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***Case Control Study***

**Estimating steatosis and fibrosis: Comparison of acoustic structure quantification with established techniques**

Karlas T *et al.* ASQ for characterization of liver disease

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**Abstract**

**AIM:** To compare ultrasound-based acoustic structure quantification (ASQ) with established non-invasive techniques for grading and staging fatty liver disease.

**METHODS:** Type 2 diabetic patients at risk for non-alcoholic fatty liver disease (*n =* 50) and healthy volunteers (*n =* 20) were evaluated with laboratory analysis and anthropometric measurements, transient elastography (TE), controlled attenuation parameter (CAP), proton magnetic resonance spectroscopy (1H-MRS; only available for the diabetic cohort), and ASQ. ASQ parameters mode, average and focal disturbance (FD) ratio were compared with (1) the extent of liver fibrosis estimated from TE and non-alcoholic fatty liver disease (NAFLD) fibrosis scores; and (2) the amount of steatosis, which was classified according to CAP values.

**RESULTS:** 47 diabetic patients (age 67.0 ± 8.6 years; body mass index 29.4 ± 4.5 kg/m²) with reliable CAP measurements and all controls (age 26.5 ± 3.2 years; body mass index 22.0 ± 2.7 kg/m²) were included in the analysis. All ASQ parameters showed differences between healthy controls and diabetic patients (*p <* 0.001, respectively). The ASQ FD ratio (logarithmic) correlated with CAP (*r =* -0.81, *p <* 0.001) and 1H-MRS (*r =* -0.43, *p =* 0.004) results. FD ratio [CAP < 250 dB/m: 107 (102–109), CAP between 250 and 300 dB/m: 106 (102–114); CAP between 300 and 350 dB/m: 105 (100-112), CAP ≥ 350 dB/m: 102 (99-108)] as well as mode and average parameters were reduced in cases with advanced steatosis (ANOVA *p <* 0.05). However, none of the ASQ parameters showed a significant difference in patients with advanced fibrosis determined by TE and NAFLD fibrosis score (*p* > 0.08, respectively).

**CONCLUSION:** ASQ parameters correlate with steatosis, but not with fibrosis in fatty liver disease. Steatosis estimation with ASQ should be further evaluated in biopsy controlled studies.

**Key words:** Transient elastography; Non-alcoholic fatty liver disease; Liver stiffness; Non-alcoholic fatty liver disease fibrosis score; Controlled attenuation parameter

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**Core tip:** Non-invasive characterization of hepatic steatosis and fibrosis is of emerging importance for the screening, diagnosis, and monitoring of patients with chronic liver diseases. This work compares acoustic structure quantification (ASQ) and established non-invasive methods for characterization of fatty liver disease. ASQ parameters differed between healthy controls and diabetic patients with fatty liver disease independent of the extent of fibrosis. The focal disturbance ratio and further ASQ parameters correlated with the severity of steatosis. Therefore, ASQ can be used for evaluation of steatosis and merits further investigation but seems to be impractical for characterizing fibrosis in patients with fatty liver disease.

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**INTRODUCTION**

Non-invasive characterization of hepatic steatosis and fibrosis is of emerging importance for the screening, diagnosis, and monitoring of patients with chronic liver diseases[1-3]. Especially fibrosis staging by means of elastography provides high accuracy for detecting advanced liver injury and has therefore been progressively implemented in clinical practice[1].

In addition to fibrosis characterization, non-invasive quantification of hepatic fat content has attracted increasing attention in the areas of both risk assessment and monitoring of patients with non-alcoholic fatty liver disease (NAFLD) and liver diseases from other etiologies[1,4]. The controlled attenuation parameter (CAP) – computed by commercial software that comes with the transient elastography device (Fibroscan) – correlates steatosis with the signal attenuation during liver stiffness measurement (LSM) and has shown high accuracy for the detection of advanced hepatic steatosis[5-9]. Moreover, magnetic resonance based techniques (*e.g.*, proton magnetic resonance spectroscopy, 1H-MRS) allow one to quantify hepatic lipid fraction, an additional parameter of the severity of steatosis[10,11]. However, these approaches are either affected by high costs and limited availability (1H-MRS) or by anthropometry (CAP)[4,8].

