

## NAFLD/NASH

**Impact of skin capsular distance on the performance of controlled attenuation parameter in patients with chronic liver disease**

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**Abstract**

**Background & Aims:** Controlled attenuation parameter (CAP) is a non-invasive method for evaluating hepatic steatosis. However, larger skin capsular distance (SCD) can affect the accuracy. The aim of this study was to investigate the impact of SCD on the diagnostic performance of CAP and liver stiffness measurement (LSM). **Methods:** Of 101 patients with non-alcoholic fatty liver disease (NAFLD) and 280 patients with chronic hepatitis B (CHB) who underwent liver biopsy were prospectively recruited. CAP, LSM and SCD were performed using FibroScan with M probe. The areas under receiver operating characteristics curves (AUROCs) were calculated to determine the diagnostic efficacy. The optimal thresholds were defined by the maximum Youden index. **Results:** SCD (B 30.34,  $P < 0.001$ ) and hepatic steatosis (B 23.04,  $P < 0.001$ ) were independently associated with CAP by multivariate analysis. The AUROCs were slightly higher for SCD  $< 25$  mm compared to those for SCD  $\geq 25$  mm for steatosis  $\geq 5\%$  (0.88 vs. 0.81),  $> 33\%$  (0.90 vs. 0.85) and  $> 66\%$  (0.84 vs. 0.72). For SCD  $< 25$  mm, the optimal CAP cut-offs for differentiating steatosis  $\geq 5\%$ ,  $> 33\%$  and  $> 66\%$  were 255.0 dB/m, 283.5 dB/m and 293.5 dB/m. However, cut-offs were elevated by approximately 60–70 dB/m for SCD  $\geq 25$  mm. When stratified by fibrosis grade, LSM was significantly affected by SCD  $\geq 25$  mm for advanced fibrosis ( $\geq F3$ ) in NAFLD, but not in CHB. **Conclusion:** CAP is a promising tool for detecting and quantifying hepatic steatosis. SCD  $\geq 25$  mm may cause overestimation of steatosis. Similarly, SCD  $\geq 25$  mm affects the detection of advanced fibrosis by LSM in NAFLD patients.

**Keywords**

controlled attenuation parameter – liver stiffness – non-alcoholic fatty liver disease – skin capsular distance – transient elastography

**Abbreviations**

BMI, body mass index; CAP, controlled attenuation parameter; CHB, chronic hepatitis B; IQR, interquartile range; LSM, liver stiffness measurement; NAFLD, non-alcoholic fatty liver disease; ROC curves and AUROCs, receiver operating characteristics curves and areas under the curves; SCD, skin capsular distance; TE, transient elastography.

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**Key points**

- SCD is independently associated with CAP. The areas under receiver operating characteristics curves (AUROCs) are slightly higher for SCD <25 mm compared to those for SCD ≥25 mm.
- For SCD <25 mm, the optimal CAP cut-offs for differentiating steatosis ≥5%, >33% and >66% are 255.0 dB/m, 283.5 dB/m and 293.5 dB/m. However, cut-offs were elevated by approximately 60–70 dB/m for SCD ≥25 mm.
- SCD ≥25 mm affects the detection of advanced fibrosis by LSM in NAFLD patients.
- A CAP algorithm for the obese population and developed specifically for the FibroScan XL probe would be welcome.

Liver stiffness measurement (LSM), using transient elastography (TE) by FibroScan, is a popular non-invasive method for diagnosing liver fibrosis. LSM has a greater accuracy in predicting bridging fibrosis and cirrhosis compared with other biomarkers (1). Although LSM is widely used in cases of chronic liver disease, high body mass index (BMI) and obesity are independent risk factors for unreliable LSM results as well as LSM failure if performed using an M probe (2). This limitation is particularly relevant in obese patients with a thicker subcutaneous fat layer, such as those with non-alcoholic fatty liver disease (NAFLD) (3, 4).

Recently, a novel physical parameter, 'controlled attenuation parameter' (CAP), has been implemented successfully based on TE and automatically estimates the ultrasonic attenuation of the liver. CAP has been shown to be highly correlated with liver steatosis grade (5). However, the failure rate of CAP measurement is increased in overweight and obese patients and can be as high as 58.4% among patients with BMI >40 kg/m<sup>2</sup> (6). The optimal cut-off, for example, to discriminate the presence of significant hepatic steatosis (steatosis involving ≥10% of hepatocytes) range from 222 dB/m (7) to 283 dB/m (5). CAP might have suboptimal accuracy for detection of severe steatosis (8).

