Optimizing the number of valid measurements for the attenuation coefficient to assess hepatic steatosis in MAFLD patients: A study of 139 patients who underwent liver biopsy

Optimierung der Anzahl gültiger Messungen des Dämpfungskoeffizienten zur Beurteilung der Lebersteatose bei Patienten mit MAFLD: Eine Studie an 139 Patienten mit Leberbiopsie

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ABSTRACT

Purpose We investigated the optimal number of valid measurements (VMs) for the attenuation coefficient (AC) to assess liver steatosis using attenuation imaging (ATI) and explored factors that may affect AC measurement in patients with metabolic dysfunction-associated fatty liver disease (MAFLD).

Materials and Methods A total of 139 patients with MAFLD who underwent ATI and liver biopsy were enrolled. Hepatic steatosis was graded as S0–3 according to the SAF scoring system. The AC values from 1, 2, 3, 5, and 7 VMs were compared with the degree of liver steatosis. The correlation between AC values from different VMs was analyzed. The diagnostic performance of AC from different VMs at each steatosis grade was compared. The factors related to AC were identified using linear regression analysis.

Results The mean AC values from 1, 2, 3, 5, and 7 VMs were not significantly different between grades S0–3 (p = n.s. for all). Bland-Altman analysis showed the mean difference in AC values of 3 VMs and 7 VMs was 0.003 dB/cm/MHz, which was smaller compared with 2 VMs, and close to 5 VMs. The intra-class correlation coefficients of AC were all >0.90 among dif-
Introduction

Metabolic dysfunction-associated fatty liver disease (MAFLD) was first defined by an international consensus in 2020. It affects almost 25% of the adult population worldwide [1]. MAFLD is diagnosed in the presence of hepatic steatosis based on histological, imaging, or blood biomarker evidence, and has one of the following three cases: overweight/obesity, type 2 diabetes mellitus (T2DM), or metabolic dysregulation [2]. Excessive fat accumulation in hepatocytes can cause hepatic steatosis [3]. Steatohepatitis develops in a subset of patients with hepatic steatosis and may progress to advanced hepatic fibrosis, eventually leading to cirrhosis or even hepatocellular carcinoma [4]. MAFLD also affects extra-hepatic organs, for instance, by increasing risks of cardiovascular and cardiac diseases, chronic kidney disease, and hypertension [5, 6, 7]. Early diagnosis and intervention for liver steatosis can prevent disease progression and improve prognosis. Although liver biopsy is the gold standard for diagnosing and quantifying hepatic steatosis, risk of bleeding and infection and sampling error make it an unreliable tool for monitoring hepatic parenchyma [8]. Ultrasound is the preferred diagnostic modality and the most widely used for the detection of liver steatosis, but its results are affected by subjective and limited sensitivity [9]. Furthermore, ultrasound has poor performance in patients with a body mass index (BMI) ≥ 40 kg/m² [2]. Noninvasive detection and quantification are important for the timely management and prevention of the progression of liver steatosis. In recent years, attenuation imaging (ATI) technology is increasingly being used due to its noninvasive and useful properties for the assessment of liver steatosis [10]. ATI is based on two-dimensional ultrasound images and enables visualization of the liver parenchyma [11]. Compared to the normal liver, the attenuation of the ultrasound beam is increased in MAFLD patients due to liver steatosis. ATI calculates the attenuation coefficient (AC), which is expressed in dB/cm/MHz, and corresponds to the change in ultrasound beam intensity. AC can be used to quantify hepatic steatosis. Burgio et al. [11] compared the diagnostic performance of AC with histopathological analysis and reported that patients with steatosis of any grade had a higher AC value than patients without liver steatosis. Furthermore, a greater AC value was correlated with a higher steatosis grade.

AC can be easily measured by placing a 2 × 4 cm region of interest (ROI) in the sampling box [12]. The reliability of the AC can be expressed by an R² value and the AC is considered valid if R² ≥ 0.80. Although the exact number of valid measurements (VMs) for the clinical use of ATI is not clear, most studies performed five VMs for the evaluation of liver steatosis [13]. As we know, no study has evaluated whether the number of VMs affects the diagnostic accuracy of ATI technology. In clinical work, an excessive number of measurements can be time-consuming and increase the burden on patients. Therefore, it is necessary to investigate the optimal number of valid AC measurements that provides good diagnostic accuracy. The aim of our study was to evaluate the effect of different numbers of valid AC measurements on the evaluation of hepatic steatosis using ATI technology, to determine the optimal number of valid measurements, and to investigate the impact factor of the AC on MAFLD patients.