Analysis of B-Mode ultrasound may represent a further option for the non-invasive grading and staging of liver damage, as conventional sonography is highly sensitive to subtle changes in tissue texture[12,13]. However, comparison of gray scale images is operator-dependent and also varies with some technical parameters[12]. Computerized analysis of acoustic tissue properties may overcome these limitations: Acoustic structure quantification (ASQ) software analyzes the characteristic intensity pattern (“speckles”) of conventional B-Mode ultrasound and compares its distribution histogram with a theoretical probability density function (PDF, Rayleigh distribution) of the echo amplitude[13,14]. Histology-based studies have reported ASQ parameters to correlate with the degree of fibrosis in patients with liver diseases of different etiologies[13-15]. Moreover, a pilot study revealed a strong agreement between ASQ values and hepatic fat accumulation in an animal model[16].

ASQ results have not yet been compared with those of established non-invasive techniques. Therefore, we prospectively compared ASQ measurements in healthy controls and diabetic patients at risk for NAFLD or associated liver injury with TE and CAP values (reference standards) as well as 1H-MRS results and serum based NAFLD fibrosis scores.

**MATERIALS AND METHODS**

***Patients and controls***

The study was reviewed and approved by the local ethics committee (University of Leipzig, register no. 358/08-B-ff and no. 419-12-17122012) and performed in accordance with the ethical guidelines of the Helsinki Declaration. Written informed consent was obtained from all participants prior to study enrollment.

Between April 2013 and March 2014, outpatients with type 2 diabetes mellitus at risk for NAFLD and without other liver diseases were recruited. For the control group, healthy volunteers without history of any chronic liver disease, diabetes mellitus, or metabolic syndrome) were recruited. Increased ultrasound echogenicity of the liver parenchyma (compared with right renal cortex) or CAP > 252 dB/m were regarded as exclusion criterion for the control group[5]. Significant alcohol intake (weekly consumption > 210 g for men and 140 g for woman, respectively) was ruled out for the total study cohort prior to inclusion[17].

For all participants, anthropometric examination, ultrasound, LSM (transient elastography, M probe), CAP assessment and laboratory parameters (patients only) were performed on the same day after a fasting period of at least three hours. 1H-MRS was performed either the same day or after 6 months during follow-up visit.

***Laboratory assessment and NAFLD fibrosis score***

Albumin, blood count, and serum levels of aminotransferases (ALT and AST) were determined for all diabetic patients. Individuals with highly elevated aminotransferases (at least five times the upper normal limit) were excluded from further examinations due to the risk of inaccurate LSM.

The NAFLD fibrosis score was calculated according to Angulo *et al*[18]: Score = –1.675 + 0.037 × age (years) + 0.094 × body mass index (kg/m2) + 1.13 × diabetes (yes 1, no 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (Gpt/l) – 0.66 × albumin (g/dl). A score of at least 0.676 was assumed to indicate a high risk of advanced liver fibrosis[18].

***Ultrasound, elastography, and controlled attenuation parameter***

All subjects underwent conventional ultrasound to rule out mechanical cholestasis. LSM was performed using the M probe of transient elastography (TE; Fibroscan, Echosens, Paris, France). According to the manufacturer’s recommendation examinations with fewer than 10 valid measurements or an interquartile range (IQR) > 30% of the median LSM value (only in cases with liver stiffness ≥ 7.1 kPa) were excluded from further analysis[19]. TE values ≥ 7.9 kPa indicated the presence of advanced fibrosis[20].

