NAFLD (p = 0.058).

Diagnostic performance and cut-off values

Sensitivity, specificity, NPV, PPV, accuracy and positive or negative LR of CAP to detect liver steatosis are listed in table 3. The ROC analyses are shown in figure 6. For the prediction of steatosis based on CAP measurements (S0 vs S1/2/3), the AUC was 0.78 (95% CI 0.72–0.84); for the differentiation between no/low and moderate/high grade steatosis (S0/1 vs S2/3, fat >33%), the was AUC 0.83 (95% CI 0.76–0.89); to predict a high grade steatosis (S0/1/2 vs S3, fat >66%), the AUC was 0.82 (95% CI 0.72–0.92). Optimal cut-off values were calculated with the maximum Youden index. The cut-off value for S0 vs S1/2/3 was 258 dB/m (95% CI 245–277), for S0/1 vs S2/3 282.5 dB/m (278–307) and for S0/1/2 vs S3 307.5 dB/m.

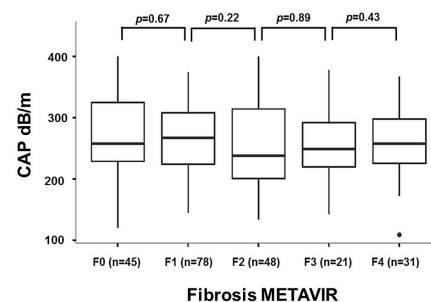
A similar analysis for each pair of individual steatosis categories is presented in table S1 and figure S1 (appendix 1).

Correlation of CAP with other clinical and histological parameters

Table 4 shows the coefficients of the factors included in the regression model together with a 95% CIs and a p-values. Multivariate analysis showed correlation with CAP value for BMI, sex, liver stiffness (kPa), presence of NAFLD and steatosis in percent. No association was found for the factor age.

The comparison of liver stiffness measurements between the steatosis groups is summarised in table S2 and figure S2 (appendix 1).

Figure 5: Distribution of controlled attenuation parameter (CAP) values according to fibrosis groups (METAVIR).



Discussion

This study investigated the performance of CAP to identify liver steatosis in a “real life” scenario in consecutive patients at an outpatient liver unit of a tertiary centre. The study included more than 10 different liver diseases. However, the subgroup with hepatitis C was disproportionately large (63%), because liver biopsy was mandatory for the treatment with direct-acting antiviral drugs during trial recruitment. This study shows that CAP performs well not

Figure 6: Receiver operating characteristic (ROC) curves of controlled attenuation parameter (CAP) in the diagnosis of: $S \geq 1$ (steatosis $\geq 5\%$), AUC 0.78; $S \geq 2$ (steatosis $\geq 34\%$), AUC 0.83; and $S \geq 3$ (steatosis $\geq 67\%$), AUC 0.82. AUC = area under the curve

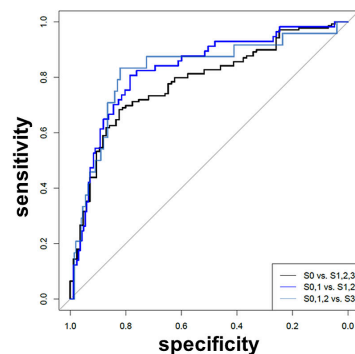


Table 4: Multivariate analysis, regression model including the 203 patients with body mass index (BMI) data available.*

Intercept	159.1 (113.7, 204.7)	$p < 0.001$
Age	0.16 (−0.3, 0.61)	$p = 0.50$
Sex: male	15.47 (1.4, 29.54)	$p = 0.03$
BMI (kg/m^2)	2.69	$p < 0.001$
Liver stiffness	−0.95 (−1.62, −0.27)	$p = 0.01$
Steatosis in %	0.96 (0.6, 1.32)	$p < 0.001$
NAFLD	21.26 (0.91, 41.61)	$p = 0.04$
Adjusted R^2	0.42	
Residual standard error	47.5 (degrees of freedom = 196)	

NAFLD = non-alcoholic fatty liver disease; NASH = non-alcoholic steatohepatitis; BMI = body mass index. Coefficients of the factors included in the regression model with a 95% CI and p-values. Null hypothesis examines whether the corresponding coefficient is equal to zero. Association with CAP is assumed if the coefficient is different from zero, p-value is small and the 95% CI excludes the null hypothesis. * An alternative regression model with all 224 patients (without BMI) is shown table S3 in appendix 1. The results do not differ significantly from the model presented here.

only in patients with metabolic syndrome or hepatitis B (as shown in several studies before), but also in patients with a broad spectrum of underlying liver diseases [23–27]. This is important for the future use of CAP as a screening tool for liver steatosis not only as an independent disease, but also as a concomitant condition with other liver diseases.