The layers of tissues that connect the skin to the liver capsule were defined as the skin capsular distance (SCD), and these layers are mainly comprised of subcutaneous fatty tissue. SCD may contribute to the beam distortion and scattering that is known to attenuate the propagation of shear waves into the liver and ultrasonic signals used to assess the speed of propagation (5, 9, 10). In patients with SCD thicker than 25 mm, the attenuation may be enhanced and lead to an overestimation of LSM, and the failure rate of the M probe was markedly increased to 33% (3). Although an XL probe has been designed for overweight and obese patients, it is not widely available (11).

Along with hepatic steatosis, subcutaneous fat can attenuate the ultrasonic signals, and thus high SCD may be an important potential barrier to the effective use of CAP. Currently, CAP measurement has only been implemented using an M probe and can still produce valid results if the SCD is ≥25 mm (5). However, the effects of SCD on both CAP and LSM have not been evaluated.

The objectives of this study were to assess the impact of SCD on the diagnostic performance of CAP and LSM, and to improve the accuracy of CAP in diagnosing hepatic steatosis in chronic liver diseases.

**Patients and methods****Study population**

A prospective multicenter study was carried out in five Chinese liver centres (Xinhua Hospital, Shanghai; Zhengxing Hospital, Fujian; The Affiliated Hospital of Hangzhou Normal University, Zhejiang; Tianjin Second People's Hospital, Tianjin; 302 Military Hospital, Beijing) between May 2012 and May 2014. Consecutive patients aged ≥18 years who underwent liver biopsies and met the diagnostic criteria for NAFLD (12) or chronic hepatitis B (CHB) (13) were prospectively recruited. We excluded patients with a history of excessive ethanol consumption (140 g per week for men, 70 g for women) in the past 12 months, as well as patients who refused to undergo liver biopsies and those with secondary causes of hepatic steatosis (such as the use of systemic corticosteroids), anti-hepatitis C virus antibodies or common contraindications for TE (e.g. pregnancy, ascites, pacemaker implanted). The Ethics Committees of Xinhua Hospital approved this study, and all patients provided informed written consent prior to enrolment.

**Clinical and laboratory assessment**

Demographic information, including age, sex, history of drinking, hypertension and type 2 diabetes mellitus, was collected. Anthropometric measurements included BMI [weight (kg)/height (m)<sup>2</sup>], waist circumference (cm) and hip circumference (cm). Laboratory tests were performed within a week of liver biopsy. Hepatitis B virus DNA (for CHB patients only), prothrombin time, platelet counts, fasting plasma glucose, alanine aminotransferase, aspartate aminotransferase, albumin, total bilirubin, total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol levels were measured at each hospital's laboratory, using a standard testing kit and techniques.

**Controlled attenuation parameter and liver stiffness measurement**

TE was performed within 1 week preceding liver biopsy. The operators had performed TE evaluations on at least

300 patients prior to this study. CAP is a new proprietary algorithm that estimates the total ultrasonic attenuation, which is expressed in dB/m, and is currently only implemented using the M probe. In this study, FibroScan-502 (Echosens, Paris, France) with M probe (3.5 MHz) was used to capture CAP and LSM simultaneously, using the same TE signals. CAP values range from 100 to 400 dB/m, while LSM values are expressed as kilopascals (kPa) and range from 1.5 to 75 kPa. Measurements were performed on the right lobe of the liver, through the intercostal spaces, with the patient lying in the dorsal decubitus position and the right hand on the head to extend the intercostal space.

A reliable measurement of LSM was defined as obtaining 10 or more valid shots, a success rate of at least 60% and an interquartile range (IQR) <30% of the median LSM values. CAP was measured only on validated measurements, according to the same criteria used for LSM. If reliable measurements were not obtained after a minimum of 10 attempts, the results were defined as failed or unreliable and were excluded from our analysis.

#### Skin capsular distance measurement

The SCD includes the layers of tissues connecting the epidermis of the skin to the liver, and can contain various proportions of fatty tissue. A fixed-position FibroScan-502 with M probe was used, and a series of temporal ultrasound radiofrequency lines were acquired to measure the subcutaneous layer thickness (<25 mm and  $\geq$ 25 mm), which was subsequently separated from the liver tissue using real-time computation during TE scanning (9, 10). The value was based on the first measurement.

#### Liver histology

Percutaneous liver biopsy was performed using the 18-gauge BARD Max-Core Disposable Core Biopsy Instrument (BARD Biopsy Systems, Tempe, AZ, USA) at the right lobe, under real-time ultrasound guidance. All samples were  $\geq$ 16 mm, and included at least six portal tracts. Liver biopsy specimens were fixed in formalin, embedded in paraffin and stained with H&E, Masson's trichrome and reticulin. The liver biopsies were interpreted by three experienced hepatopathologists who were blinded to clinical data; discrepancies were resolved by consensus. For all samples, liver steatosis was categorized by visual assessment, according to the NAFLD activity score as S0 (<5%), S1 (5–33%), S2 (34–66%) or S3 (>66%) (14).