Different VM groups. AC values from different VMs all significantly predicted steatosis grade ≥S1, ≥S2, and S3 without significant statistical differences (p = n.s. for all). The multivariate analysis showed that the hepatic steatosis grade and triglyceride level were factors independently associated with AC.

Conclusion Three valid measurements of AC may be adequate to ensure the accuracy and reproducibility of hepatic steatosis assessment. The degree of liver steatosis and the triglyceride level significantly affected AC values.

ZUSAMMENFASSUNG

Ziel Wir untersuchten die optimale Anzahl gültiger Messungen („valid measurements”, VMs) des Dämpfungskoeffizienten („attenuation coefficient”, AC) zur Beurteilung der Lebersteatose mittels Attenuation-Imaging (ATI) und untersuchten Faktoren, die bei Patienten mit metabolisch-assoziierten Fettlebererkrankung (MAFLD) die AC-Messung beeinflussen können.


Ergebnisse Die mittleren AC-Werte von 1, 2, 3, 5 und 7 VMs unterschieden sich nicht signifikant zwischen den Graden S0–3 (p = n.s. für alle). Die Bland-Altman-Analyse zeigte, dass der mittlere Unterschied der AC-Werte von 3 VMs und 7 VMs 0,003 dB/cm/MHz betrug, was im Vergleich zu 2 VMs geringer war und nahe an 5 VMs lag. Die Intraklassen-Korrelationskoeffizienten der AC-Werte waren alle > 0,90 zwischen den verschiedenen VMs-Gruppen. Die AC-Werte der verschiedenen VMs sagten alle signifikant den Steatosegrad ≥S1, ≥S2 und S3 voraus, ohne signifikante statistische Unterschiede (p = n.s. für alle). Die multivariante Analyse zeigte, dass der Grad der Lebersteatose und der Triglyzeridspiegel Faktoren waren, die unabhängig voneinander mit dem AC assoziiert waren.

Schlussfolgerung Drei gültige Messungen des AC können ausreichen, um die Genauigkeit und Reproduzierbarkeit der Bewertung der Lebersteatose zu gewährleisten. Der Grad der Lebersteatose und der Triglyzeridspiegel beeinflussten die AC-Werte signifikant.
Materials and methods

Patients

Between June 2021 and January 2022, 319 consecutive adult patients undergoing bariatric surgery, as per the standard National Institute of Health (NIH) criteria, were enrolled in this study. All patients fulfilled the diagnostic criteria for MAFLD. Patients were excluded as follows: (1) loss of ATI data; (2) no liver biopsy within 3 days after ATI examination; (3) patients with malignant tumors (such as hepatocellular carcinoma or cholangiocarcinoma) or less than seven valid AC measurements. In total, 139 patients were included. ▶ Fig. 1 presents the flowchart of study patients.

The prospective study and its protocol were approved by the Ethics Committee of our institute, and all participants provided written informed consent.

Attenuation imaging

ATI examinations were performed using an Aplio i900 (Canon Medical Systems, Tochigi, Japan) and an i8CX1 convex probe by a single physician with 5 years of experience performing abdominal ultrasound. The patients fasted for at least 6 hours before the ATI examination, and the operator was blinded to the clinical information. The patient was placed in a supine position with their right upper limb lifted. The probe was placed on the skin, between two ribs in the intercostal space overlying the right lobe of the liver. When the ATI mode was selected, the patient was instructed to hold his or her breath for 5 s.

After the ATI mode was initiated, a sampling box was placed over the hepatic parenchyma more than 2 cm below the liver capsule (▶ Fig. 2). Then, a 2 × 4 cm ROI, the default setting for AC measurement, was placed within the middle portion of the sampling box. The AC value (measured in dB/cm/MHz) was displayed in the bottom left corner. Each AC measurement yielded a quality measurement coefficient (goodness-of-fit) value. The reliability coefficient of the measurement result was expressed by an R² value and categorized as poor (R² < 0.80), good (0.80 ≤ R² < 0.90), and excellent (R² ≥ 0.90). An AC value with R² ≥ 0.80 was considered valid. If the following conditions were met, the measurement was considered successful: (1) At least five valid data points collected. (2) The valid rate was over 60 %. (3) In valid data points, the interquartile range was less than 30 % of the median AC value. In the patients collected from June 2021 to September 2021, at least five valid AC measurements were collected; in the patients from October 2021 to January 2022, at least seven valid measurements were collected. We selected the first 1, 2, 3, 5, and 7 VMs and calculated the mean AC values of the first 1, 2, 3, 5, and 7 VMs. The skin-to-liver-capsule distance was also measured.

