Diagnostic performance of a novel ultrasound-based quantitative method to assess liver steatosis in histologically identified nonalcoholic fatty liver disease

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Abstract
Aims: To investigate the diagnostic performance of ultrasound-guided attenuation parameter (UGAP) for the detection of hepatic steatosis in nonalcoholic fatty liver disease (NAFLD) cohorts using histopathology as the reference standard and comparing it with that of various imaging modalities. Materials and methods: A total of 87 subjects who underwent UGAP, controlled attenuation parameter (CAP), and magnetic resonance imaging-based proton density fat fraction (MRI-PDFF) between December, 2020 and January, 2022 were enrolled. Of these patients, 38 patients had NAFLD. The association between UGAP and clinical and imaging parameters was assessed using Pearson’s or Spearman’s correlations. The area under the receiver operating characteristic curve (AUROC) was used to evaluate the diagnostic performance. Results: The UGAP and MRI-logPDFF demonstrated strong positive correlations (correlation coefficient= 0.704, P <0.0001). UGAP showed excellent diagnostic performance for distinguishing steatosis grade ≥1 with an AUROC of 0.821 (95% confidence interval [CI], 0.729–0.913), which was comparable to that of MRI-PDFF (0.829, 95%CI, 0.723–0.936). The AUROCs of BUSG (B-mode ultrasound) (0.766, 95% CI, 0.767–0.856) and CAP (0.788, 95% CI, 0.684–0.891) were slightly lower than those of UGAP. The AUROCs of UGAP, MRI-PDFF, CAP, and BUSG for detecting steatosis grade ≥2 were 0.796 (95% CI, 0.616–0.975), 0.971 (95% CI, 0.936–1.000), 0.726 (95% CI, 0.561–0.891) and 0.774 (95% CI, 0.612–0.936), respectively. Conclusion: UGAP may be a valuable potential screening tool as a first-line assessment of liver steatosis in patients with NAFLD. Keywords: ultrasound-guided attenuation parameter; pathology; magnetic resonance imaging; non-alcoholic fatty liver disease; prospective studies

Introduction
The prevalence of non-alcoholic fatty liver disease (NAFLD) has been increasing along with increasing rates of obesity, diabetes, and metabolic syndrome worldwide [1]. Although liver fibrosis is a key driver of morbidity and mortality in chronic liver disease, early detection and monitoring of simple steatosis are also important because identifying fatty liver is the first step in the screening of hepatic fibrosis in the general population. Furthermore, it has been reported that coexistence of steatosis accelerates the disease progression to hepatic fibrosis [2,3].

Various imaging techniques have been developed for NAFLD diagnosis. In routine clinical practice, ultrasound (US) is the most commonly used tool for the evaluation of fatty liver, owing to its easy accessibility and cost-effectiveness. However, there are several obstacles to conventional B-mode US; for example, the results
are highly operator dependent and subjective. Moreover, it performs poorly in cases of mild steatosis [4].

Several US-based methods have been proposed for quantitative assessment of steatosis. The controlled attenuation parameter (CAP) using transient elastography is one of the techniques that enables the quantification of liver fat. However, it can only be performed in the A-mode using a dedicated probe without visual guidance.

Recently, a new method for attenuation coefficient measurements using an US-guided attenuation parameter (UGAP) was introduced to quantify hepatic steatosis. For this technique to be widely used as a screening tool for fatty liver, validation studies using gold standard methods must precede it. Thus far, there have been limited validation studies on UGAP, including the heterogeneous etiology of chronic liver disease [5-9]. The usefulness of the UGAP in specific population groups, particularly in patients with NAFLD, has not yet been fully established. Validation studies in patients with NAFLD using liver biopsy as the reference standard are difficult to establish, because liver biopsies in asymptomatic patients with NAFLD are rarely available.

This study aimed to investigate the diagnostic performance of UGAP in the diagnosis of hepatic steatosis in a cohort of adults with biopsy-confirmed NAFLD. In addition, magnetic resonance imaging-based proton density fat fraction (MRI-PDFF) has emerged as a leading quantitative imaging method for the assessment of steatosis. Hence, the diagnostic performance of UGAP was compared with that of MRI-PDFF, using histopathology as the reference standard.