The AUC values of 0.78 (for the diagnosis of steatosis $\geq 5\%$), 0.83 ($\geq 33\%$) and 0.82 ($\geq 66\%$) are comparable to data from previous studies such as those of de Lédinghen et al. [26] (in patients with known NAFLD) or Chen et al. [25] and Ferraioli et al. [20] (in chronic viral hepatitis). Despite several existing studies with different populations, there is still no consensus on the cut-off values for CAP measurements. Compared to previous studies, the values proposed here are in the middle range, except for the value for S_0 vs S_{1-3} . This value, at 258.5 dB/m, was relatively high compared with other findings [20, 23, 25–27, 36, 37]. This may be due to the more heterogeneous patient population. However, even in this population of patients with a variety of liver diseases, the cut-off values were clearly separated from each other. Additional studies will have to establish whether these cut-off values can be applied in patients with a broad spectrum of liver diseases.

From the comparisons between the BMI groups ($\geq 25 \text{ kg}/\text{m}^2$ vs $< 25 \text{ kg}/\text{m}^2$) and the groups with confirmed NAFLD/NASH versus all other liver diseases, it can be assumed that patients with a high BMI and patients with a diagnosis of NAFLD/NASH present with higher CAP values. This is confirmed by the following results: 280 dB/m and 229 dB/m for the BMI $\geq < 25 \text{ kg}/\text{m}^2$ subgroups; 324.5 dB/m and 239.5 dB/m for NAFLD/NASH versus other liver diseases groups. However, it is interesting to note that the CAP values in the groups for the comparison of NAFLD/NASH with others are significantly higher than the values for the comparison of BMI $\geq < 25 \text{ kg}/\text{m}^2$, which is in line with the data presented by Karlas et al. [38]. Similarly, the presence of T2DM also results in increased CAP values (288 vs 253 dB/m) in comparison with non-T2DM patients. This finding could be useful for screening for NAFLD/NASH in populations at risk, such as patients with metabolic syndrome and obesity.

This study found no significant difference between CAP values of patients with steatohepatitis and those without an inflammatory component. The presence of steatohepatitis was quantified with the SAF score. Based on our findings, we assume that the inflammatory cells and ballooning have no relevant physical influence on the attenuation

Table 3: Performance of CAP on the basis of the area under receiver operating characteristics curves (AUROC).

	S0 vs S1,2,3		S0,1 vs S2,3		S0,1,2 vs S3	
	Value	95% CI	Value	95% CI	Value	95% CI
AUC	0.78	0.72–0.84	0.83	0.76–0.89	0.82	0.72–0.92
Threshold	258.50	244.5–276.5	282.5	277.5–306.5	307.50	286.0–323.5
Specificity	0.82	0.73–0.93	0.78	0.70–0.90	0.82	0.71–0.88
Sensitivity	0.68	0.56–0.78	0.81	0.67–0.91	0.83	0.67–0.96
NPV	0.61	0.55–0.69	0.92	0.88–0.96	0.98	0.96–0.99
PPV	0.86	0.81–0.93	0.55	0.48–0.72	0.36	0.27–0.47
Accuracy	0.73	0.68–0.80	0.78	0.73–0.86	0.82	0.73–0.88
Positive LR	3.79	–	3.62	–	4.63	–
Negative LR	0.39	–	0.25	–	0.20	–

NPV = negative predictive value; PPV = positive predictive value; LR = likelihood ratio; CI = confidence interval. Performance measures of ROC and for CAP cut-offs which maximise the distance from the diagonal (max. Youden index)

of the sonic wave. In this context it would be interesting to compare CAP values in a larger group of patients with pure steatosis (without ballooning, inflammation and fibrosis) with CAP values in NASH patients. However, it is unlikely that CAP will have the same prognostic impact as the components fibrosis and inflammation in NAFLD patients [39, 40]. Nevertheless, steatosis is a relevant co-factor in liver diseases such as chronic hepatitis C and even after sustained virological response steatosis remains a relevant risk factor for fibrosis progression [41–47]. Therefore the use of CAP has a role as an additional prognostic tool.