Pathological evaluation

Within 3 days after ATI examination, the liver biopsy samples were obtained from the left lobe using an 18-gauge Tru-Cut needle during bariatric surgery under laparoscopic vision. The median time between ATI examination and liver biopsy was 2 days. The specimen was fixed in paraffin wax for histopathologic evaluation. According to the steatosis, activity, and fibrosis (SAF) score [14], steatosis (S) was graded from 0 to 3 (S0, < 5 %; S1, 5–33 %; S2, 34–66 %; S3, > 66 %) based on the percentage of hepatocytes with intracytoplasmic lipid droplets. Ballooning and inflammation of hepatocytes was graded from 0 to 2. Fibrosis (F) was categorized as follows: without fibrosis (F0), mild fibrosis (F1), moderate fibrosis (F2), severe fibrosis (F3), and cirrhosis (F4). Non-alcoholic steatohepatitis (NASH) was defined as steatosis with both ballooning and inflammation. Fibrotic NASH was defined as a total score of steatosis, ballooning, and inflammation ≥ 4, and fibrosis stage ≥F2 [15].

Statistical analysis

The patient characteristics are presented as mean ± standard deviation for continuous variables and absolute figures with percentages for categorical variables. The independent t test, analysis of variance, and the Kruskal–Wallis test were performed to analyze continuous variables, whereas the χ² test was performed for categorical variables. Bland-Altman analysis was performed to measure the agreement in AC values between other VMs and 7 VMs. The intraclass correlation coefficient (ICC) was calculated to express the correlation of AC from different VM groups and reflected the consistency between groups. The ICC was graded...
as poor (ICC = 0–0.5), moderate (ICC = 0.5–0.75), good (ICC = 0.75–0.90), and excellent (ICC = 0.90–1) consistency [16]. A receiver operating characteristic curve (ROC) was constructed to estimate the performance of the AC values acquired from a different number of measurements for the assessment of liver steatosis grade. Delong’s test was used to compare the areas under the ROC curve (AUROC). Linear regression was used to identify the factors significantly related to AC values. Binary logistic regression was used to analyze the related factors for fibrotic NASH. A p-value < 5 % was considered significant. The tests were performed using SPSS (version 20.0) or MedCalc (version 15.2.2) software.

Results

Patient characteristics

Of 319 MAFLD patients, 316 had ATI data. The success rate of AC measurement was 93.4 % in 316 patients. Of 319 patients, 25 withdrew their consent before the surgery and were excluded. Of the remaining 294 subjects, 124 did not consent to liver biopsy. Of the remaining 170 subjects, 31 were excluded because of less than 7 valid AC measurements which were all derived from the cohort of June–September 2021. The study included 139 patients.

The baseline demographic, clinical, biochemical, and pathological data of the patients are summarized in Table 1. The patients had an average age of 31 ± 8 years and 31 (22.3 %) were males. The mean BMI was 37.5 ± 6 kg.m\(^{-2}\). Based on the WHO class of obesity [17], 9 (6.4 %), 45 (32.4 %), 45 (32.4 %), and 40 (28.8 %) patients were overweight, class I, class II, and class III obesity, respectively. 57 patients had diabetes. According to the pathological analysis of hepatic steatosis grades, 16 (11.5 %), 56 (40 %), 51 (37 %), and 16 (11.5 %) patients had S0, S1, S2, and S3 grades, respectively. 9 (6.5 %), 34 (24.5 %), 69 (49.6 %), 27 (19.4 %), and 0 (0 %) patients were categorized as F0, F1, F2, F3, and F4, respectively. Furthermore, 16 (11.5 %), 53 (38.1 %), and 70 (50.4 %) patients had no steatosis, simple steatosis, and NASH, respectively. 35 (25.2 %) patients had fibrotic NASH.

