HEPATOBILIARY-PANCREAS

Assessment of hepatic steatosis by using attenuation imaging: a quantitative, easy-to-perform ultrasound technique

Jae Seok Bae 1,2 · Dong Ho Lee 1,2 · Jae Young Lee 1,2,3 · Haeryoung Kim 4 · Su Jong Yu 5 · Jeong-Hoon Lee 5 · Eun Ju Cho 5 · Yun Bin Lee 5 · Joon Koo Han 1,2,3 · Byung Ihn Choi 6

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Abstract
Objectives To evaluate the diagnostic performance of attenuation imaging (ATI) in the detection of hepatic steatosis compared with a histopathology gold standard.
Methods We prospectively enrolled 108 consecutive patients (35 males; median age, 54.0 years) who underwent percutaneous liver biopsy for evaluation of diffuse liver disease between January 2018 and November 2018 in a tertiary academic center. Grayscale ultrasound examination with ATI was performed just before biopsy, and an attenuation coefficient (AC) was obtained from each patient. The degree of hepatic steatosis, fibrosis stage, and necroinflammatory activity were assessed on histopathologic examination. The significant factor associated with the AC was found by a linear regression analysis, and the diagnostic performance of the AC for the classification into each hepatic steatosis stage was evaluated by receiver operating characteristic (ROC) analysis.
Results The distribution of hepatic steatosis grade on histopathology was 53/11/22/16/6 for none/mild (< 10%)/mild (≥ 10%)/moderate/severe steatosis, respectively. The area under the ROC curve, sensitivity, specificity, and optimal cutoff AC value for detection of hepatic steatosis ranged from 0.843–0.926, 74.5–100.0%, 77.4–82.8%, and 0.635–0.745, respectively. Multivariate analysis revealed that the degree of steatosis was the only significant determinant factor for the AC.
Conclusions The AC from ATI provided good diagnostic performance in detecting the varying degrees of hepatic steatosis. The degree of steatosis was the only significant factor affecting the AC, whereas fibrosis and inflammation were not.

Key Points
- Attenuation imaging (ATI) is based on two-dimensional grayscale ultrasound images that can incorporate into routine ultrasound examinations with less than 2 min of acquisition time.
- ATI provided good diagnostic performance in detecting the varying degrees of hepatic steatosis with an area under the ROC curves ranging from 0.843 to 0.926, and there was no technical failure in this study indicating high applicability of this technique.
- The degree of hepatic steatosis was the only significant factor affecting the result of ATI examination.

Keywords Fatty liver · Ultrasonography · Biopsy · Sensitivity and specificity · Linear models

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**Abbreviations**

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<tr>
<th>Abbreviation</th>
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<tr>
<td>AC</td>
<td>Attenuation coefficient</td>
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<td>ATI</td>
<td>Attenuation imaging</td>
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<tr>
<td>AUC</td>
<td>Area under the receiver operating characteristic curve</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CAP</td>
<td>Controlled attenuation parameter</td>
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<tr>
<td>MR</td>
<td>Magnetic resonance</td>
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<tr>
<td>NAFLD</td>
<td>Nonalcoholic fatty liver disease</td>
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<td>NASH</td>
<td>Nonalcoholic steatohepatitis</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>SCD</td>
<td>Skin-capsular distance</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TE</td>
<td>Transient elastography</td>
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<td>US</td>
<td>Ultrasound</td>
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</table>

**Introduction**

Hepatic steatosis is the excessive intracellular accumulation of triglyceride in hepatocytes and is common, with an estimated prevalence of more than 30% in an urban population [1]. It is most commonly associated with nonalcoholic fatty liver disease (NAFLD) and also with alcoholic fatty liver disease, viral hepatitis, or drug-induced liver injury [2]. Hepatic steatosis can potentially progress into cirrhosis, liver failure, or hepatocellular carcinoma [3, 4]. Currently, the incidence of nonalcoholic steatohepatitis (NASH), which is defined as hepatic steatosis with combined inflammation, is increasing. Nowadays, NASH is the most common cause of liver transplantation for women in North America [5]. Hepatic steatosis is associated with cardiovascular disease, as well as metabolic syndrome [6], and can be improved by treatment [7]. This explains that steatosis is increasingly screened in these high-risk patients [8, 9]. Therefore, it is important to detect and quantify hepatic steatosis for a timely management as well as to prevent it from progressing to NASH and liver cirrhosis.