The controlled attenuation parameter (CAP) gives additional information about the attenuation of ultrasonic signals during TE examination. CAP computation is included in the TE software, and results (CAP, LSM) are shown together[21]. CAP was only considered when TE measurements were valid and reliable[5,21]. Because CAP served as reference method, only cases with valid CAP results were included in the final analysis. CAP values ≥ 252 dB/m indicated fatty liver disease[5], and values ≥ 300 dB/m were regarded as advanced steatosis[8]. Because no patient had values between 250 and 252 dB/m, diabetic subjects were classified into four groups to better present the data: (1) CAP < 250 dB/m; (2) CAP ≥ 250 and < 300 dB/m; (3) CAP ≥ 300 and < 350 dB/m; and (4) CAP ≥ 350 dB/m.

***Acoustic structure quantification***

**Analytical method:** The ASQ software analyzes the linear raw data from ultrasound B-mode images. It provides a number of tissue parameters that are related to the scattering of echoes in user-defined region of interests (ROI). The histogram of the measured intensity distribution in the B-mode image ROI is essentially compared with the PDF of an ideal, homogenous scatterer (Rayleigh distribution)[22]. ASQ subdivides the primary ROI into a large number of (up to 1000) secondary ROIs and displays the so-called C2-histogram of the distribution of frequency ratios given by frequency of the ratio

C2 = 2/R2

where  and R stand for the standard deviations of measured and estimated (Rayleigh) PDF, respectively[13]. In the liver, ASQ uses an empirical cut-off parameter  and reports slightly modified parameters  m and Cm2 instead to minimize the influence of individual strong scatterers[13].

Mode (value with highest appearance, “peak value”), average and standard deviation (SD) are then derived from the C2 or Cm2 histogram. A more recent ASQ implementation computes two curves (displayed in red and blue) depending on whether the variation in quantitatively changes the parameter by less (depicted in red) or more (depicted in blue) than an empirical percentage (20%), respectively. The focal disturbance (FD) ratio is then defined as the ratio of the areas under the curve (AUC) between red and blue curve[16].

**ASQ imaging:** In the present study, B-mode images of the right liver lobe were recorded in the region of LSM and CAP acquisition using a curved array transducer (PVT-375 BT 3.5 MHz) using an Aplio 500 ultrasound scanner (Toshiba Medical Systems, Otawara, Japan). Display depth and focus were fixed at 10 and 5.5 cm, respectively. The ultrasound signal gain was set to 90%. A raw data loop of 2-3 s was recorded using the ASQ preset mode and files were exported to a PC workstation in DICOM format. ASQ was analyzed with the vendor's software (PC-ASQR, Aplio500, Version V1.01R000. Mode, average and FD ratio were computed in four ROIs covering most of the liver parenchyma but avoiding larger vessels and bile ducts (Figure 1). The respective mean ASQ values of five separate frames were computed to obtain more reliable measures for each subject.

***Magnetic resonance spectroscopy***

The majority of patients (79%) underwent 1H-MRS on the same or following day of ASQ and TE examinations. In 21% of the cases, the time interval between ultrasound examination and 1H-MRS was 6.5 mo(range: 6.2–7.3 mo) for technical reasons.

1H-MRS was performed as described previously with some technical modifications[8]. In brief, T2-corrected, single-voxel MR spectra were acquired on a 1.5-T scanner (Achieva XR, Philips Healthcare, Best, Netherlands) using local shimming and a stimulated-echo acquisition method (STEAM). Voxels sized 20 mm x 20 mm x 20 mm were placed in liver segment VII avoiding larger bile ducts and vessels. Spectroscopic data were acquired without water suppression using the following sequence parameters: repetition time, T*R =* 3.000 ms; number of echoes, 5; echo times 10-50 ms; 2048 data points; bandwidth 1.000 Hz/pixel; 40 averages; total acquisition time 270 s. MR spectra were analyzed with a commercial tool (LCModel 6.3, Oakville, Canada) that determines the relative hepatic lipid concentrations. Calculated areas of water and fat peaks were corrected for T2 relaxation (using MR spectra at different echo times) and were used for calculating the hepatic fat fraction[8].