Comparison of AC values from different valid measurements at each hepatic steatosis grade in MAFLD patients

There was no significant difference in the mean AC values obtained from 1, 2, 3, 5, and 7 VMs at each liver steatosis grade in MAFLD patients (from S0 to S3, p = 0.999, 0.976, 0.746, and 0.999, respectively; Table 2). In Fig. 3. Bland-Altman analysis showed that the mean difference in AC values between 1 VM and 7 VMs was 0.010 dB/cm/MHz for all steatosis grades (95 % CI: –0.090, 0.109). The mean difference between 2 VMs and 7 VMs was –0.008 dB/cm/MHz (95 % CI: –0.067, 0.051). The mean difference between the mean of 3 VMs and 7 VMs was –0.003 dB/cm/MHz (95 % CI: –0.039, 0.049). The mean difference between the mean of 5 VMs and 7 VMs was –0.002 dB/cm/MHz (95 % CI: –0.026, 0.022).

Correlation among different valid measurements of AC values at each hepatic steatosis grade

At different pathological liver steatosis grades (S0-S3) and all grades, the ICCs of AC values from different VMs were 0.950 (95 % CI: –0.900, 0.980), 0.949(95 % CI: –0.926, 0.967), 0.929 (95 % CI: –0.895, 0.955), 0.960 (95 % CI: –0.921, 0.984), and 0.960 (95 % CI: –0.949, 0.969), respectively (Table 3).
Table 2 Comparison of attenuation coefficient values from different valid measurements at each hepatic steatosis grade in MAFLD patients.

<table>
<thead>
<tr>
<th>Grade</th>
<th>n</th>
<th>1 VM (dB/cm/MHz)</th>
<th>2 VMs (dB/cm/MHz)</th>
<th>3 VMs (dB/cm/MHz)</th>
<th>5 VMs (dB/cm/MHz)</th>
<th>7 VMs (dB/cm/MHz)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>16</td>
<td>0.72 ± 0.14</td>
<td>0.74 ± 0.16</td>
<td>0.73 ± 0.15</td>
<td>0.73 ± 0.15</td>
<td>0.73 ± 0.15</td>
<td>0.999</td>
</tr>
<tr>
<td>S1</td>
<td>56</td>
<td>0.84 ± 0.14</td>
<td>0.86 ± 0.14</td>
<td>0.85 ± 0.13</td>
<td>0.85 ± 0.13</td>
<td>0.85 ± 0.13</td>
<td>0.976</td>
</tr>
<tr>
<td>S2</td>
<td>51</td>
<td>0.93 ± 0.10</td>
<td>0.95 ± 0.10</td>
<td>0.93 ± 0.10</td>
<td>0.94 ± 0.10</td>
<td>0.94 ± 0.09</td>
<td>0.746</td>
</tr>
<tr>
<td>S3</td>
<td>16</td>
<td>0.99 ± 0.15</td>
<td>1.00 ± 0.14</td>
<td>0.98 ± 0.14</td>
<td>0.99 ± 0.14</td>
<td>0.99 ± 0.14</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Note: VM = valid measurement

Fig. 3 Box plot graphs showing the comparison of the attenuation coefficient (AC) values of 1, 2, 3, 5, and 7 valid measurements (VMs) for liver steatosis groups (A) S0, (B) S1, (C) S2, and (D) S3, respectively. Boxes represent the 25th and 75th percentiles and outlier dots.
Comparison of diagnostic performance of AC values from different valid measurements in distinguishing between steatosis grades in MAFLD patients

Fig. 5 and Table 4 showed the diagnostic performance and corresponding ROC curves for predicting steatosis grade ≥ S1, ≥ S2, and S3 using AC values obtained from different VM groups. There was no statistically significant difference in AUROC values between the groups (p = n.s. for all).

Distribution of AC values from three valid measurements at different grades of hepatic steatosis in MAFLD patients

Although there was no difference in the diagnostic performance with respect to predicting the steatosis grade between the average AC values of 1, 2, 3, 5, and 7 VMs, the mean difference in AC values of 3 VMs and 7 VMs was smaller than 1 VM and 7 VMs, as well as 2 VMs and 7 VMs. Three valid measurements were used for further analysis. According to the liver steatosis grade, the mean

Table 3 Correlation among different valid measurements of attenuation coefficient values at each hepatic steatosis grade.

