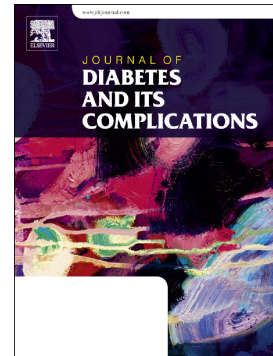# Accepted Manuscript

Controlled attenuation parameter in NAFLD identifies risk of suboptimal glycaemic and metabolic control

Preya Janubhai Patel, Fabrina Hossain, Leigh Ula Horsfall, Xuan Banh, Kelly Lee Hayward, Suzanne Williams, Tracey Johnson, Nigel Neil Brown, Nivene Saad, Patricia Casarolli Valery, Katharine Margaret Irvine, Andrew Donald Clouston, Katherine Anne Stuart, Anthony William Russell, Elizabeth Ellen Powell

Please cite this article as: Preya Janubhai Patel, Fabrina Hossain, Leigh Ula Horsfall, Xuan Banh, Kelly Lee Hayward, Suzanne Williams, Tracey Johnson, Nigel Neil Brown, Nivene Saad, Patricia Casarolli Valery, Katharine Margaret Irvine, Andrew Donald Clouston, Katherine Anne Stuart, Anthony William Russell, Elizabeth Ellen Powell , Controlled attenuation parameter in NAFLD identifies risk of suboptimal glycaemic and metabolic control. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Jdc(2018), doi:10.1016/j.jdiacomp.2018.05.008

Controlled attenuation parameter in NAFLD identifies risk of suboptimal glycaemic and metabolic control

Short Title: Utility of CAP in identifying metabolic risk

Preya Janubhai Patel[1,2], Fabrina Hossain[3], Leigh Ula Horsfall[1,2], Xuan Banh[2], Kelly Lee Hayward[2], Suzanne Williams[3], Tracey Johnson[3], Nigel Neil Brown[4], Nivene Saad[5,6], Patricia Casarolli Valery[7], Katharine Margaret Irvine[2,8], Andrew Donald Clouston[2], Katherine Anne Stuart[1], *Anthony William Russell[6,9], *Elizabeth Ellen Powell[1,2]

[1]Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane, Australia; [2]Centre for Liver Disease Research, Translational Research Institute, Faculty of Medicine and Biomedical Science, The University of Queensland, Brisbane, Australia; [3]Inala Primary Care, Brisbane, Australia; [4]Pathology Queensland, Brisbane, Australia; [5]Department of Radiology, Princess Alexandra Hospital, Brisbane, Australia; [6]School of Medicine, University of Queensland, Brisbane Australia; [7]QIMR Berghofer Medical Research Institute, Brisbane, Australia; [8]Mater Research, Translational Research Institute, The University of Queensland, Brisbane, Australia; [9]Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Australia

* Equal contribution as Senior Authors.

Corresponding author:

Prof Elizabeth Powell, e.powell@uq.edu.au

Department of Gastroenterology and Hepatology

Princess Alexandra Hospital, Brisbane

Ipswich Rd

Woolloongabba, QLD 4102

Conflicts of interest:

The authors have no conflicts of interest to declare.

Word Count:

Abstract: 208

Main Text: 4005

Abbreviations

NAFLD, non-alcoholic fatty liver disease; MetS, metabolic syndrome; T2DM, type 2 diabetes mellitus; CAP, Controlled Attenuation Parameter; LSM, liver stiffness measurement; IQR, interquartile range; BMI, body mass index; cIQR, Controlled Attenuation Parameter interquartile range; SD, standard deviation; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; HbA1c, glycated haemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; AUROC, area under the receiver-operating characteristic curve; MRI-PDFF, magnetic resonance imaging proton-density-fat-fraction.

Abstract

Aims:

To examine the relationship between steatosis quantified by controlled attenuation Parameter (CAP) values and glycaemic/metabolic control.

Methods:

230 patients, recruited from an Endocrine clinic or primary care underwent routine Hepatology assessment, with liver stiffness measurements and simultaneous CAP. Multivariable logistic regression was performed to identify potential predictors of Metabolic Syndrome (MetS), HbA1c≥7%, use of insulin, hypertriglyceridaemia and CAP≥300dB/m.

Results:

Patients were 56.7 ± 12.3 years of age with a high prevalence of MetS (83.5%), T2DM (81.3%), and BMI≥40kg/m^2(18%). Median CAP score was 344dB/m, ranging from 128 to 400dB/m. BMI (aOR 1.140 95%CI 1.068-1.216), requirement for insulin (aOR 2.599 95%CI 1.212-5.575), and serum ALT (aOR 1.018 95%CI 1.004-1.033) were independently associated with CAP≥300dB/m. Patients with CAP interquartile range<40 (68%) had a higher median serum ALT level (p=0.029), greater prevalence of BMI≥40kg/m^2 (p=0.020) and higher median CAP score (p<0.001). Patients with higher CAP scores were more likely to have MetS (aOR 1.011 95%CI 1.003-1.019), HBA1c≥7 (aOR 1.010 95%CI 1.003-1.016), requirement for insulin (aOR 1.007 95%CI 1.002-1.013) and hypertriglyceridemia (aOR 1.007 95%CI 1.002-1.013).

Conclusions:

Our data demonstrate that an elevated CAP reflects suboptimal metabolic control. In diabetic patients with NAFLD, CAP may be a useful point-of-care test to identify patients at risk of poorly controlled metabolic comorbidities or advanced diabetes.