The grades and stages of the liver samples were dependent on the liver disease aetiology. The proposed NAFLD activity score  $\geq$ 5 was considered indicative of non-alcoholic steatohepatitis (14). Fibrosis of NAFLD was staged as follows: F0, absence of fibrosis; F1, perisinusoidal or periportal fibrosis; F2, perisinusoidal and portal/periportal fibrosis; F3, septal or bridging fibrosis; and F4, cirrhosis. The METAVIR classification was used

for CHB patients (15). Given the different diagnostic criteria, METAVIR grades  $\geq$ 2 in patients with CHB, and NAFLD activity scores  $\geq$ 5, were classified as moderate to severe inflammation. Significant fibrosis or advanced fibrosis was defined as  $\geq$ F2 or  $\geq$ F3 respectively.

#### Statistical analyses

All continuous variables and patient characteristics were expressed as medians (interquartile range (IQR)) or *n* (%), as appropriate. Chi-square or Fisher's exact tests were used to compare categorical data. The Mann-Whitney *U* test and Kruskal–Wallis test was used to compare two or more groups. Correlations between CAP and continuous variables were assessed using Spearman correlation coefficients ( $\rho$ ). Parameters that were significantly associated with CAP were subsequently entered into a multiple linear regression models (Stepwise method).

Receiver operating characteristic (ROC) curves were plotted, and areas under the curves (AUROCs) with 95% confidence interval (CI) were calculated to determine the diagnostic efficacy. The accuracy of CAP at optimal thresholds was defined by the maximum Youden index. For each optimal cut-off value, the sensitivity, specificity, positive predictive values (PPVs) and negative predictive values (NPVs), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were calculated. The general coincidence rate was calculated as (true positive + true negative)/overall patients.

All statistical analyses were performed using SPSS version 16.0 for WINDOWS (SPSS, Chicago, IL, USA) and MEDCALC statistical software version 11.0 (MedCalc, Mariakerke, Belgium). Two-sided *P*-values <0.05 were considered statistically significant.

## Results

#### Patients' characteristics and histological characteristics

Four hundred and thirty-four patients who underwent liver biopsy and TE using FibroScan were recruited. Twelve patients were excluded because of liver biopsy length <16 mm or insufficient portal tracts. Forty-one of the remaining 422 patients (9.7%) were excluded because of failed or unreliable LSM and/or CAP values. Thus, the remaining 381 patients included 332 with SCD <25 mm and 49 with SCD  $\geq$ 25 mm, who were evaluated in this study (Fig. S1).

The characteristics of this study population are outlined in Table 1 and histology features in Table S1. One hundred and one (26.5%) were NAFLD patients and 280 (73.5%) were CHB patients. The majority (70.3%) were men and the median age was 37.0 (IQR 29.0–46.0) years. In the SCD  $\geq$ 25 mm group, male gender, the prevalence of hypertension and type 2 diabetes mellitus, BMI, waist circumference, hip circumference, albumin, low-density lipoprotein cholesterol, measurements times, CAP value and rate of CAP/median were