Liver biopsy, the former reference standard for the diagnosis and quantification of hepatic steatosis, has drawbacks like sampling error, bleeding risk, and interreader variability [10–12] and is not anymore a monitoring tool due to poor acceptance, risk, and cost. Therefore, noninvasive methods have been investigated. Magnetic resonance (MR) spectroscopy and MR fat fraction offer quantification of liver fat with a diagnostic accuracy that may exceed that of liver biopsy [13, 14], however with the limitation of cost and availability.

Ultrasound (US) is a screening imaging tool for patients with suspected liver disease and has merits like noninvasiveness, low cost, and availability [15]. However, grayscale US has drawbacks: subjective nature of examination, low accuracy, and intra/interobserver variability [16, 17]. Attenuation imaging (ATI) assesses the characteristics of US beam attenuation in certain tissues. In the liver, the attenuation is determined by the acoustic characteristics of the hepatic parenchyma [18]. In the case of steatosis, the attenuation increases, particularly in deeper areas [18]. Although ATI shares with transient elastography, the controlled attenuation parameter (CAP), ATI is based on two-dimensional US images and is performed during grayscale ultrasound examination, avoiding large vessel or focal hepatic lesion. ATI yields an attenuation coefficient (AC), which corresponds to the change of US beam intensity with depth on US images. To obtain pure intensity change for the calculation of AC, systemic influence such as gain control or focus-dependent US beam profile is removed from the observed intensity. Therefore, ATI has the potential to quantitatively assess the degree of steatosis. However, clinical usefulness has not been evaluated.

Therefore, the purpose of this study was to prospectively investigate the use of ATI in the detection and grading of hepatic steatosis compared with a histopathology gold standard.

**Materials and methods**

**Patient enrollment**

This prospective, single-institution study was performed in compliance with the Health Insurance Portability and Accountability Act. The Korean Food and Drug Administration and our institutional review board approved this study (H-1712-163-914) and we obtained written informed consent from all patients.

The patients were consecutively enrolled from the outpatient clinics of the Department of Surgery and Internal Medicine in the Seoul National University Hospital between January 2018 and November 2018. Patients with elevated liver function test results or with clinical suspicion of having diffuse liver disease were referred to percutaneous US-guided liver biopsy for the evaluation of etiology and disease activity (Fig. 1). The inclusion criteria were the following: (1) informed consent was obtained, (2) aged between 20 years old and 85 years old, and (3) patients without significant bleeding risk (platelet ≥ 80,000/μL, prothrombin time international normalized ratio ≤ 1.5). The exclusion criteria were the following: (1) age < 20 years old or > 85 years old and (2) patients with bleeding risk (platelet < 80,000/μL, prothrombin time international normalized ratio > 1.5).