This study has several limitations. Because of incomplete data sets due to too long intervals between liver biopsy and laboratory tests, we decided not to evaluate the laboratory data. A correlation with certain laboratory values and certain indices (e.g., fatty liver index) would be interesting, since according to previous studies there is still some disagreement about the association of CAP with gamma-glutamyltransferase, alkaline phosphatase, aspartate or alanine transaminase, or bilirubin [24, 25, 27, 48].

A recurring problem is the use of the M or XL probe [49–52]. For this study we decided to use the algorithm suggested by FibroScan® Touch 502. However, this algorithm refers primarily to the elastography. It is therefore unclear whether it can be used in the same way for steatosis measurement or whether the XL probe should be used according to a separate algorithm such as BMI or thickness of the subcutaneous fat tissue.

Another methodological limitation is the introduction of a potential bias by the exclusion of patients who did not fulfil the reliability criteria. For this study, we chose the rather restrictive criteria of Castera et al. (≥ 10 valid measurements, IQR/med $< 30\%$ in elastography and success rate $> 60\%$) [30]. This resulted in a total of 39 exclusions, in which NAFLD patients ($n = 21$, 54%) were overrepresented. This is a consequence of the more difficult and therefore more error-prone examination in obese patients.

In this study we could not use $^1\text{H-MRS}$ because it is not available in our centre. It would be desirable to involve the $^1\text{H-MRS}$ in future studies. Because the $^1\text{H-MRS}$ seems to be an ideal (but expensive) method for the quantification of liver steatosis and in particular takes account of the partly inhomogeneous fat distribution in the liver [16].

In conclusion, this study shows, that CAP offers a quantitative measurement of hepatic steatosis with excellent accuracy when compared with histology. This is an important advantage as it qualifies CAP not only as a noninvasive screening tool, but also for assessing the course and severity of steatosis. In addition, CAP is readily available with the well-established elastography and cheaper than biopsy or MR spectroscopy [16, 53]. This makes CAP measurement to a potential standard tool for steatosis screening.

This study was presented orally in abstract form at: Jahreskongress Schweizerische Gesellschaft für Gastroenterologie 2017, Interlaken; and United European Gastroenterology (UEG) Week 2017, Barcelona.

Disclosure statement

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Appendix 1

Supplementary data

Figure S1: Receiver operating characteristic (ROC) curves for pairwise steatosis grades.

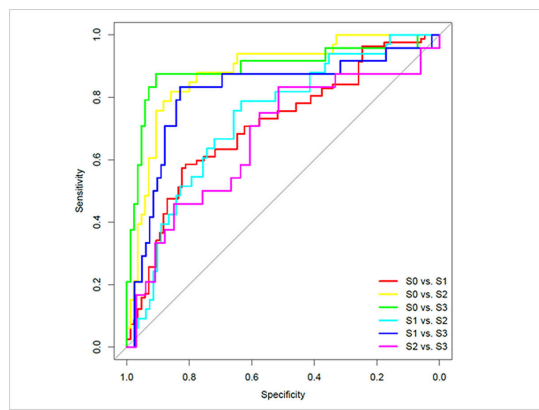


Figure S2: Boxplots for liver stiffness (FibroScan) according to steatosis grade (histological).

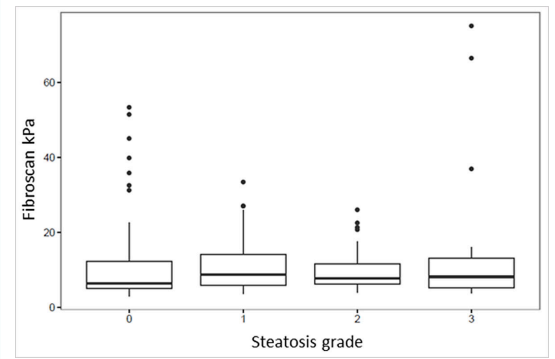


Figure S3: Histogram for controlled attenuation parameter (CAP) values according to steatosis grade.

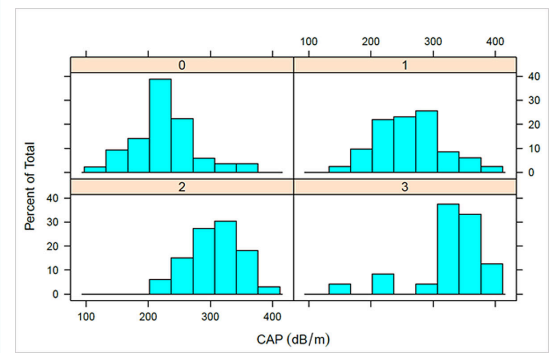


Table S1: Performance of controlled attenuation parameter (CAP) using area under receiver operating characteristics curve (AUROC) for each steatosis category.