**Table 1.** Baseline characteristics of study patients

Characteristics	All patients (n = 381)	Skin capsular distance		P-value
		<25 mm (n = 332)	≥25 mm (n = 49)	
<b>Liver disease aetiology</b>				
NAFLD, n (%)	101 (26.5)	67 (20.2)	34 (69.4)	<0.001
CHB, n (%)	280 (73.5)	265 (79.8)	15 (30.6)	
<b>Demographics</b>				
Male gender, n (%)	268 (70.3)	228 (68.7)	40 (81.6)	0.042
Age (years)	37.0 (29.0–46.0)	37.0 (29.0–45.8)	38.0 (30.0–48.5)	0.512
Hypertension, n (%)	36 (9.4)	30 (9.0)	6 (12.2)	0.046
Type 2 diabetes mellitus, n (%)	27 (7.1)	20 (6.0)	7 (14.4)	0.041
<b>Anthropometrics</b>				
Body mass index (kg/m <sup>2</sup> )	24.8 (22.3–27.5)	24.3 (21.9–26.5)	27.7 (25.1–29.6)	<0.001
<18.5	17 (4.5)	16 (4.8)	1 (2.0)	
18.5–24.9	192 (50.4)	181 (54.5)	11 (22.4)	
25.0–29.9	137 (36.0)	110 (33.1)	27 (55.1)	
≥30	35 (9.2)	25 (7.5)	10 (20.4)	
Waist circumference (cm)	89.0 (82.0–95.0)	88.0 (81.0–95.0)	92.5 (88.3–100.0)	0.001
Hip circumference (cm)	96.0 (92.0–102.0)	95.0 (91.0–101.0)	101.0 (96.0–105.0)	<0.001
<b>Biochemistry</b>				
Platelets (× 10 <sup>9</sup> /L)	190.0 (156.0–228.0)	188.5 (154.8–226.3)	208.5 (164.3–234.5)	0.092
Fasting blood glucose (mmol/L)	5.1 (4.7–5.7)	5.2 (4.7–5.7)	5.0 (4.6–6.1)	0.845
Alanine aminotransferase (U/L)	44.2 (23.1–74.1)	43.0 (23.1–77.2)	48.1 (26.9–68.9)	0.971
Aspartate aminotransferase (U/L)	33.0 (23.2–58.5)	34.0 (23.1–58.0)	33.0 (21.2–47.0)	0.358
Albumin (g/L)	44.0 (41.0–46.4)	44.0 (41.0–46.3)	44.1 (42.4–47.4)	0.041
Total bilirubin (μmol/L)	14.0 (10.6–18.3)	13.7 (10.6–18.3)	14.6 (10.8–18.4)	0.834
Prothrombin time (s)	11.9 (11.0–12.6)	11.9 (11.1–12.7)	11.6 (10.7–12.3)	0.057
Total cholesterol (mmol/L)	4.9 (4.2–5.6)	4.9 (4.1–5.6)	5.0 (4.3–5.4)	0.456
Triglycerides (mmol/L)	1.3 (0.9–2.0)	1.2 (0.9–2.0)	1.6 (1.0–2.1)	0.064
High-density lipoprotein cholesterol (mmol/L)	1.3 (1.1–1.5)	1.3 (1.1–1.5)	1.3 (1.1–1.5)	0.846
Low-density lipoprotein cholesterol (mmol/L)	2.8 (2.3–3.4)	2.8 (2.2–3.3)	2.9 (2.7–3.7)	0.009
<b>Transient elastography</b>				
Success rate	99.0 (98.0–100.0)	100 (100–100)	96 (91.5–99.0)	0.133
Time (s)	99.0 (80.0–141.5)	97.0 (79.0–137.3)	131.0 (91.0–201.5)	0.001
CAP (dB/m)	254.0 (210.0–305.0)	241.0 (206.0–290.8)	345.0 (308.0–360.5)	<0.001
Interquartile range /median <sup>CAP</sup>	12.3% (8.2–18.4%)	9.1% (6.0–12.3%)	13.7% (8.9–18.5%)	<0.001
LSM (kPa)	7.6 (5.5–11.8)	7.4 (5.5–11.5)	8.7 (5.7–11.8)	0.183
Interquartile range /median <sup>LSM</sup>	13.8% (9.2–18.5%)	13.8% (8.5–19.2%)	13.9% (9.9–18.7%)	0.531

All data are expressed as medians (interquartile range), or n (%), as appropriate. BMI, high body mass index; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

significant higher than that in the SCD <25 mm group (all  $P < 0.05$ ). The median HBV-DNA (log<sub>10</sub>) was 5.2 (IQR 3.0–7.2) IU/ml for CHB patients.

The median specimen length of liver biopsy was 20.0 mm (IQR 17.0–21.5), and the median number of portal tracts was 10 (IQR 8–15).

#### Factors associated with controlled attenuation parameter values

The median CAP measurements for NAFLD patients and CHB patients in S1, S2 and S3 were 273.0 dB/m and 273.0 dB/m, 327.0 dB/m and 335.5 dB/m and 346.5 dB/m and 309.0 dB/m respectively. There was no significant difference between the NAFLD and CHB group for each hepatic steatosis grade (all  $P > 0.05$ ) (Fig. S2). CAP values were strongly correlated with steatosis grade ( $\rho = 0.71$ ,  $P < 0.001$ ) and moderately

correlated with high SCD ( $\rho = 0.44$ ,  $P < 0.001$ ). Old age, male sex, high BMI, waist circumference, hip circumference, platelet count, fasting blood glucose, aspartate aminotransferase, albumin, total bilirubin, total cholesterol, triglyceride, low-density lipoprotein cholesterol levels and moderate to severe inflammation were also significantly associated with CAP values by univariate analysis. Multiple linear regression (Stepwise method) revealed that SCD (B 30.34, 95% CI 21.18–39.49,  $P < 0.001$ ) and hepatic steatosis (B 23.04, 95% CI 19.74–34.34,  $P < 0.001$ ) were independently positively associated with CAP values (Table S2).