<table>
<thead>
<tr>
<th>Grade</th>
<th>n</th>
<th>ICC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>16</td>
<td>0.950</td>
<td>0.900–0.980</td>
</tr>
<tr>
<td>S1</td>
<td>56</td>
<td>0.949</td>
<td>0.926–0.967</td>
</tr>
<tr>
<td>S2</td>
<td>51</td>
<td>0.929</td>
<td>0.895–0.955</td>
</tr>
<tr>
<td>S3</td>
<td>17</td>
<td>0.960</td>
<td>0.921–0.984</td>
</tr>
<tr>
<td>All</td>
<td>139</td>
<td>0.960</td>
<td>0.949–0.969</td>
</tr>
</tbody>
</table>

Note: ICC = intraclass correlation coefficient; CI = confidence interval
AC values were 0.73 ± 0.15 dB/cm/MHz, 0.85 ± 0.13 dB/cm/MHz, 0.93 ± 0.10 dB/cm/MHz, and 0.98 ± 0.14 dB/cm/MHz, respectively, for S0, S1, S2, and S3 (Table 2). The AC values were significantly different between the grades of liver steatosis (S0 vs. S1–3, p < 0.05; S1 vs. S2 and S3, p < 0.05; S2 vs. S3, p = n.s.; Fig. 6). The AUROC of the AC was 0.812 (95% CI: 0.679–0.945) for predicting ≥S1, 0.758 (95% CI: 0.677–0.839) for predicting ≥S2, and 0.719 (95% CI: 0.588–0.850) for predicting S3 (Fig. 5 and Table 5). The cut-off values for predicting ≥S1, ≥S2, and S3 were 0.73 dB/cm/MHz, 0.90 dB/cm/MHz, and 0.92 dB/cm/MHz, respectively.

Factors associated with AC values in MAFLD patients

The univariate regression analysis showed that the triglyceride, HDL cholesterol, fasting glucose, fasting insulin, and hepatic steatosis levels significantly affected the AC value (Supplementary Table 1). The multivariate regression analysis showed that the triglyceride level and hepatic steatosis grade were associated with the AC value. The AC value was not significantly affected by age, BMI, waist circumference, HbA1c level, or fibrosis stage (p = n.s. for all). The difference in AC values between patients with and without T2DM was marginally significant (p = 0.050; Supplementary Table 2). However, there was no difference in AC values among overweight, class I obesity, class II obesity, and class III obesity (p = 0.765; Supplementary Table 2). There was no significant difference in AC values between simple steatosis and NASH (p = 0.220; Supplementary Table 2).

Factors associated with fibrotic NASH

The AUROC of the AC was 0.637 (95% CI: 0.535–0.739) for predicting fibrotic NASH. The cut-off value for predicting fibrotic NASH was 0.98 dB/cm/MHz. As shown in Supplementary Table 3, patients with high fasting glucose or a high AC had 1.166-fold (95% CI: 1.015–1.340; p = 0.030) and 48.775-fold (95% CI: 2.480–959.160; p = 0.011) increased likelihoods for fibrotic NASH. T2DM (OR, 4.694; 95% CI, 2.058–10.706; p < 0.001) was strongly associated with increased odds for fibrotic NASH.
Discussion

MAFLD is diagnosed based on “positive criteria” for diagnosis, which ensures that it is a clear and unique entity [1]. These criteria are based on the detection of steatosis in different investigations (imaging, blood biomarkers, or histology) and fulfillment of one of the three conditions: overweight or obesity, diabetes, or evidence of metabolic abnormalities [18]. Recently metabolic dysfunction-associated steatotic liver disease (MASLD) was adopted as the new nomenclature and new diagnostic criteria that are widely supported were introduced in order to improve awareness of liver steatosis [19]. The term MAFLD has been updated as MASLD. ATI is a quantitative imaging technology for the noninvasive diagnosis of liver steatosis, with easy accessibility that makes it suitable for the detection and monitoring of liver parenchyma diseases. Previous ATI studies have mainly focused on the diagnostic performance for the assessment of hepatic steatosis [10, 11, 20]. A recent study by Sugimoto et al. [21] suggested that the ROI for AC measurement should be placed in the middle or at the bottom of the sampling box, rather than at the upper edge of the sampling box. Many previous studies recommend five VMs for assessment, but no study has analyzed the number of VMs required to obtain reliable AC values without significant loss of diagnostic accuracy. The purpose of our study was to compare the effect of AC values from different numbers of VMs to assess the steatosis grade in MAFLD patients. We evaluated the AC values from 1, 2, 3, 5, and 7 VMs.