**ATI examination**

All ATI examinations were performed by two radiologists (with 13 and 6 years of experience in abdominal US imaging, respectively) using a US scanner (Apio i900, Canon Medical Systems) with a 1–8-MHz convex probe. The patients fasted for at least 6 h before ATI, and both operators were blinded to the clinical details of the patients. At first, we evaluated liver...
parenchyma on B-mode images to detect any focal liver lesion. After that, ATI mode was activated, and examinations were performed in the right liver lobe through an intercostal window with the transducer perpendicular to the skin. ATI examination was conducted where liver biopsy was likely to be performed. A fan-shaped sampling box was positioned in the hepatic parenchyma while the breath was held. The sampling box was as large as possible to obtain more average data, but it was at least 2 cm below the liver capsule to avoid reverberation artifacts (Fig. 2). Details of ATI examination are described in the Supplementary material. Structures other than hepatic parenchyma such as large vessels were regarded as heterogeneous structures and were automatically excluded on the sampling box by using intensity variance. Thereafter, a 2 × 4 cm or more sized fan-shaped region of interest (ROI) for measurement was placed within the sampling box. The AC value, which is dB/cm/MHz, was displayed on the lower left corner. Additionally, the reliability of the result was displayed as an $R^2$ value. The $R^2$ values were categorized into poor ($R^2 < 0.80$), good ($0.80 \leq R^2 < 0.90$), and excellent ($R^2 > 0.90$). ACs with $R^2 \geq 0.80$ were regarded as valid measurements and we placed the ROIs in the middle portion of the sampling box to reduce the intraobserver variability. ATI examinations were performed until five valid measurements were obtained and the median value among the obtained results was used. The skin-capsular distance (SCD), which was defined as the distance between the skin surfaces to the liver capsule, was measured during the ATI examinations as well. The length of the ATI examination was measured from the activation of ATI mode to the acquisition of five valid measurements. In each patient, all measurements were performed at the same intercostal sonic window while a patient holds his or her breath.
Histopathologic analysis

After performing ATI, a percutaneous liver biopsy using an 18-G automatized gun (TSK laboratory) was conducted under US guidance in all patients. After liver biopsy, electronic medical records of all patients were reviewed to detect adverse events related to biopsy.

From liver biopsy, two pieces of 22-mm-length liver specimens were obtained and fixed in a formalin-alcohol-acetate solution. After being embedded in paraffin, 4-mm-thick slices were cut for staining with hematoxylin-eosin. One expert pathologist (with 18 years of experience in liver pathology) who was blinded to the US examination results analyzed all specimens. Masson’s trichrome staining was also performed for the assessment of hepatic fibrosis. The degree of steatosis was determined according to the histological scoring system for NAFLD as follows: S0 (< 5%, none); S1 (5–33%, mild); S2 (> 33–66%, moderate); and S3 (> 66%, severe) [19]. In addition, S1 was further divided into < 10% and ≥ 10%, which is the criterion used for screening potential liver donors [20].

Fibrosis and necroinflammatory activity in the liver were evaluated by the standardized guidelines established by the Korean Study Group for the Pathology of Digestive Diseases, which is similar to the META VIR scoring system [21, 22]. Fibrosis was graded on a 0–4 scale as follows: F0 (no fibrosis), F1 (portal fibrosis without septa), F2 (portal fibrosis and few septa), F3 (numerous septa without cirrhosis), and F4 (cirrhosis). The necroinflammatory activity consisted of lobular activity and porto-periportal activity, and both were graded on a 0–4 scale as follows: score 0 (none), score 1 (minimal), score 2 (mild), score 3 (moderate), and score 4 (severe).

Statistical analysis

Data were expressed as the mean ± standard deviation (SD), median (IQR), or absolute figures (percentages), as appropriate. Spearman correlation coefficients were used to assess the correlations between each variable. The Kruskal-Wallis tests and the Mann-Whitney U tests were performed to compare the AC between the groups of different degrees of hepatic steatosis. Receiver operating characteristic curves were calculated to evaluate the diagnostic performance of the AC for detecting hepatic steatosis using the area under the receiver operating characteristic curve (AUC) and to identify the optimal cutoff values. Univariate linear regression analyses were performed to evaluate the relationship between each factor and the AC, followed by a multivariate analysis to determine the factors that significantly affect the AC, including all variables with \( p < 0.05 \) on a univariate analysis. All statistical analyses were performed with statistical software (MedCalc version 18.9; MedCalc Software, and IBM SPSS Statistics for Windows, Version 23.0; IBM Corp.). A difference with a \( p \) value <0.05 was considered to be statistically significant.