#### The impact of skin-liver capsule on the diagnostic performance of controlled attenuation parameter

Median CAP values significantly increased with steatosis grade for both SCD <25 mm and SCD ≥25 mm (all

**Table 2.** Distribution of CAP values for different steatosis grades following stratification of SCD

Steatosis grade	CAP (dB/m)			P*
	Total subjects (n = 381)	SCD <25 mm (n = 332)	SCD ≥25 mm (n = 49)	
S0 (<5%)	216.5 (191.8–246.5)	215.0 (190.0–241.0)	303.0 (266.5–334.0)	<0.001
S1 (5–33%)	273.0 (248.5–304.5)	269.0 (244.5–298.5)	318.0 (280.5–341.8)§	<0.001
S2 (34–66%)	331.0 (301.5–359.0)	315.0 (293.0–349.0)	350.5 (341.8–366.3)	0.001
S3 (>66%)‡	344.0 (308.0–359.0)	318.0 (298.0–346.5)	362.5 (342.3–375.8)	0.009
P†	<0.001	<0.001	<0.001	

Variables were given as medians (interquartile range).

\*Comparison between CAP in skin capsular distance <25 mm and ≥25 mm, within the same steatosis grade.

†Correlation between CAP and the grade of hepatic steatosis.

‡P > 0.05, S3 vs. S2.

§P > 0.05, S1 vs. S0.

P < 0.001, Table 2). After adjustment, CAP values were significantly higher in patients with SCD ≥25 mm compared to those with SCD <25 mm, for the same steatosis grade (Table 2).

To evaluate the ability of CAP to discriminate the grading of hepatic steatosis, ROC curves and the corresponding AUROCs and 95% CIs were calculated. Among subjects with SCD <25 mm or SCD ≥25 mm, the AUROCs for steatosis grades of ≥5%, >33% and >66% were 0.88 and 0.81 (P = 0.406), 0.90 and 0.85 (P = 0.381) and 0.84 and 0.72 (P = 0.303) respectively (Fig. 1).

#### The optimal cut-off values of controlled attenuation parameter for estimation of hepatic steatosis

The optimal CAP cut-offs for the diagnosis of hepatic steatosis grade, stratified by SCD are outlined in Table 3. At each steatosis grade, the optimal CAP cut-offs were much higher for SCD >25 mm compared to those for SCD <25 mm. Results for the diagnostic accuracy of CAP are also outlined in Table 3.

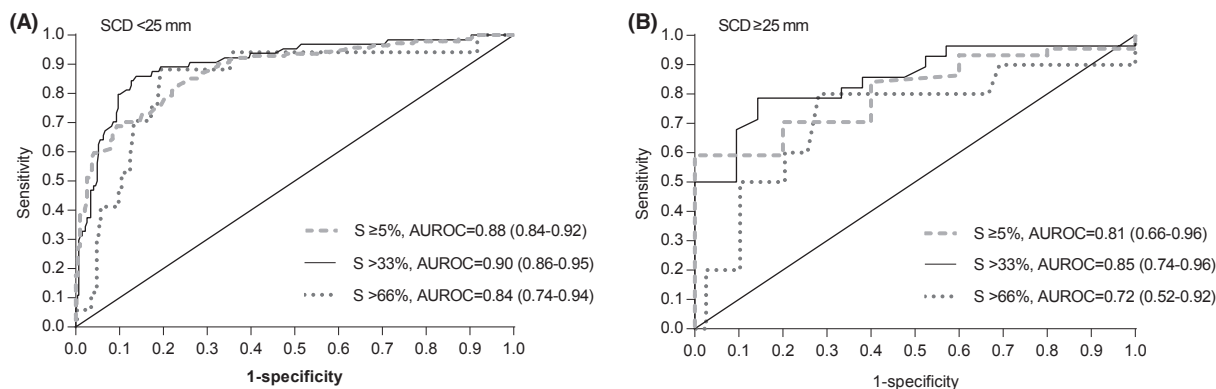
The general coincidence rate was used to determine the correct rate. For SCD <25 mm, the general coinci-

dence rate of CAP discriminating S1, S2 and S3 was 81.6%, 86.1% and 81.0% respectively. For SCD ≥25 mm, the general coincidence rate was 63.3%, 81.6% and 73.5% respectively.