Our results showed that, for each steatosis grade from S0 to S3, the mean AC values between the different VMs were not statistically different. For all VM groups, the ICCs of AC values at each liver steatosis grade reached up to 0.90, which correlated with excellent consistency. It is essential to minimize the presence of non-homogeneous areas (e.g., portal vein) in the sampling box and avoid areas producing strong reverberant signals. The sampling box was placed more than 2 cm below the liver capsule and the ROI for AC measurement needed to be kept away from the upper edge of the sampling box. These measurements may allow the calculation of AC values from a small number of VMs that are close to those obtained by multiple VMs, thereby indicating good repeatability.

The accuracy of ATI for the diagnosis of liver steatosis based on the mean AC values was similar between the five VM groups. The AUROCs for each group were similar with no statistically significant differences based on Delong’s test. Therefore, we speculated that AC data can be obtained from 1, 2, and 3 VMs of detection that have performance equivalent to AC data obtained from 7 VMs. In clinical practice, it is not recommended to obtain the reliable result from a single AC value, and the result of Bland-Altman analysis showed that the mean difference in AC values of 3 VMs and 7 VMs was 0.003 dB/cm/MHz, which was smaller compared with 2 VMs, and close to 5 VMs. Therefore, we suggest that three VMs of the AC should be obtained to assess liver steatosis without a significant reduction in diagnostic accuracy. This can reduce the workload for doctors, simplify the process for patients, and improve testing efficiency.

The mean AC values from three VMs differed significantly between different steatosis grades. Although there was no difference in the AC values between S2 and S3, we found a significant increase in AC values with increasing grade of liver steatosis. Further...
thermore, AUROCs of the AC for the diagnosis of ≥ S1, ≥ S2, and S3 were 0.812, 0.758, and 0.719 in MAFLD patients, corresponding to moderate diagnostic value. The cut-off values for predicting ≥ S1, ≥ S2, and S3 were 0.73 dB/cm/MHz, 0.90 dB/cm/MHz, and 0.92 dB/cm/MHz, respectively. Some MAFLD patients have higher proportions of metabolic comorbidities (diabetes, hypertension), and higher fasting glucose levels and lipid levels [18]. The linear analysis showed that the degree of liver steatosis and the triglyceride level were independently associated with the AC value. Therefore, the AC value may increase with an increasing degree of liver steatosis and triglyceride level. However, our results showed that the stage of fibrosis was not related to the AC value, which was consistent with the findings of Bae et al. [12]. The possible explanations for these results could be the uneven distribution of patients across the fibrosis stages. In our study, more than 90% (130 of 139) of patients were categorized as F1, F2, and F3, whereas only 6% (9 of 139) were categorized as F0, and none were categorized as F4. No patient had cirrhosis, probably because of the younger age in our cohort. Furthermore, the AC value was not significantly influenced by age, BMI, waist circumference, and HbA1c level.

Patients with fibrotic NASH correspond to patients with active steatohepatitis and stage 2 fibrosis or higher. Identifying fibrotic NASH in patients remains a high priority in clinical practice, since such patients are candidates for therapeutic clinical trials with novel agents. The diagnostic capability of the AC for predicting fibrotic NASH was not particularly high. A model combining multiple parameters to improve predictive properties is to be explored in further studies.

This study had some limitations. The ATI examination was performed over the right lobe of the liver, rather than at the liver biopsy site (i.e., the left lobe), which may have introduced bias into our results. Further studies are needed to compare the results across different detection sites. In addition, we only analyzed patients with a single etiology of MAFLD and excluded those with other liver diseases. Furthermore, patients who were diagnosed with MAFLD were largely overweight and suffered from metabolic syndrome, which may have caused a population selection bias. Therefore, a larger study, possibly a multicenter trial, of patients with multiple chronic liver diseases should be conducted to confirm our findings.

In conclusion, our results suggest that adequate repeatability and accuracy of measurement results can be achieved by adopting three valid measurements for AC values to assess hepatic steatosis without significant loss of diagnostic accuracy in MAFLD patients. The degree of liver steatosis and triglyceride level were significant factors affecting the AC value.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

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