Keywords: steatosis, metabolic syndrome, controlled attenuation parameter, transient elastography, non-alcoholic fatty liver disease

Introduction

The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing in association with the widespread presence of metabolic syndrome (MetS), obesity and type 2 diabetes (T2DM).[1, 2,] Diagnosis of NAFLD requires demonstration of steatosis in >5% of hepatocytes in the presence of metabolic risk factors and the exclusion of significant alcohol consumption (≥20g/day) or other chronic liver diseases.[3] In clinical practice, the presence of steatosis is usually determined by liver ultrasound, as this method is widely available, simple and inexpensive.[4] For the detection of moderate to severe steatosis (>33% steatotic hepatocytes), liver ultrasound has a sensitivity and specificity of 84.8% and 93.6%, respectively.[4] Although earlier studies suggested ultrasound had limited ability in detecting mild steatosis, more recent studies have determined that using a combination of different echographic parameters and a semi-quantitative ultrasonographic score, lesser amounts of steatosis (10 – 12.5%) may be detected.[5, 6] In view of this ultrasonography remains the first line imaging technique investigating NAFLD.[3]

More recently the controlled attenuation parameter (CAP) has been evaluated for detection and quantitation of steatosis by measuring the degree of ultrasound attenuation during simultaneous acquisition of liver stiffness measurements (LSM). This rapid, point-of-care test provides a numerical value that correlates with the histological grade of steatosis[7, 8] although discordance has been reported in approximately 15% of cases.[9] Performance of CAP with the XL probe has shown similar results to the M probe and the same cut-off values have been applied in some[10] but not all studies.[11] The optimal threshold of CAP for the detection of steatosis is reported to be around 250dB/m, whereas a cut-off of 280dB/m identifies moderate to severe steatosis.[9, 10] CAP measurements are only obtained when the associated LSM are valid, however additional validity criteria for CAP remain poorly defined. Two recent studies found that accuracy of CAP for detection of steatosis was more reliable when the interquartile range (IQR) of CAP was <30dB/m[11] or <40dB/m.[12]

In addition to assessing steatosis, recent studies suggest that CAP also provides information about the MetS.[13, 14] NAFLD has a complex, bi-directional relationship with the MetS[15] and previous studies have shown that NAFLD is closely linked with increased BMI, waist circumference, hypertriglyceridemia and hyperuricemia[16] as well as poor metabolic control in type 2 diabetes.[17] CAP measurements have been shown to increase progressively with

the number of MetS components[14, 18] prompting speculation that CAP may be an objective tool to evaluate the MetS.[10, 13, 18] We hypothesized that an elevated CAP score may also have a role in identifying patients with type 2 diabetes at risk of suboptimal glycaemic or metabolic control and that this relationship may be independent of traditional risk factors such as body mass index (BMI) or age.

Therefore the purpose of this study was to assess the performance of CAP in a cohort of NAFLD patients with a high prevalence of T2DM and MetS, and to examine the relationship between CAP values, parameters of the MetS and glycaemic/metabolic control. In addition we aimed to assess factors associated with the CAP IQR (cIQR) quality indicator (cIQR<40dB/m).

Methods

Subjects

This is a cross-sectional analysis of a prospective study involving patients identified with NAFLD between October 2015 and August 2017.

The source population included (i) consecutive patients attending the Diabetes clinic at the Princess Alexandra Hospital, Brisbane, and (ii) all patients referred to secondary care from primary care with a history of fatty liver, T2DM or MetS during the study period. All eligible patients were invited to attend the liver clinic at the Princess Alexandra Hospital, Brisbane, for further clinical assessment.

Patients were eligible for inclusion if they had attended the liver clinic and had a diagnosis of NAFLD defined by demonstration of hepatic steatosis by liver ultrasound in the presence of metabolic risk factors and the exclusion of significant alcohol consumption (≥20g/day) or other chronic liver diseases (including a prior history of alcohol-related liver disease).[3] Patients were excluded if they had stage 5 chronic kidney disease, renal replacement therapy, history of organ transplant or if other causes of hepatic steatosis were suspected. Thirty-five per cent of the primary care patients were recruited from a single General Practice (Inala Primary Care) that delivers an integrated primary-secondary care diabetes service.

Informed written consent was obtained from each eligible patient. The protocol was approved by the Metro South Health and The University of Queensland Human Research Ethics Committees (HREC/15/QPAH/301; UQ2015001047).

Clinical assessment

Medical history was obtained regarding lifetime alcohol consumption,[19] previously diagnosed liver disease, other medical conditions, and use of medications.[20] Clinical assessment included anthropometric measurements (weight, height, girth), laboratory tests (routine biochemical, haematological, serological assays), transient elastography and liver ultrasound. Definition of obesity based on BMI was adjusted according to ethnicity for patients from South Asia (BMI 23-27.4kg/m^2 = overweight, BMI 27.5-30.0kg/m^2 = Class 1 obese).[21] Metabolic syndrome was defined as central obesity (waist circumference: Europid male ≥ 94 cm, South Asian male ≥ 90 cm, female ≥ 80 cm), plus any two of the following

four factors: raised fasting plasma glucose or previously diagnosed T2DM, elevated blood pressure or treatment of previously diagnosed hypertension, dyslipidaemia (defined as raised triglycerides or reduced HDL cholesterol or specific treatment for these lipid abnormalities).[22]

Transient elastography

CAP and LSM were performed after a 3 hour fast using FibroScan® technology (Echosens, Paris, France) with the standard M or XL probes in accordance with the manufacturer's instructions. Recommended standard FibroScan® operating procedures were followed along with adherence to criteria for definition of reliable LSM. The XL probe was used when the skin-capsule depth was ≥2.5cm. CAP scores ranging between 100 and 400dB/m were only obtained when the associated LSM were valid (IQR-to-median ratio of 10 acquisitions ≤0.3). Quality indicators to assist with interpretation of CAP data are still being defined. The cIQR was calculated, and factors associated with cIQR<40dB/m[12] were determined.