	AUC	Threshold	Specificity	Sensitivity	NPV	PPV	Accuracy	Positive LR	Negative LR
S0 vs S1	0.70	256.0	0.80	0.59	0.66	0.74	0.69	2.89	0.52
2.5%	0.62	229.5	0.62	0.46	0.61	0.64	0.63		
97.5%	0.78	268.5	0.90	0.77	0.75	0.84	0.76		
S0 vs S2	0.88	277.5	0.88	0.79	0.91	0.72	0.85	6.62	0.24
2.5%	0.81	237.0	0.68	0.70	0.88	0.53	0.74		
97.5%	0.95	283.5	0.94	0.97	0.99	0.85	0.91		
S0 vs S3	0.89	285.5	0.90	0.88	0.96	0.72	0.90	9.19	0.14
2.5%	0.80	281.5	0.86	0.75	0.92	0.62	0.85		
97.5%	0.99	314.5	0.98	1.00	1.00	0.91	0.96		
S1 vs S2	0.73	282.5	0.66	0.76	0.87	0.47	0.69	2.22	0.37
2.5%	0.63	240.0	0.44	0.48	0.80	0.40	0.58		
97.5%	0.83	322.0	0.89	0.94	0.96	0.68	0.81		
S1 vs S3	0.81	306.5	0.83	0.83	0.94	0.59	0.83	4.88	0.20
2.5%	0.70	287.5	0.72	0.67	0.90	0.47	0.75		
97.5%	0.93	322.5	0.91	0.96	0.99	0.74	0.91		
S2 vs S3	0.67	307.5	0.52	0.83	0.81	0.56	0.65	1.72	0.32
2.5%	0.52	303.0	0.42	0.33	0.65	0.50	0.58		
97.5%	0.82	354.5	0.97	0.96	0.95	0.89	0.81		

Performance measures – area under the curve (AUC), negative and positive predictive values (NPV, PPV) and positive (pos.) and negative (neg.) likelihood ratios (LR) of ROC – and CAP cut-offs (thresholds) that maximise the distance from the diagonal (maximum Youden index)

Table S2: The p-values and 95% confidence intervals (CI) from Mann-Whitney U-tests comparing liver stiffness between steatosis grade groups.

	p-value	95% CI
S0 vs S1–3	0.04	–2.2 to 0.0
S0,1 vs S2,3	0.62	–1.5 to 0.9
S0–2 vs S3	0.78	–2.2 to 1.5
S0 vs S1	0.05	–2.6 to 0.0
S1 vs S2	0.57	–1.1 to 2.2
S2 vs S3	0.99	–2.9 to 2.2

Table S3: Regression models with and without body mass index (BMI) data.

	Estimate	2.5%	97.5%	p-value
Model (without BMI) including 224 patients				
Intercept	221.66	196.46	246.85	<0.001
Age	0.24	–0.2	0.69	0.28
Liver stiffness (kPa)	–0.90	–1.57	–0.23	0.01
Sex: male vs female	14.9	1.28	28.51	0.03
NAFLD: yes vs no	23.65	3.25	44.06	0.02
Steatosis (%)	1.17	0.83	1.52	<0.001
Adjusted R ²	0.38			
Residual standard error	48.4 (df = 218)			
Model without BMI but including only the 203 patients with BMI data available				
Intercept	226.38	199.73	253.04	<0.001
Age	0.16	–0.31	0.62	0.50
Liver stiffness (kPa)	–0.84	–1.53	–0.15	0.02
Sex: male vs. female	12.87	–1.53	27.27	0.08
NAFLD: yes vs no	24.72	3.88	45.57	0.02
Steatosis (%)	1.16	0.8	1.51	<0.001
Adjusted R ²	0.39			
Residual standard error	48.9 (df = 197)			
Model with BMI including the 203 patients with BMI data available				
Intercept	159.21	113.71	204.7	<0.001
Age	0.16	–0.3	0.61	0.50
Liver stiffness (kPa)	–0.95	–1.62	–0.27	0.01
Sex: male vs. female	15.47	1.4	29.54	0.03
NAFLD: yes vs no	21.26	0.91	41.61	0.04
Steatosis (%)	0.96	0.6	1.32	<0.001
BMI (mg/m ²)	2.69	1.19	4.18	<0.001
Adjusted R ²	0.42			
Residual standard error	47.5 (df = 196)			