#### The impact of skin capsular distance on the diagnostic performance of liver stiffness measurement

There was no significant correlation between SCD and LSM values in NAFLD subjects ( $\rho = 0.15$ , P = 0.144) or CHB subjects ( $\rho = 0.06$ , P = 0.343). We also evaluated fibrosis stage by dividing cases into significant fibrosis (≥F2) and advanced fibrosis (≥F3). Among subjects without significant fibrosis (F0–F1) or with significant fibrosis (F2–F4), there was a trend where LSM values were slightly higher for SCD ≥25 mm compared to those for SCD <25 mm among both NAFLD (Fig. 2A) and CHB subjects (Fig. 2B).

Similar results were obtained for subjects without advanced fibrosis (F0–F2) among NAFLD (Fig. 2C) or CHB (Fig. 2D) subjects. However, the only statistically significant difference in LSM was detected among NAFLD patients with advanced fibrosis (F3–F4), where the median LSM value was 18.5 kPa (IQR, 12.8–23.8) for



**Fig. 1.** Receiver operating characteristic curves and area under the curves (AUROCs) for the detection of steatosis grades ≥5%, >33% and >66% for skin-liver capsule (SCD) distance <25 mm (A) and SCD ≥25 mm (B).

**Table 3.** Diagnostic performance of controlled attenuation parameter (CAP) for the detection of steatosis grade

Steatosis	SCD (mm)	Optimal cut-off* (dB/m)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	PLR (95% CI)	NLR (95% CI)
S0 vs. S1–3	<25	255.0	0.75 (0.67–0.82)	0.82 (0.75–0.87)	0.75 (0.67–0.82)	0.82 (0.75–0.87)	4.10 (3.00–5.62)	0.30 (0.23–0.41)
	≥25	326.5	0.71 (0.55–0.83)	0.80 (0.30–0.99)	0.97 (0.82–0.99)	0.24 (0.09–0.50)	3.52 (0.60–20.55)	0.37 (0.21–0.64)
S0–1 vs. S2–3	<25	283.5	0.86 (0.74–0.93)	0.86 (0.81–0.90)	0.60 (0.49–0.70)	0.96 (0.93–0.98)	6.23 (4.54–8.53)	0.16 (0.09–0.30)
	≥25	344.0	0.79 (0.59–0.91)	0.86 (0.63–0.96)	0.88 (0.68–0.97)	0.75 (0.53–0.89)	5.50 (1.90–15.96)	0.25 (0.12–0.52)
S0–2 vs. S3	<25	293.5	0.88 (0.62–0.98)	0.81 (0.76–0.85)	0.20 (0.12–0.31)	0.99 (0.97–0.99)	4.56 (3.43–6.06)	0.15 (0.04–0.54)
	≥25	350.5	0.80 (0.44–0.97)	0.72 (0.55–0.85)	0.42 (0.21–0.66)	0.93 (0.77–0.99)	2.84 (1.57–5.11)	0.28 (0.08–0.98)

95% CI, 95% confidence interval; PPV and NPV, positive and negative predictive values; PLR and NLR, positive and negative likelihood ratio; SCD, skin capsular distance.

\*Optimal cut-offs were defined by the maximal sum of sensitivity and specificity.

SCD  $\geq 25$  mm, which was significant higher than that for SCD  $< 25$  mm (median 8.8 kPa; IQR, 5.3–10.8;  $P = 0.045$ ) (Fig. 2C).