***Data processing and statistical methods***

All parameters were recorded in a spreadsheet file (Microsoft Excel, Microsoft). Statistical testing was carried out using commercial software (MedCalc 14.12, MedCalc Software, Ostend, Belgium). Data were expressed either as mean ± standard deviation or median and range, as appropriate.

Fisher’s exact test and chi-square tests were used to test for the association of variables. Nonparametric tests (Mann-Whitney *U* test, Kruskal-Wallis test) were used for comparison of median values of independent samples, where post-hoc pair-wise comparisons are performed according to Conover[23]. For mean values the t-test was applied. The Pearson correlation coefficient was calculated to analyze the degree of association between two variables. *P*-values < 0.05 indicated a significant difference. Diagnostic performance of ASQ parameters was analyzed using receiver operating characteristic (ROC) curves.

The statistical methods of this study were reviewed by PD Dr. David Petroff (IFB AdiposityDiseases, Leipzig University Medical Center/Clinical Trial Center, University of Leipzig, Leipzig, Germany).

**RESULTS**

***Clinical characteristics of the study cohort***

50 patients with type 2 diabetes mellitus and 20 healthy volunteers were recruited. TE and CAP were available in 47 of the diabetic patients (94%): three subjects had an invalid measurement (two males, all cases with fewer than ten valid shots) and where excluded from further analysis. 1H-MRS was available for *n =* 43 diabetic patients due to contraindications and technical reasons in four cases. Characteristics of the analyzed cohort are displayed in Table 1.

***Non-invasive fibrosis estimation with ASQ vs TE and NAFLD score***

LSM values (logarithmic) showed good correlation with NAFLD fibrosis scores: *r =* 0.46 (0.20; 0.66), *p =* 0.0012. None of the healthy controls had elevated TE whereas advanced hepatic fibrosis was considerable in the diabetic cohort: ten patients (21%) had LSM > 7.9 kPa and 12 patients (26%) had NAFLD scores > 0.676.

The association of ASQ parameters mode, average, and FD ratio was analyzed according to the risk of hepatic fibrosis. All parameters showed differences between healthy controls and diabetic patients (Figures 1 and 2). No significant association of mode and average for both (blue and red) C2-histogram curves was observed in diabetic patients independent of the presence of fibrosis as defined by TE (Figure 2) or NAFLD score (*p*-values >0.08, respectively). In addition, FD ratios did not differ between both groups of diabetic patients: 0.060 (0.044; 0.076) *vs* 0.053 (0.037; 0.089) (TE cut-off, *p =* 0.640) and 0.059 (0.030; 0.125) *vs* 0.057 (0.047; 0.071) (NAFLD fibrosis score cut-off, *p =* 0.946), respectively.

***Non-invasive steatosis characterization with ASQ vs CAP and 1H-MRS***

Diabetic patients were classified according to the degree of fatty liver disease defined by CAP values: only *n =* 6 cases were at low risk for fatty liver (CAP <250 dB m) whereas *n =* 33 were above the highly specific cut-off level (CAP > 300 dB/m) for advanced hepatic steatosis[8]. Gender distribution did not differ between healthy controls and the four diabetes subgroups, whereas BMI was increased in patients with advanced hepatic steatosis (Table 2). Furthermore, 1H-MRS revealed a positive correlation between CAP and hepatic fat fraction [*n =* 43; *r =* 0.5 (0.31; 0.73), *p <* 0.001] (cf. also table 2).

ASQ parameters mode and average of both C2-histogram curves and FD ratio differed between healthy controls and diabetic patients (*p*-values <0.001). They also showed a stepwise decrease according to the CAP-defined classification of steatosis in diabetic patients (Table 2, Figure 3). ROC analysis for the detection of advanced steatosis (defined by CAP > 300 dB/m) in diabetic patients by the FD ratio revealed an area under the curve (accuracy) of 0.76(0.61; 0.87) (sensitivity 97%, specificity 50%) at a cut-off value of 0.092. With the addition of control subjects, the accuracy increased to 0.89 (sensitivity 97%, specificity 78%) with the same cut-off.