 The optimal threshold of CAP for the detection of steatosis is reported to be between 246 and 288dB/m.[10, 11] Previous studies have identified moderate/severe steatosis is very likely with CAP values >300–310dB/m (positive predictive value 80%).[10, 23] In accordance with these CAP thresholds, patients were separated into 3 groups: low CAP<250dB/m, intermediate 250–300dB/m and high >300dB/m.[10]

Liver biopsy was performed in a proportion of patients with advanced fibrosis or cirrhosis based on the FibroScan® examination, discordant results of investigations for cirrhosis, or interest in participating in a clinical therapy trial. Liver histology was assessed by a single experienced pathologist (ADC) who was blinded to the clinical data and histological scoring was performed according to the system of Kleiner et al.[24]

Data analysis

Participant socio-demographic and clinical characteristics were described using frequency and percentage for categorical variables, mean and standard deviation (SD) for continuous data normally distributed, and median and IQR for non-normally distributed data. Correlation between continuous variables was assessed using Pearson correlation or Spearman's rho correlation (non-parametric case). The univariate relationship between two categorical variables was assessed using Pearson's Chi-square test or Fisher's exact test

where appropriate. Post-hoc testing was conducted using a partitioning Chi-square when required. The comparison of a continuous variable between two groups was tested using the independent T-test or Mann-Whitney U test (non-parametric). When comparing more than 2 groups one-way ANOVA or Kruskal-Wallis test (non-parametric case) was used. Univariate logistic regression was performed to identify potential predictors of MetS, HbA1c≥7% (53mmol/mol), requirement for insulin, hypertriglyceridaemia and CAP≥300dB/m. All variables from the univariate analysis with p-values less than 0.2 were included in a multiple logistic regression model with backward stepwise selection to identify factors influencing the outcome. Odds ratios (OR), adjusted odds ratios (aOR) and 95% confidence interval (CI) were reported. All p values were 2-sided and statistical significance was set at alpha=0.05. Data analysis was conducted using SPSS Inc version 24.0 (College Station TX: StatCorp LP; 2013).

Results

Characteristics of the study population

Of the 252 patients with NAFLD reviewed in the Hepatology Clinic, 230 (91.3%) had reliable LSM by either the XL probe (76.5%) or M probe (23.5%) and were included in the CAP examination.

The mean age of subjects was 56.7±12.3 years and 54.8% were male. The majority (77.0%) were Caucasian, with a mean BMI of 34.3±7.8kg/m^2 and mean girth 115.4±18.0cm. Overall, patients had a high prevalence of MetS (83.5%), T2DM (81.3%), and more than 18% of the cohort had ≥class 3 obesity (BMI≥40 kg/m^2). Median LSM was 6.1kPa with a range from 2.5 to 63.9kPa.

Factors associated with CAP score

The median CAP score was 344dB/m with a range from 128 to 400dB/m. 74.3% of patients had a CAP≥300dB/m consistent with moderate/severe steatosis. The demographic and clinical characteristics of the cohort according to CAP threshold category are summarized in Table 1. There was an increase in BMI, girth and prevalence of MetS with increasing CAP score category (p<0.001), along with increasing prevalence of suboptimal glycaemic control (HbA1c≥7%, p=0.025), hypertriglyceridemia (p=0.012) and requirement for insulin (p=0.006). Although LSM increased across the CAP categories (p<0.001), the median values were low.

Liver histology was available for 47 patients and showed grade 1 steatosis in 11 patients, grade 2 in 8, grade 3 in 28. All patients with grade 2 or 3 (moderate/severe) steatosis on liver histology had CAP≥300dB/m.

Multivariable logistic regression analysis was used to determine the factors associated with CAP≥300dB/m compared to CAP<300dB/m. The final model identified BMI, LSM, requirement for insulin, serum ALT level and presence of dyslipidaemia as significant factors influencing CAP ≥300 dB/m. BMI, requiring insulin, and serum ALT remained independently associated with CAP≥300dB/m (Table 2).

Influence of CAP on presence of the MetS, glycaemic control and dyslipidaemia

Multivariable logistic regression analysis was used to determine the factors associated with presence of the MetS, glycaemic control (specifically HbA1c≥7% and requirement for insulin) and hypertriglyceridemia (defined as serum triglycerides ≥1.7mmol/L) (Table 3). These analyses identified that, independent of BMI and age, for every 1dB/m increase in CAP score, there was a 1.011-fold increase (95%CI 1.003-1.019; p=0.009) in risk of having the MetS. Patients with a higher CAP were more likely to have a HbA1c≥7% (aOR 1.007 95%CI 1.002-1.013), and require insulin (aOR1.010 95%CI 1.002-1.013). Multivariable logistic regression analysis also identified CAP as a significant factor influencing the presence of hypertriglyceridaemia, however BMI, age and gender were included in the model as variables of interest. For every 1 dB/m increase in CAP score, there was a 1.007-fold increase (95%CI 1.002-1.013) in risk of having hypertriglyceridaemia.

Factors associated with CAP IQR

The relationship between cIQR and CAP score is illustrated in Figure 1a. In contrast to LSM (Figure 1b), cIQR had a moderate inverse correlation with CAP (Spearman's Rho=-0.467, p<0.001). Of the 230 patients with reliable LSM, 157 (68.3%) had CAP scores with cIQR<40dB/m. Demographic and clinical characteristics according to CAP scores with cIQR< or ≥40dB/m are summarized in Table 4. Patients with cIQR<40 (68%) had a higher prevalence of BMI≥40kg/m^2 (p=0.020), higher median serum ALT level (p=0.029), and higher median CAP score (p<0.001).