Results

Patient characteristics

A total of 115 consecutive patients were subjected for liver biopsy for the following reasons: liver function test abnormality (n = 46), positive serum autoantibody (n = 31), liver function test abnormality combined with positive serum autoantibody (n = 10), fatty liver (n = 6), fatty liver with liver function test abnormality (n = 4), liver donor (n = 4), liver cirrhosis of unknown etiology (n = 4), and increased stiffness on transient elastography (n = 3). Seven patients were excluded because of the patients’ refusal to participate in the study. The patients’ characteristics are shown in Table 1. Among the 108 patients, 25 (23.1%) and 46 (42.6%) patients were categorized as overweight (body mass index [BMI] 23–25 kg/m²) and obese (BMI > 25 kg/m²), respectively, according to the modified BMI criteria for Asians [23]. No patient had an adverse event related to the percutaneous liver biopsy.

AC from ATI according to the histopathologic findings

ATI yielded valid AC measurements in all 108 patients and there was no technical failure. The median and mean length of ATI examinations were less than 2 min for both.

There was a significant correlation between the degree of steatosis and AC (\( \rho = 0.660, 95\% \text{ confidence interval [CI]} 0.538–0.755, p < .001 \)). AC increased as the degree of hepatic steatosis increased (Fig. 3) and there was a significant difference in the AC between each group of different degrees of hepatic steatosis (\( p < 0.05 \)), except for between the patients with moderate steatosis and with severe steatosis (\( p = 0.06 \)). In contrast, fibrosis stage and necroinflammatory activity were not associated with the AC (\( \rho = 0.149 \) and 0.169, \( p = 0.125 \) and 0.081, respectively).

The diagnostic performance of AC in the evaluation of hepatic steatosis

The AUC of AC for the detection of hepatic steatosis was progressively increased from ≥ S1, ≥ 10%, ≥ S2 to ≥ S3 (0.843, 95% CI, 0.761–0.906; 0.876, 95% CI, 0.799–0.931; 0.886, 95% CI, 0.811–0.939; 0.926, 95% CI, 0.860–0.968) (Fig. 4). The cutoff AC values for the detection of hepatic steatosis of ≥ S1, ≥ 10%, ≥ S2 to ≥ S3 was also increased (0.635, 0.660, 0.700, and 0.745, respectively). The corresponding sensitivity, specificity, positive predictive value, negative predictive value, and accuracy are shown in Table 2. The cutoff AC value of 0.560 and 0.585 yielded a negative predictive value of 92.9% and 92.1% for the detection of hepatic steatosis of ≥ S1 and ≥ 10%, respectively.
Factors associated with AC obtained from ATI

For the univariate regression analysis, BMI, SCD, and hepatic steatosis were significant factors affecting the AC (Table 3). Although BMI and SCD were significantly correlated with one another in our study population ($\rho = 0.816$, 95% CI 0.741–0.871, $p < 0.001$), both variables were used in the multivariate analysis because the variance inflation factors of BMI and SCD were only 3.453 and 3.466, respectively, and the issue of multicollinearity was considered not significant. Multivariate regression analysis revealed that only the grade of hepatic steatosis is associated with the AC ($p < 0.001$).