As with the CAP results (Figure 3B), ASQ parameters were negatively correlated with 1H-MRS in the diabetic cohort (table 2), *e.g.*, *r =* -0.43 (-0.65; -0.15), *p =* 0.004 for the FD ratio. Agreement between both methods increased when only cases with a hepatic fat fraction < 25% were considered (*n =* 36): *r =* -0.64 (-0.80; -0.40), *p <* 0.001.

**DISCUSSION**

The present study provides a comprehensive comparison of the ASQ technique with established non-invasive methods for the grading and staging of fatty liver disease. Our data underline the value of ASQ for quantification of liver fat: the FD ratio shows a strong correlation with the CAP method, which in turn achieves high diagnostic accuracy for the grading of liver steatosis when compared with liver histology[1,4-9]. Furthermore, the FD ratio also correlates with the hepatic lipid fraction quantified by 1H-MRS, especially in cases with low to moderate hepatic fat content. These results confirm data from a mice model, which demonstrated the potential value of the refined ASQ algorithm for non-invasive liver fat quantification[16]. Our findings are also in line with preliminary observations on the regression of FD ratios in NAFLD patients treated for morbid obesity[24]. ASQ may therefore become a novel tool for the estimation of hepatic steatosis equally accurate than other non-invasive methods[4].

ASQ was originally developed as an alternative to liver biopsy for fibrosis staging. Three biopsy-controlled, cross-sectional studies have shown a correlation of ASQ parameters (mode and average) with the extent of liver fibrosis[13-15]. However, these ASQ parameters correlated with CAP results and thus with the degree of liver steatosis in our cohort (Table 2). All ASQ parameters also differed between healthy controls and patients at risk of fatty liver disease independent of the extent of fibrosis. In contrast, none of these parameters were associated with either the NAFLD fibrosis score or liver tissue stiffness, although both approaches represent well established surrogates of liver fibrosis[3,4]. We therefore assume that anthropometric factors and advanced steatosis, which was associated with a higher frequency of increased liver stiffness in our cohort, interfere with fibrosis in the B-mode speckle pattern and thus limit the prognostic value of ASQ for fibrosis in such cases. The three previous studies where ASQ correlated with fibrosis were performed either in lean cohorts without advanced steatosis and cirrhosis[13], or in patients with homogeneous steatosis for all fibrosis stages[14], or did not analyze the impact of steatosis on ASQ results in more detail[15]. Furthermore, the study of Ricci *et al*[15] reported low diagnostic accuracy of ASQ for fibrosis detection (AUROC 0.71 for any grade) in patients with viral hepatitis, which may be associated with steatosis especially for cases with progressive fibrosis[25]. Our results may therefore demonstrate a limited value of ASQ for characterizing fibrosis in the presence of steatosis. This finding is supported by recent data from a histology-controlled cohort where ASQ proved imprecise in assessing liver fibrosis[26].

Our study had some limitations: Quantitative analysis of ASQ parameters is still an experimental approach and the method has not yet been standardized (*e.g.*, position and size of B-Mode ROIs, number of measurements, technical ultrasound parameters). Most previous ASQ studies did not adequately describe their data acquisition[13,15], which prevents a proper comparison with our results. Another source of discrepancy in fibrosis detection is a vendor's update to the ASQ data processing[16] that came with the introduction of the FD ratio[13-15]. Furthermore, we only correlated ASQ results with other non-invasive detection techniques because liver histology was ethically prohibited in this pilot study and only provides a semi-quantitative estimate of steatosis[8]. However, an impact of the non-invasive reference methods on our study findings cannot be ruled out. As has already been discussed previously[8], anthropometric factors as well as the extent of liver fat can alter liver stiffness, CAP and MR spectroscopic results[27-31]. Especially MR techniques are susceptible to fibrosis-related iron deposits and may not properly discriminate between advanced grades of hepatic steatosis[27,28]. This could explain the moderate correlation between 1H-MRS and ASQ in our cohort, where advanced steatosis and fibrosis was highly prevalent. In this line, the findings of our pilot study should be further verified in histology-controlled studies.