Multivariable logistic regression analysis used to determine the factors associated with CAP≥300dB/m compared to CAP<300dB/m was repeated in those patients with cIQR<40

(N=157). The final model obtained identified BMI (aOR1.189 95%CI 1.086-1.298), and requiring insulin (aOR4.319 95%CI 1.354-13.778), as significant factors influencing CAP≥300dB/m.

Discussion

In this cohort of patients with NAFLD and a high prevalence of T2DM and MetS, CAP was independently associated with presence of the MetS, and with the severity of metabolic abnormalities. These results support previous studies which demonstrated that presence of the MetS was independently associated with elevated CAP score, along with insulin resistance and increased serum uric acid.[14] In addition, our study showed that increasing CAP was associated with poorer diabetic control (defined by HbA1c≥7%, increasing number of diabetic medications prescribed, and requiring insulin) and hypertriglyceridemia. This information may add value to results provided by the clinical, laboratory and imaging studies performed routinely in patients with NAFLD, and suggests that CAP may be a useful surrogate marker of more advanced diabetes or metabolic disease duration.

The accuracy of CAP for detection of steatosis ≥10% and differentiation between steatosis grades at least 2 grades apart, is reported to be good, and is independent of histological fibrosis stage, activity grade and liver disease etiology.[8, 25-27] However clinical use of this tool has been hampered by the lack of validity criteria for CAP and knowledge of the factors associated with inaccurate CAP measurements. For LSM, the most important validity indicator is the IQR-to-median ratio.[28] The IQR of LSM increases with higher median LSM values and therefore normalization of IQR to the median LSM is necessary to allow its use as a quality indicator across a broad range of LSM.[12] In contrast, we and others[12] have shown that cIQR decreases with increasing median CAP value, possibly related to the relatively high number of patients scoring close to the maximum CAP value of 400dB/m. Wong and colleagues showed that the accuracy of CAP for the diagnosis of fatty liver by M probe is lower if the cIQR is ≥40dB/m.[12] In their derivation cohort, the area under the receiver-operating characteristic curve (AUROC) for CAP diagnosis of fatty liver was 0.86, 0.89 and 0.76 in patients with cIQR of <20, 20-39 and ≥40dB/m, respectively, and was similar in a validation cohort of 414 patients.[12] Similarly, in a smaller study of 119 subjects who

underwent MRI-PDFF, the diagnostic accuracy of CAP increased when the cIQR was <30dB/m (AUROC 0.92 and 0.70 for cIQR < or ≥30dB/m respectively).[11]

Compared with LSM, the proportion of patients with CAP measurements that meet proposed validity criteria is lower. In our study 68% patients with reliable LSM had cIQR<40dB/m. This proportion is similar to previous studies in which 67% of a derivation cohort and 66% of a validation cohort had cIQR<40dB/m[12] and 50% of another cross-sectional study had cIQR<30dB/m.[11] In our cohort, patients with cIQR<40dB/m had a higher median serum ALT level and a higher prevalence of BMI≥40kg/m^2, possibly reflecting more severe steatosis and a higher CAP score. There were no other differences in clinical or demographic factors between patients with and without cIQR<40dB/m, consistent with reports that the usual factors affecting LSM performance (such as high liver enzymes and severe obesity), have little impact on CAP accuracy.[12] Importantly, the factors associated with CAP≥300dB/m were comparable in both the smaller cohort with cIQR<40dB/m and the overall patient cohort. Similarly, use of cIQR cut-off <40dB/m did not appear to impact the relationship between CAP score and presence of the MetS. Further studies are necessary to determine whether cIQR cut-offs have a clinically relevant impact on diagnostic accuracy or grading of steatosis severity, and if so, what strategies may increase the proportion of patients with a valid cIQR.

Since CAP has been shown to accurately identify the extent of steatosis due to various causes,[9, 18, 29, 30] it is not surprising that CAP has been shown to be independently associated with metabolic risk factors for the development of NAFLD, including increasing BMI, girth and presence of the MetS, in the present study as in foregoing reports. As liver histology was only available in a small number of patients, we were unable to determine if the association between these factors and CAP was independent of steatosis severity. However in a recent study of patients with fatty liver (diagnosed by liver biopsy) and healthy controls with intrahepatic triglyceride content <5% (by proton-magnetic resonance spectroscopy), obesity was associated with higher CAP values following adjustment for grade of steatosis.[12] Patients with BMI≥30kg/m^2 had higher CAP values (M probe) than those with BMI<30kg/m^2 at each steatosis grade.[12] Similarly, in a study using the XL probe, a high CAP was positively associated with waist circumference in multivariate analysis.[10] The mechanism remains

unclear but may be due to increased subcutaneous fat and skin capsular distance attenuating the ultrasonic signals,[31] and further assessment using the XL probe is warranted.