Discussion

In our study, the AUC, sensitivity, specificity, and optimal cutoff value of AC obtained from ATI for the detection of hepatic steatosis ranged from 0.843–0.926, 74.5–100.0%, 77.4–82.8%, and 0.635–0.745, respectively. Moreover, there was no patient who failed to obtain AC from ATI, indicating high applicability of this technique in clinical practice. The AC value was significantly correlated with the degree of hepatic steatosis ($\rho = 0.660$, $p < 0.001$) and showed a high negative predictive value for the detection of any or clinically meaningful degree of hepatic steatosis ($\geq S1$ or $\geq 10\%$, respectively) by using a cutoff value of 0.560 and 0.585, respectively. Therefore, ATI could be used as a screening examination for the selection of liver donor candidate, in whom $< 10\%$ of hepatic steatosis is required. In addition to good diagnostic performance, the ease-of-use of ATI renders it as a promising screening and surveillance examination tool for the detection of hepatic steatosis. In our study, less than 2 min was required to obtain five valid measurements for the AC. Additionally, ATI can be easily incorporated into routine ultrasound
examinations because it is based on two-dimensional gray-scale ultrasound images. ATI also enables the placement of an ROI to avoid unreliable areas, such as large hepatic vessels or bile ducts, and provides a reliability index ($R^2$), which could result in the absence of technical failure of ATI in our study. Moreover, the sample volume of ATI is at least 8 cm$^2$, which is two times as large as that of controlled attenuation parameter (CAP) in transient elastography (TE), and it would be another potential benefit of ATI in terms of the measurement reliability and reproducibility [24]. Indeed, the ability to provide routine B-mode US examination in the ATI examination is another merit of this technique over CAP in TE.

![Fig. 4](image)

**Fig. 4** The diagnostic performance of the attenuation coefficient for detecting varying degrees of hepatic steatosis on histopathology. The receiver operating characteristic curves and their area under the curves are presented for the detection of mild ($\geq 5\%$) (a), mild ($\geq 10\%$) (b), moderate (c), and severe (d) steatosis.

<table>
<thead>
<tr>
<th>Grade of hepatic steatosis</th>
<th>Cutoff value</th>
<th>AUC</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 5%$</td>
<td>$&gt; 0.635$</td>
<td>$0.843$ (0.761, 0.906)</td>
<td>74.5 (61.0, 85.3)</td>
<td>77.4 (63.8, 87.7)</td>
<td>77.4 (67.0, 85.2)</td>
<td>74.5 (64.6, 82.5)</td>
<td>75.9 (82/108)</td>
</tr>
<tr>
<td>$\geq 10%$</td>
<td>$&gt; 0.660$</td>
<td>$0.876$ (0.799, 0.931)</td>
<td>79.5 (64.7, 90.2)</td>
<td>82.8 (71.3, 91.1)</td>
<td>76.1 (64.5, 84.8)</td>
<td>85.5 (64.5, 84.8)</td>
<td>81.5 (88/108)</td>
</tr>
<tr>
<td>&gt; 33%</td>
<td>$&gt; 0.700$</td>
<td>$0.886$ (0.811, 0.939)</td>
<td>86.4 (65.1, 97.1)</td>
<td>81.4 (71.6, 89.0)</td>
<td>54.3 (42.5, 65.6)</td>
<td>95.9 (89.0, 98.5)</td>
<td>82.4 (89/108)</td>
</tr>
<tr>
<td>&gt; 66%</td>
<td>$&gt; 0.745$</td>
<td>$0.926$ (0.860, 0.968)</td>
<td>100.0 (54.1, 100.0)</td>
<td>82.4 (73.6, 89.2)</td>
<td>25.0 (18.0, 33.6)</td>
<td>100.0</td>
<td>83.3 (90/108)</td>
</tr>
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</table>

*ATI*, attenuation imaging; *AUC*, area under the curve
In our study, ATI clearly demonstrated a comparable diagnostic performance for the evaluation of hepatic steatosis to that of CAP from the literature. A recent meta-analysis by Kalras et al has reported that CAP has AUCs of 0.823 and 0.865 for the detection of steatosis of ≥S1 and ≥S2, respectively, in patients whose distribution of steatosis was 51%/27%/16%/6% for S0/S1/S2/S3, which closely resembles that of our study [25]. In addition, the subgroup analysis among patients with mild steatosis according to the criteria of 10% steatosis also showed good diagnostic performance. Hepatic steatosis < 10% is considered as the threshold for living donor liver transplantation to minimize the risk of a graft failure in the recipient and to reduce complication in the donor [26]. In our study, ATI showed an AUC of 0.876, which is slightly higher than the AUC of 0.790 by CAP from a large population-based study [27]. Considering these results, ATI might be useful as a screening tool for selecting potential liver donors, although a further prospective study with a large population number would be needed to validate our study results.