In conclusion, our results provide first evidence that ASQ FD ratio can be used for non-invasive evaluation of hepatic steatosis in patients at risk for fatty liver disease and merits further investigation. In its current implementation, the ASQ algorithm seems to be impractical for characterizing fibrosis in patients with fatty liver disease. There is also a need for biopsy-controlled studies to further validate ASQ parameters and evaluate critical factors like data acquisition or patient anthropometry.

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**COMMENTS**

***Background***

Non-invasive characterization of hepatic fat content and fibrosis is of emerging importance for the care of patients with chronic liver diseases. Among different approaches, liver stiffness measurement combined with ultrasound attenuation analysis represents the current non-invasive references standards for estimation of fibrosis and steatosis.

***Research frontiers***

Computerized analysis of acoustic tissue properties may represent a further alternative for grading and staging of liver damage. In this line, acoustic structure quantification (ASQ) software analyzes the intensity pattern of B-Mode ultrasound. Several ASQ parameters showed a correlation with the extent of fibrosis in histology-controlled studies. However, ASQ has not yet been compared with established non-invasive techniques and the impact of hepatic steatosis on ASQ has not yet been studied.

***Innovations and breakthroughs***

These results provide first evidence that ASQ parameters correlate with the extent of hepatic steatosis. However, ASQ seems to be impractical for characterizing fibrosis in patients with fatty liver disease.

***Applications***

If further proved in biopsy-controlled studies ASQ may complement ultrasound assessment of patients with chronic liver diseases, especially for the non-invasive estimation of hepatic fat content.

***Terminology***

Non-alcoholic fatty liver disease (NAFLD): obesity, nutrition and further factors can lead to fat deposition in hepatocytes called NAFLD. This process may lead to hepatic inflammation and ultimately to fibrosis and cirrhosis; Elastography: measurement of tissue stiffness by means of mechanical and ultrasound impulses. Liver stiffness correlates with the extent of fibrosis.

***Peer-review***

The authors compared ultrasound-based ASQ with established non-invasive techniques for grading and staging fatty liver disease. The topic is a relevant because NAFLD is perhaps the commonest liver disease… The authors claim that this study provides first evidence that ASQ FD ratio can be used for non-invasive evaluation of hepatic steatosis in patients at risk for fatty liver disease. Overall, this is a well written manuscript.

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**Table 1 Clinical characteristics of the study cohort *n* (%)**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Healthy controls** | **Type 2 diabetes mellitus** |
| sex (f/m) | 10/10 | 23/24 |
| age (yr) | 26.5 ± 3.2 | 67.0 ± 8.6b |
| body mass index (kg/m²)< 25 kg/m²25–30 kg/m²> 30 kg/m² | 22.0 ± 2.719 (95)1 (5)- | 29.4 ± 4.5b7 (15)21 (45)19 (40) |
| waist-to-hip ratio< 1.0≥ 1.0 | 0.85 ± 0.0819 (95)1 (5) | 0.97 ± 0.08b27 (57%)20 (43%) |
| CAP (dB/m)< 250 dB/m250–300 dB/m300–350 dB/m> 350 dB/m | 192 (151-237)20 (100)--- | 329 (100-396)d6 (13)10 (21)19 (40)12 (26) |

b*p<* 0.01 *vs* controls; d*p <* 0.01 *vs* controls by design. Controls were excluded if CAP > 252 dB/m; CAP: controlled attenuation parameter.