Importantly, we showed that increasing CAP scores are associated with suboptimal glycaemic control and treatment escalation in patients with NAFLD, independently of BMI. These data confirm previous studies showing that a high CAP was independently associated with serum triglyceride level,[10, 32] fasting plasma glucose[14, 32] and elevated HOMA-IR score.[14] Although the severity of steatosis does not appear to impact liver disease progression in NAFLD, cardiovascular diseases are the leading cause of morbidity and mortality in these patients, and optimal diabetic control reduces the long term risk of cardiovascular events.[33] In a recent study of 4,282 patients (1,542 with NAFLD), neither the presence nor the severity of steatosis as measured by CAP predicted liver-related events, non-hepatocellular carcinoma or cardiovascular events over a median follow-up period of 26 months.[34] In contrast to this short-term study, a 10 year follow-up study of patients with NAFLD (n=91) and matched controls (n=182) showed that steatosis (hazard ratio 1.99, 95% CI 1.01 – 3.94) and presence of plaques (hazard ratio 5.08, 95% CI 2.56 – 10.96) were the strongest predictors by multivariate analysis for cardiovascular events.[35] In addition, grade of steatosis (evaluated by liver histology at enrolment), and liver enzyme levels were higher in NAFLD patients who developed cardiovascular events.[35] It is also unknown whether an increased CAP score is a predictor of response to bariatric surgery. Currently patients with poorly controlled diabetes and obesity are offered radical bariatric surgery with ~60% achieving diabetes remission. A better predictor of those who develop remission and the ability to cease insulin would be beneficial.

Strengths of our study include the prospective recruitment of unselected "real-world" subjects from both diabetes and primary care clinics. Our findings are further evidence for NAFLD as a systemic condition,[36] however the mechanisms underlying the association between CAP and metabolic derangement remain to be clarified. The study has a number of limitations. In particular, liver biopsy was only performed in a subset of the patients who were selected based on increased likelihood of advanced disease or patient interest in participating in clinical therapeutic trials. Due to the lack of availability of magnetic resonance imaging[37] or semi-quantitative ultrasonographic indices[38] we were unable to validate the CAP measurements. This was a small single-centre study with preferential

recruitment of patients with MetS and diabetes. Therefore, our findings may not be representative of the wider population with NAFLD.

Our data illustrate an association between CAP score, poor glycaemic control, requirement for insulin therapy and hypertriglyceridaemia. CAP is a rapid, point-of-care test providing a numerical value that correlates with the histological grade of steatosis[7, 8, 26, 27] and with the severity of metabolic abnormalities[14, 18]. When assessing a diabetic patient with NAFLD, a higher CAP score may immediately identify patients at risk of poorly controlled metabolic comorbidities or advanced diabetes. The value of CAP in predicting liver and overall clinical outcomes will require ongoing prospective evaluation, as CAP technology has only been available for a short period of time. Nevertheless, our data demonstrate that an elevated CAP score reflects suboptimal metabolic control.

References

1. Brunt EM, Wong VW, Nobili V, et al. Nonalcoholic fatty liver disease. *Nature reviews Disease primers.* 2015;1: 15080.

2. Bjornsson E, Angulo P. Non-alcoholic fatty liver disease. *Scand J Gastroenterol.* 2007;42(9): 1023-1030.

3. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol.* 2016;64(6): 1388-1402.

4. Hernaez R, Lazo M, Bonekamp S, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology.* 2011;54(3): 1082-1090.

5. Bril F, Ortiz-Lopez C, Lomonaco R, et al. Clinical value of liver ultrasound for the diagnosis of nonalcoholic fatty liver disease in overweight and obese patients. *Liver Int.* 2015;35(9): 2139-2146.

6. Ballestri S, Nascimbeni F, Baldelli E, et al. Ultrasonographic fatty liver indicator detects mild steatosis and correlates with metabolic/histological parameters in various liver diseases. *Metabolism.* 2017;72: 57-65.

7. Sasso M, Beaugrand M, de Ledinghen V, et al. Controlled attenuation parameter (CAP): a novel VCTE guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: preliminary study and validation in a cohort of patients with chronic liver disease from various causes. *Ultrasound Med Biol.* 2010;36(11): 1825-1835.

8. Chon YE, Jung KS, Kim SU, et al. Controlled attenuation parameter (CAP) for detection of hepatic steatosis in patients with chronic liver diseases: a prospective study of a native Korean population. *Liver Int.* 2014;34(1): 102-109.

9. Karlas T, Petroff D, Sasso M, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. *J Hepatol.* 2017;66(5): 1022-1030.

10. de Ledinghen V, Hiriart JB, Vergniol J, Merrouche W, Bedossa P, Paradis V. Controlled Attenuation Parameter (CAP) with the XL Probe of the Fibroscan((R)): A Comparative Study with the M Probe and Liver Biopsy. *Dig Dis Sci.* 2017;62(9): 2569-2577.

11. Caussy C, Alquiraish MH, Nguyen P, et al. Optimal threshold of controlled attenuation parameter with MRI-PDFF as the gold standard for the detection of hepatic steatosis. *Hepatology.* 2017.

12. Wong VW, Petta S, Hiriart JB, et al. Validity criteria for the diagnosis of fatty liver by M probe-based controlled attenuation parameter. *J Hepatol.* 2017;67(3): 577-584.

13. Romero-Gomez M, Cortez-Pinto H. Detecting liver fat from viscoelasticity: How good is CAP in clinical practice? The need for universal cut-offs. *J Hepatol.* 2017;66(5): 886-887.

14. Mikolasevic I, Milic S, Orlic L, Stimac D, Franjic N, Targher G. Factors associated with significant liver steatosis and fibrosis as assessed by transient elastography in patients with one or more components of the metabolic syndrome. *J Diabetes Complications.* 2016;30(7): 1347-1353.

15. Lonardo A, Nascimbeni F, Mantovani A, Targher G. Hypertension, diabetes, atherosclerosis and NASH: Cause or consequence? *J Hepatol.* 2018;68(2): 335-352.

16. Lonardo A, Nascimbeni F, Maurantonio M, Marrazzo A, Rinaldi L, Adinolfi LE. Nonalcoholic fatty liver disease: Evolving paradigms. *World J Gastroenterol.* 2017;23(36): 6571-6592.