Regarding the significant determinant factors for the AC, we found that the degree of hepatic steatosis was the only factor independently associated with the AC on multivariate analysis. This result is concordant with a previous study by Šasso et al on CAP, a similar attenuation-based fat quantification technique, which reported that CAP is only associated with the degree of steatosis [28]. Theoretically, the attenuation of the US beam is dependent on fibrosis, although it is less pronounced than on steatosis [29]. However, our study showed that the stage of fibrosis was not associated with AC, which is similar to the results of CAP [28, 30, 31]. Possible explanations for this result include a skewed distribution of patients with respect to the stage of fibrosis and heterogeneous study population. In our study, more than 66% (71 of 108 patients) belonged to F0 or F1 and less than 20% (20 of 108 patients) belonged to F3 or F4. Additionally, the inclusion of patients with varying degrees of underlying liver conditions that ranged from normal to chronic hepatitis/cirrhosis might have obscured the effect of fibrosis on the AC. To elucidate the influence of other factors, including hepatic fibrosis on the AC, further studies with a homogeneous study population regarding the underlying liver disease (for example, NAFLD) with a large sample size are warranted.

There are a few limitations in our study. First, our study population was heterogeneous regarding the etiology of liver disease, was not focused on the NAFLD, and lacked the information on the NAFLD-related variables, such as waist circumference or blood pressure. Further studies focused on patients with NAFLD are required to assess the diagnostic performance of the ATI examination in the evaluation of NAFLD, which would be quite important in clinical practice. Second, all ATI examinations were performed by one of two operators and therefore, interobserver agreement could not be assessed. However, a recent study by Han et al has shown that AC was reproducible across two different US platforms, although AC was obtained from post-processing in that study [32].

In conclusion, ATI showed a good diagnostic performance in the detection of varying degrees of hepatic steatosis, and the degree of hepatic steatosis was the only significant determinant factor for the AC in the multivariate analysis.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Factors associated with ATI</th>
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<tr>
<td></td>
<td>Univariate analysis</td>
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<tr>
<td></td>
<td>Coefficient 95% CI p value</td>
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<tr>
<td>Age</td>
<td>-0.090 -0.232, 0.052 0.214</td>
</tr>
<tr>
<td>Female gender</td>
<td>-3.131 -8.087, 1.824 0.213</td>
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<tr>
<td>BMI</td>
<td>1.306 0.854, 1.758 &lt; 0.001***</td>
</tr>
<tr>
<td>AST</td>
<td>0.023 -0.012, &lt;0.001 0.051</td>
</tr>
<tr>
<td>ALT</td>
<td>-1.529 -9.819, 6.760 0.715</td>
</tr>
<tr>
<td>GGT</td>
<td>0.001 -0.001, 0.028 0.941</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.509 -0.341, 3.358 0.109</td>
</tr>
<tr>
<td>Platelet</td>
<td>1.995 -0.198, 4.188 0.074</td>
</tr>
<tr>
<td>SCD</td>
<td>9.033 7.101, 10.965 &lt; 0.001***</td>
</tr>
<tr>
<td>Fibrosis stage</td>
<td>1.509 -0.341, 3.358 0.109</td>
</tr>
<tr>
<td>Necroinflammatory activity</td>
<td>1.995 -0.198, 4.188 0.074</td>
</tr>
<tr>
<td>Steatosis grade</td>
<td>9.033 7.101, 10.965 &lt; 0.001***</td>
</tr>
</tbody>
</table>

Values are multiplied by 100. ***p < 0.001. ATI, attenuation imaging; CI, confidence interval; BMI, body mass index; AST, aspartate transaminase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; SCD, skin-capsular distance.
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Compliance with ethical standards

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Informed consent Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval Institutional Review Board approval was obtained.

Methodology

• Prospective
• Diagnostic or prognostic study
• Performed at one institution

References


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