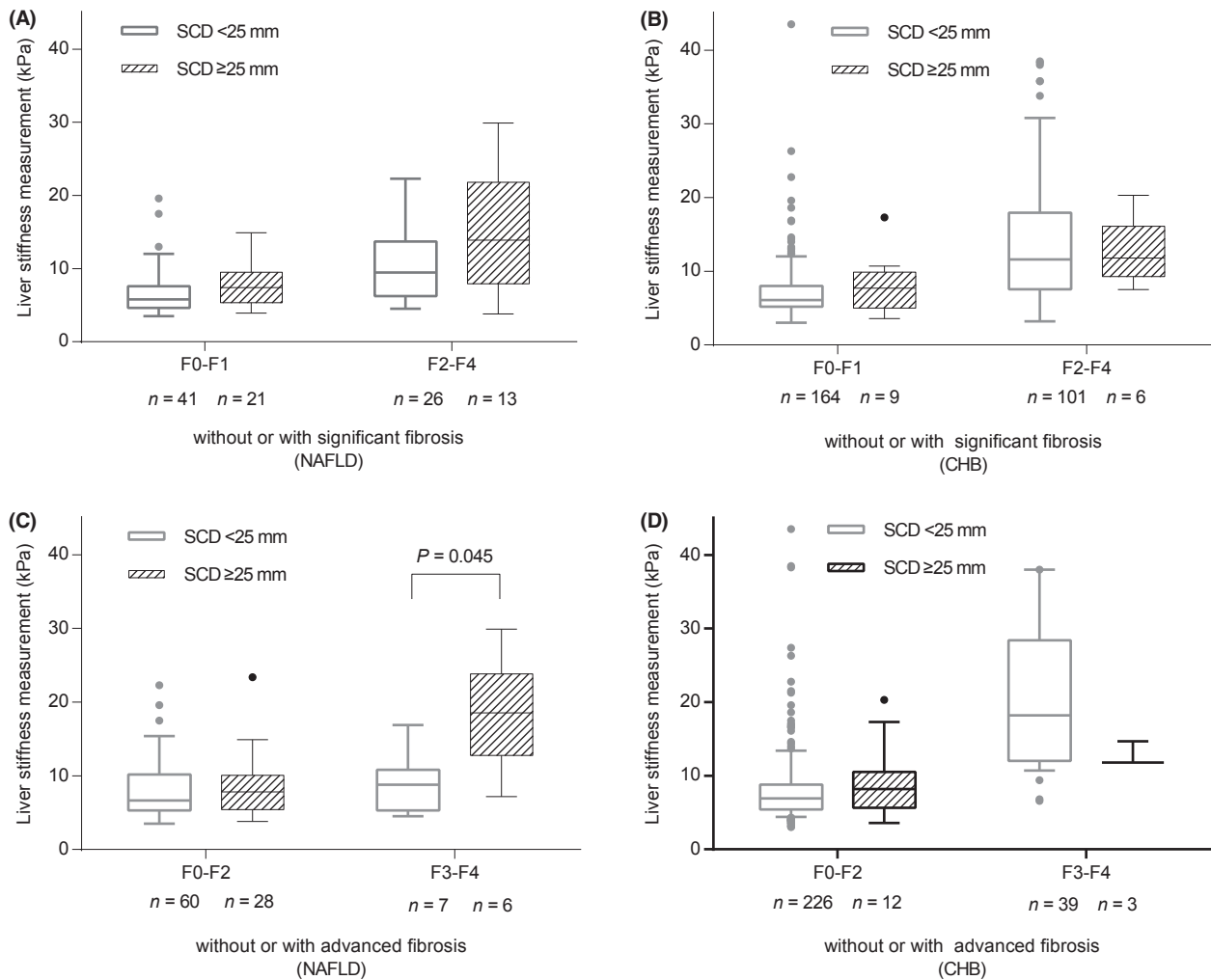
For NAFLD subjects, the optimal LSM cut-off for the diagnosis of advanced fibrosis varied according to SCD. For SCD  $< 25$  mm, an optimal cut-off of 8.7 kPa was appropriate, with an AUROC of 0.62 (95% CI 0.52–0.84), sensitivity of 0.71 (95% CI 0.60–0.95) and specificity of 0.77 (95% CI 0.58–0.82). For SCD  $\geq 25$  mm, an optimal cut-off of 12.9 kPa was appropriate, with an AUROC of 0.88 (95% CI 0.70–0.99), sensitivity of 0.83 (95% CI 0.37–0.99) and specificity of 0.89 (95% CI 0.71–0.97).

## Discussion

In this multicenter study, we found that CAP could be used to detect hepatic steatosis with good diagnostic accuracy, regardless of aetiology, as has been previously reported (16). However, SCD was an independently factor influence the CAP values and always overestimated for SCD  $\geq 25$  mm. Although LSM was slightly higher for SCD  $\geq 25$  mm in CHB subjects after adjusting for the liver fibrosis stage, this result was not statistically significant. In contrast, LSM was significantly affected by SCD in advanced fibrosis ( $\geq F3$ ) among NAFLD subjects.

Liver biopsy is prone to sampling error, higher risk of complications and is difficult to repeat; hence, non-invasive tests are urgently needed. One such method is FibroScan, which can measure the ultrasound attenuation of hepatic fat, and can simultaneously evaluate fibrosis (by LSM). However, the accuracy of this widely used tool has been challenged. LSM may be limited by various factors, including the cause of chronic hepatitis, acute liver parenchymal injury, central venous pressure or extrahepatic cholestasis (17–19). Higher BMI and SCD can also lead to unreliable or failed LSM measurements with the M probe (3, 4).