17. Targher G, Lonardo A, Byrne CD. Nonalcoholic fatty liver disease and chronic vascular complications of diabetes mellitus. *Nat Rev Endocrinol.* 2018;14(2): 99-114.

18. de Ledinghen V, Vergniol J, Capdepont M, et al. Controlled attenuation parameter (CAP) for the diagnosis of steatosis: a prospective study of 5323 examinations. *J Hepatol.* 2014;60(5): 1026-1031.

19. Patel PJ, Smith D, Connor JP, et al. Alcohol Consumption in Diabetic Patients with Nonalcoholic Fatty Liver Disease. *Canadian journal of gastroenterology & hepatology.* 2017;2017: 7927685.

20. Patel PJ, Hayward KL, Rudra R, et al. Multimorbidity and polypharmacy in diabetic patients with NAFLD: Implications for disease severity and management. *Medicine (Baltimore).* 2017;96(26): e6761.

21.   World Health Organisation Expert Consultation . Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet.* 2004;363(9403): 157-163.

22.   Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med.* 2006;23(5): 469-480.

23.   de Ledinghen V, Wong GL, Vergniol J, et al. Controlled attenuation parameter for the diagnosis of steatosis in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol.* 2016;31(4): 848-855.

24.   Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology.* 2005;41(6): 1313-1321.

25.   Sasso M, Tengher-Barna I, Ziol M, et al. Novel controlled attenuation parameter for noninvasive assessment of steatosis using Fibroscan((R)): validation in chronic hepatitis C. *J Viral Hepat.* 2012;19(4): 244-253.

26.   de Ledinghen V, Vergniol J, Foucher J, Merrouche W, le Bail B. Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography. *Liver Int.* 2012;32(6): 911-918.

27.   Shen F, Zheng RD, Mi YQ, et al. Controlled attenuation parameter for non-invasive assessment of hepatic steatosis in Chinese patients. *World J Gastroenterol.* 2014;20(16): 4702-4711.

28.   Boursier J, Zarski JP, de Ledinghen V, et al. Determination of reliability criteria for liver stiffness evaluation by transient elastography. *Hepatology.* 2013;57(3): 1182-1191.

29.   Thiele M, Rausch V, Fluhr G, et al. Controlled attenuation parameter and alcoholic hepatic steatosis: Diagnostic accuracy and role of alcohol detoxification. *J Hepatol.* 2018.

30.   Cardoso AC, Beaugrand M, de Ledinghen V, et al. Diagnostic performance of controlled attenuation parameter for predicting steatosis grade in chronic hepatitis B. *Ann Hepatol.* 2015;14(6): 826-836.

31.   Shen F, Zheng RD, Shi JP, et al. Impact of skin capsular distance on the performance of controlled attenuation parameter in patients with chronic liver disease. *Liver Int.* 2015;35(11): 2392-2400.

32.   Kwok R, Choi KC, Wong GL, et al. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. *Gut.* 2016;65(8): 1359-1368.

33.   Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ.* 2000;321(7258): 405-412.

34.   Liu K, Wong VW, Lau K, et al. Prognostic Value of Controlled Attenuation Parameter by Transient Elastography. *Am J Gastroenterol.* 2017;112(12): 1812-1823.

35.   Fracanzani AL, Tiraboschi S, Pisano G, et al. Progression of carotid vascular damage and cardiovascular events in non-alcoholic fatty liver disease patients compared to the general population during 10 years of follow-up. *Atherosclerosis.* 2016;246: 208-213.

36.   Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut.* 2017;66(6): 1138-1153.

37.   Idilman IS, Keskin O, Celik A, et al. A comparison of liver fat content as determined by magnetic resonance imaging-proton density fat fraction and MRS versus liver histology in non-alcoholic fatty liver disease. *Acta Radiol.* 2016;57(3): 271-278.

38.   Ballestri S, Romagnoli D, Nascimbeni F, Francica G, Lonardo A. Role of ultrasound in the diagnosis and treatment of nonalcoholic fatty liver disease and its complications. *Expert Rev Gastroenterol Hepatol.* 2015;9(5): 603-627.

Table 1: Demographic and clinical characteristics of the cohort according to CAP threshold category