**Table 2 Distribution of anthropometry, hepatic lipid content, fibrosis risk and acoustic structure quantification parameters in different grades of fatty liver *n* (%)**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Controls** | **Type 2 diabetes mellitus** |
|  |  | CAP < 250 dB/m | CAP 250–< 300 dB/m | CAP 300–< 350 dB/m | CAP ≥ 350 dB/m |
| Sex (f/m) | 10/10 | 2/4 | 6/4 | 8/11 | 7/5 |
| Body mass index (kg/m²)b | 22.0 ± 2.7 | 26.7 ± 4.6 | 28.3 ± 5.1 | 29.2 ± 4.3 | 32.1 ± 3 |
| Waist-to-hip ratiob | 0.85 ± 0.08 | 1.00 ± 0.03 | 0.91 ± 0.13 | 0.98 ± 0.06 | 1.01 ± 0.05 |
|  |  |  |  |  |  |
| Liver stiffness (kpa)a≥ 7.9 kpac | 4.9 (2.9-6.8)0 (0) | 3.5 (3-6.1)0 (0) | 5.1 (3.5-11.7)1 (10) | 5.7 (3.4-12.3)5 (26) | 6.9 (4.1-70.6)4 (33) |
| NAFLD fibrosis score≥ 0.676 | - | -0.68 (-2.45-1.25)1 (17) | 0.29 (-0.3-1.26)3 (30) | -0.4 (-3.04-1.1)2 (11) | 0.11 (-1.68-2.28)4 (33) |
| 1H-MR spectroscopy(relative lipid signal, %) | - | 4.19 (2.69-16.62) | 9.77 (1.56-15.71) | 18.99 (5.66-35.55) | 18.38 (9.42-41.11) |
| **Asq** |  |  |  |  |  |
| Mode 1 (red histogram)b | 114 (105-121) | 107 (102-109) | 106 (102-114) | 105 (100-112) | 102 (99-108) |
| Average 1 (red histogram) a | 117 (109-125) | 110 (104-116) | 108 (106-114) | 106 (103-112) | 105 (102-112) |
| Mode 2 (blue histogram)b | 126 (110-135) | 117 (114-126) | 119 (105-125) | 114 (107-119) | 114 (105-121) |
| Average 2 (blue histogram)b | 132 (116-149) | 122 (116-139) | 123 (114-130) | 118 (109-134) | 115 (106-123) |
| Focal disturbance (FD) ratiob | 0.34 (0.12-0.71) | 0.16 (0.03-0.36) | 0.07 (0.03-0.18) | 0.06 (0.02-0.09) | 0.04 (0.02-0.13) |

 a*p <* 0.05 (Kruskal-Wallis test), b*p <* 0.01 (Kruskal-Wallis test) *vs* healthy controls; c*p <* 0.05 (exact test for count data), *vs* healthy controls. ASQ: Acoustic structure quantification; CAP: Controlled attenuation parameter.



**Figure 1 acoustic structure quantification analysis of liver tissue.** Four separate region of interests were drawn to cover most of the parenchyma avoiding large vessels. The acoustic structure quantification software automatically calculates the parameters mode, average and standard deviation for both curves of the histogram (green frame) as well as the ratio of the areas under both curves (focal disturbance ratio, red frame).



**Figure 2 Correlation of acoustic structure quantification parameters with liver stiffness.** Acoustic structure quantification (ASQ) parameters mode and average of the "red" (A, C) and "blue" C2-histogram (B, D) show no significant differences between diabetic patients with and without increased liver stiffness (transient elastography, TE). The differences of all parameters between both patient groups and healthy controls (b*p <* 0.01, *vs* control, Mann-Whitney *U* test) suggest an influence of diabetes-related anthropometric and metabolic factors, *e.g.*, steatosis, on ASQ analysis.



**Figure 3 Correlation of acoustic structure quantification focal disturbance ratio and controlled attenuation parameter in healthy controls and diabetic patients.** Acoustic structure quantification (ASQ) focal disturbance (FD) ratio is decreased in patients with advanced steatosis compared to healthy controls and patients with mild steatosis (a*p <* 0.05, *vs* control, Kruskal-Wallis test) (A). Logarithmic ASQ values correlate strongly with controlled attenuation parameter (CAP) values (B).