A recent study has reported that the diagnosis accuracy of CAP is higher than that of the Hepatic Steatosis Index and the Fatty Liver Index (5) and is even more accurate than  $^1\text{H}$ -magnetic resonance spectroscopy (20). However, in a small sample study, we have reported that BMI was the most relevant factor that influenced CAP (21). In the present study, we observed that SCD  $\geq 25$  mm had a more significant impact on CAP than BMI. As reported by Myers *et al.* (5), who noted that the AUROC for differentiating steatosis  $\geq 10\%$  was higher among patients with an SCD  $< 25$  mm (0.82 vs. 0.69). Our study observed that the CAP performance was good to excellent in differentiating steatosis for SCD  $< 25$  mm, and poor for SCD  $\geq 25$  mm. Among reliable measurements, CAP was grossly overestimated in subjects with SCD  $\geq 25$  mm and this highlights the need for accurate CAP cut-off values. Thus, we recommend considering SCD thickness prior to selecting the cut-off values for CAP when evaluating hepatic steatosis.



**Fig. 2.** Distribution of liver stiffness measurement (LSM) values for skin-liver capsule distance (SCD) <25 mm or ≥25 mm, according to (A) non-alcoholic fatty liver disease (NAFLD) and (B) chronic hepatitis B (CHB) with or without significant fibrosis and (C) NAFLD and (D) CHB with and without advanced fibrosis. ●Outlier: any patient result lying outside the upper or lower whiskers.

For SCD <25 mm, we selected 255.0 dB/m, 283.5 dB/m and 293.5 dB/m as optimal cut-offs to identify steatosis of ≥5%, >33% and >66% respectively. Our optimal CAP cut-off value for the diagnosis of hepatic steatosis ≥5% is similar to previously reported values, including 250 dB/m (8, 22) and 263 dB/m (23). However, Myers *et al.* (5) have proposed a higher cut-off values (283 dB/m) for the differentiation of steatosis ≥10%. In that study, patients had an BMI of ≥28 kg/m<sup>2</sup> and 16% of subjects with SCD ≥25 mm, which might have resulted in an overestimated cut-off. For patients with SCD ≥25 mm, we observed that the reference cut-off values were elevated by approximately 60–70 dB/m. We also note that the M probe used for these patients may be unsuitable, as the reference values were too similar to distinguish the degree of fatty liver.

Different LSM cut-off values have been reported in previous studies of patients with chronic liver disease of

various aetiologies (24) and it remains unclear whether SCD affects LSM. In the present study, we observed that NAFLD patients with SCD ≥25 mm had increased LSM values, especially in advanced fibrosis (≥F3). Unlike CAP, LSM was not significantly influenced by SCD in CHB patients. When evaluating NAFLD patients using FibroScan with M probe, we propose that the optimal LSM cut-offs for the diagnosis of advanced fibrosis (≥F3) is 8.7 kPa for SCD <25 mm, which is similarly to previously reported values of 8.0 kPa (25) and 8.7 kPa (26). Although higher AUROC was observed for SCD ≥25 mm, the risk of misclassification of advanced fibrosis in NAFLD patients is increased, and thus, the use of the XL probe is recommended.

Although fat-mimicking materials affect the shear waves (10), SCD seems not to have a significant effect on LSM values. However, SCD does affect ultrasonic attenuation, and can lead to overestimation of CAP

values. As the M probe is still the only measurement tool for CAP, the current optimal cut-off values are unsuitable for patients with SCD  $\geq 25$  mm.

Our study has several limitations. First, chronic liver disease patients, including those with chronic hepatitis C (CHC), were not included in our study. However, as TE is validated in CHC, there is limited data available for CHB and NAFLD patients (25). For CHB, hepatic steatosis is typically associated with obesity, rather than CHC.

Second, our study did not analyse the factors that caused the failure of CAP or LSM measurements, as the primary aim of this study was to determine whether SCD affected the reliability of TE. We also note that such analysis would be complicated by the small number of patients that experience failed measurements. Third, we did not compare our results to those obtained using other imaging modalities (e.g. ultrasonography), given their low sensitivity (5, 27). Last, we propose new cut-off values for CAP, but it is needed more validation cohort.

In conclusion, CAP presents several diagnostic advantages (simplicity, operator-independence, sensitivity and immediate assessment of steatosis) when used simultaneously with LSM to stage hepatic fibrosis. Given the limitations of the M probe, CAP is appropriate for patients with SCD  $< 25$  mm, and not for those with SCD  $\geq 25$  mm unless using SCD-adjusted cut-off value. Moreover, the SCD-modified cut-off value of LSM might be used for diagnosis of advanced fibrosis in NAFLD patients with larger SCD. As NAFLD is strongly associated with obesity, a CAP algorithm for the obese population and developed specifically for the FibroScan XL probe, would be welcome.

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