		Low <250 dB/m N=24	Intermediate 250-299 dB/m N=35	High ≥300 dB/m N=171	P value
Age (years)[†]		55.5 (53.0-66.3)	61.0 (50.0-68.0)	58.0 (49.0-65.0)	0.605
Male Gender, N (%)[‡]		13 (54.2)	17 (48.6)	96 (56.1)	0.713
BMI (kg/m^2)[†]		**25.6 (24.0-29.9)**	**30.9 (29.3-34.0)**	**35.0 (30.4-39.5)**	**<0.001**
Girth (cm)[†]		**94.0 (88.5-100.8)**	**111.0 (102.0-117.0)**	**120.0 (107.0-131.0)**	**<0.001**
Type 2 Diabetes, N (%)[‡]		19 (79.2)	26 (74.3)	142 (83.0)	0.462
Metabolic syndrome, N (%)[‡]		**13 (54.2)**	**29 (82.9)**	**150 (87.7)**	**<0.001**
HbA1c ≥7%, N (%)[‡]		**9 (37.5)**	**15 (42.9)**	**104 (60.8)**	**0.025**
Number of diabetic	0	7 (29.2)	12 (34.3)	41 (24.0)	
medications,	1-2	15 (62.5)	17 (48.6)	87 (50.9)	0.277
N (%)[§]	3+	2 (8.2)	6 (17.1)	43 (25.1)	
Requirement for Insulin, N (%)		**5 (20.8)**	**8 (22.9)**	**78 (45.6)**	**0.006**
Dyslipidaemia[§], N (%)		22 (91.7)	31 (88.6)	165 (96.5)	0.077
Triglycerides (mmol/L)[†]		**1.4 (0.9-2.0)**	**1.7 (1.0-2.4)**	**1.9 (1.4-2.8)**	**0.012**
Hypertension[‡], N (%)		14 (58.3)	26 (74.3)	128 (74.9)	0.229
OSA[†], N (%)		2 (8.3)	11 (31.4)	52 (30.4)	0.062
	ALT (IU/ml)	**22.0 (19.0-36.0)**	**27.0 (18.0-36.0)**	**34.0 (24.0-57.0)**	**0.002**
Liver tests[†]	**AST (IU/ml)**	**19.5 (15.3-28.8)**	**18.0 (14.0-28.0)**	**25.0 (18.0-37.3)**	**0.010**
	GGT (IU/ml)	**21.5 (14.3-69.0)**	**24.0 (14.3-55.0)**	**37.0 (23.0-63.0)**	**0.021**
	1	**2 (100)**	**2 (100)**	**7 (16.3)**	
Steatosis Grade[§]	**2**	**0 (0)**	**0 (0)**	**8 (18.6)**	**0.009**
	3	**0 (0)**	**0 (0)**	**28 (65.1)**	
LSM (kPa)[†]		**4.4 (3.6-6.2)**	**5.2 (4.4-6.3)**	**6.8 (5.1-11.3)**	**<0.001**

[†]Data presented continuously (median and interquartile range) and analysed using the Kruskal-Wallis test [‡]Data presented categorically and analysed using Pearson's χ^2. [§]Data presented categorically and analysed using the Fisher's Exact test.

Table 2: Factors associated with CAP < or ≥ 300 dB/m

		Univariate P value	Univariate Odds Ratio (95% CI)	Adjusted P value	Adjusted Odds Ratio[†] (95% CI)
Age (years)		0.337	0.988 (0.964-1.013)	0.739	0.995 (0.967-1.026)
Male Gender		0.482	1.237 (0.684-2.239)	0.483	1.278 (0.644-2.535)
BMI (kg/m^{2})		**<0.001**	**1.173 (1.101-1.250)**	**<0.001**	**1.140 (1.068-1.216)**
BMI category	**normal**	**<0.001**		**0.003**	
	Cat 1-2	**0.001**	**3.094 (1.627-5.882)**	**0.027[‡]**	**2.205 (1.094-4.443)[‡]**
	Cat 3 plus	**0.001**	**35.469 (4.614-272.532)**	**0.003[‡]**	**25.537 (3.005-217.024)[‡]**
Girth[b] (cm)		**<0.001**	**1.058 (1.035-1.081)**	**<0.001[§]**	**1.046 (1.021-1.072)[§]**
Diabetes		0.252	1.523 (0.741-3.132)	0.830	0.902 (0.352-2.310)
Hypertension		0.293	1.414 (0.741-2.698)	0.139	0.539 (0.238-1.222)
Metabolic syndrome		0.004	2.891 (1.400-5.971)	0.794	1.138 (0.431-3.003)
OSA		0.220	1.546 (0.770-3.103)	0.362	0.670 (0.284-1.584)
HbA1c ≥7%		0.008	2.264 (1.238-4.139)	0.507	1.291 (0.607-2.746)
Requirement for Insulin		**0.002**	**2.968 (1.496-5.889)**	**0.014**	**2.599 (1.212-5.575)**
Dyslipidaemia		0.058	3.113 (0.963-10.062)	0.081	3.892 (0.847-17.886)
ALT (IU/ml)		**0.017**	**1.017 (1.003-1.029)**	**0.018**	**1.018 (1.004-1.033)**
AST (IU/ml)		0.027	1.023 (1.003-1.045)	0.593	0.989 (0.951-1.029)
Albumin (g/L)		0.908	1.005 (0.920-1.098)	0.083	1.105 (0.987-1.237)
Platelets (x10^{9})		0.613	0.999 (0.994-1.003)	0.463	0.998 (0.992-1.004)
LSM (kPa)		0.002	1.188 (1.066-1.324)	0.146	1.068 (0.977-1.166)

[†]All variables adjusted for BMI, LSM, requirement for insulin, serum ALT and presence of dyslipidaemia unless otherwise reported. [‡]Adjusted for LSM, requirement for insulin, serum ALT and presence of dyslipidaemia. [§]Adjusted for LSM, requirement for insulin, serum ALT and presence of dyslipidaemia.

Table 3: Factors identified by multivariable backward stepwise logistic regression associated with presence of the metabolic syndrome, glycaemic control, use of insulin and dyslipidaemia

	Presence of Metabolic syndrome		HbA1c ≥7		Use of insulin		Hypertriglyceridemia	
	aOR (95%CI)	P value	aOR (95%CI)	P value	aOR (95%CI)	P value	aOR (95%CI)	P value
CAP (db/m)	1.011 (1.003-1.019)	0.009	1.007 (1.002-1.013)	0.012	1.010 (1.003-1.016)	0.006	1.007 (1.002-1.013)	0.011
Age (years)	1.091 (1.050-1.133)	<0.001	-	-	-	-	1.003 (0.980-1.026)	0.804
BMI (kg/m^2)	1.105 (1.018-1.200)	0.018	-	-	0.959 (0.915-1.019)	0.086	0.995 (0.954-1.038)	0.815
Gender	-	-	1.739 (0.955-3.166)	0.070	-	-	0.983 (0.560-1.724)	0.951
Hypertension	-	-	3.163 (1.606-6.229)	0.001	2.348 (1.019-5.410)	0.045	-	-
Albumin (g/L)	0.869 (0.758-0.997)	0.046	0.850 (0.767-0.943)	0.002	0.798 (0.711-0.895)	<0.001	-	-
ALT (IU/ml)	-	-	0.989 (0.979-1.000)	0.043	0.988 (0.976-1.001)	0.072	-	-
On treatment for dyslipidaemia	-	-	-	-	2.976 (1.412-6.273)	0.004	-	-
LSM (kPa)	-	-	-	-	1.038 (0.999-1.079)	0.056	-	-

Table 4: Demographic and clinical characteristics of the cohort according to CAP IQR < or ≥40

	CAP IQR <40 N=157	CAP IQR ≥40 N=73	p value
Age (years)[†]	57 (49-64)	61 (52-70)	0.063
Male Gender, N (%)[‡]	89 (56.7)	37 (50.7)	0.395
Caucasian[‡]	121 (77.1)	56 (76.7)	0.952
BMI (kg/m^2)[†]	33.0 (29.3-39.3)	31.8 (29.0-36.2)	0.172
BMI ≥40.0 (kg/m^2), N (%)[‡]	**35 (22.3)**	**7 (9.6)**	**0.020**
Waist Circumference (cm)[¶]	117±19	113±16	0.106
T2DM, N(%)[‡]	130 (82.8)	57 (78.1)	0.393
Metabolic Syndrome, N (%)[‡]	133 (84.7)	59 (80.8)	0.459
ALT (IU/mL)[†]	**34 (23-55)**	**29 (19-51)**	**0.029**
AST (IU/mL)[†]	25 (18-37)	21 (15-36)	0.145
GGT(IU/mL)[†]	37 (22-63)	27 (18-55)	0.064
Platelets (x10^9)[¶]	250±63	240±67	0.329
Serum Albumin (g/L)[†]	41 (39-43)	42(40-44)	0.469
HbA1c ≥7, N (%)[‡]	93 (59.2)	35 (47.9)	0.109
LSM (kPa)[†]	6.2 (4.9-8.9)	6.1 (4.4-10.2)	0.936
LSM IQR[†]	0.7 (0.4-1.0)	0.7 (05-1.3)	0.395
LSM ≥8.2, N (%)[‡]	47 (29.9)	25 (34.2)	0.512
Quality of LSM Reading[§] Poor	3 (1.9)	1 (1.4)	
Reasonable	31 (19.7)	17 (23.3)	0.838
Good	123 (78.3)	55 (75.3)	
CAP (dB/m)[†]	**355 (319-390)**	**308 (267-352)**	**<0.001**
XL Probe, N (%)[‡]	**112 (71.3)**	**64 (87.7)**	**0.007**

[†]Data presented continuously (median + range) and analysed using the Mann-Whitney U test [‡]Data presented categorically and analysed using Pearson's Chi Squared. [§]Data presented categorically and analysed using the Fisher's Exact test. [¶]Data presented continuously and analysed using an independent T test.

Figure Legends:

Figure 1: The relationship between a) CAP and CAP IQR and b) LSM and LSM IQR

Highlights:

Controlled attenuation parameter in NAFLD identifies risk of suboptimal glycaemic and metabolic control

- Patients with an elevated CAP Score were more likely to have metabolic syndrome.

- Elevated CAP scores reflect suboptimal metabolic control in terms of elevated HbA1c, requirement for insulin and hypertriglyceridaemia. CAP measurement may be a useful surrogate marker of more advanced diabetes or metabolic disease duration.

- Validity criteria for CAP measurements remain poorly defined. However our data did not identify any differences when the CAP interquartile range <40 or ≥40, in factors independently predicting the likelihood of having a high CAP score.
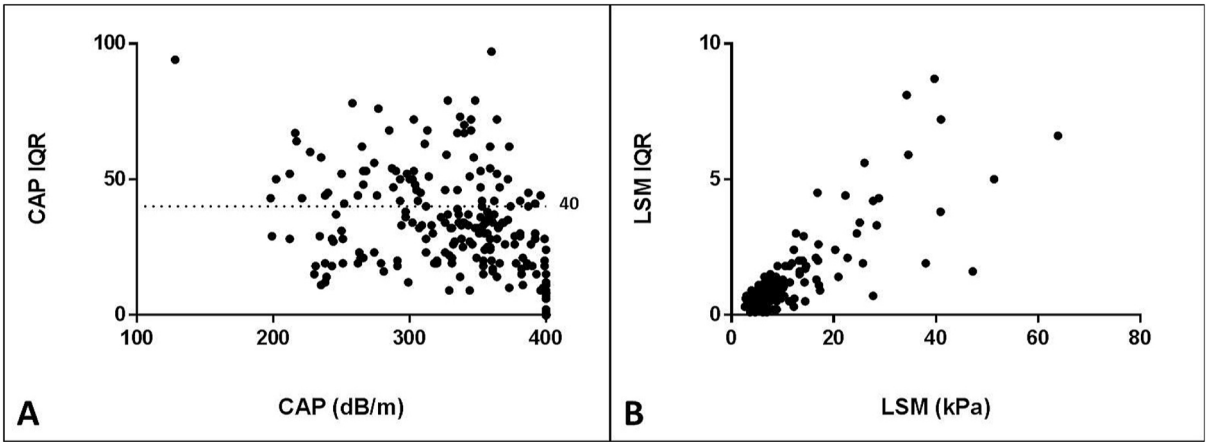
Figure 1