Materials and methods

Study population and design

The study population was obtained from a cohort of patients prospectively collected from two different hospitals in Korea. This cohort study was conducted among adults aged ≥20 years, who underwent cholecystectomy for benign gall bladder disease. Most of these cases involved patients with cholecystitis. This prospective study aimed to investigate the association between NAFLD and bile acid alterations in the general population. The diagnosis of NAFLD was based on the detection of hepatic steatosis by histology together with the absence of the secondary causes of hepatic steatosis. To exclude other identifiable cases of secondary hepatic steatosis and minimize the effect of cholecystitis on hepatotoxicity, we excluded participants with the following criteria: 1) moderate-to-severe acute cholecystitis according to the Tokyo guidelines 2018 (grade II or III) [10]; 2) significant biomarker abnormalities, defined as aspartate transaminase and alanine aminotransferase five times the upper limit of normal (ULN), total bilirubin three times the ULN, or platelet counts <50×10⁹/mm³; 3) alcohol intake of 210 g/week for men and 140 g/week for women [11]; 4) positive serologic markers for hepatitis B or C; and 5) past or current use of medications associated with NAFLD, such as valproate, amiodarone, methotrexate, tamoxifen or corticosteroids. After applying the exclusion criteria, liver biopsy was performed during cholecystectomy in all the patients enrolled in the study. The study protocol was approved by the institutional review board of each hospital.

The study population comprised 92 consecutive patients who underwent UGAP, CAP, and MRI-PDFF at Kangbuk Samsung Hospital from December 2020 to January 2022. This study was approved by the institutional review board, and all participants signed an informed consent form. Of these patients, five were excluded because of incomplete self-administered questionnaires about alcohol consumption (n=2) or incomplete clinical data (n=3). Ultimately, 87 patients were enrolled in this study (fig 1).

US examination

All US examinations were performed 1 or 2 days before surgery using the LOGIQ E10 US machine (GE Healthcare, Wauwatosa, WI, USA), which was equipped with UGAP. Two experienced abdominal radiologists with 20 and 7 years of post-fellowship experience performed the examinations using a C1-6 convex array probe. All measurements were performed after at least 4 hours of fasting.

Before the UGAP measurements, qualitative scoring of fatty liver was performed using B-mode ultrasonogra-
phy (BUSG) based on known standard criteria [12]. Fatty liver degree was scored as follows: 1) mild steatosis, diffuse increase of liver parenchymal echoes compared with the kidney; 2) moderate steatosis, impaired visualization of wall echogenicity of the main portal vein; and 3) severe steatosis, blurring of the diaphragm.

For the UGAP measurements, a single fixed-size region of interest (ROI) was placed on the right liver, avoiding large vascular structures, with a fixed depth of 4–8 cm using an intercostal scan. A quality map was used to obtain high-quality signals. The UGAP measurements were considered reliable when at least 10 valid measurements had an interquartile range (IQR)/median lower than 30%. The success rate of obtaining reliable measurements using UGAP was 100%, irrespective of body mass index (BMI).

**CAP measurements**

Immediately after the UGAP measurements, CAP measurements were performed using intercostal scan of the right liver using FibroScan502 (Echosens, Paris, France) and M probe (3.5 MHz). Because the reliability criteria for CAP measurements are not yet well-defined, CAP measurements with 10 successful acquisitions were considered reliable [13,14]. Of the 87 patients, 5 were excluded from the evaluation of diagnostic performance because of failed measurements with the M probe.

**MRI-PDFF**

Preoperative magnetic resonance cholangiopancreatography (MRCP) was performed to detect unexpected common bile duct stones in 77 patients using 3 T MRI (Ingenia CX, Philips Healthcare, Best, Netherlands). The mean interval between operation and MRCP was 7.6±27.3 days. The MRCP examination included in- and opposed phases and T2* maps (mDIXON Quant) to obtain the PDFF maps with the following parameters: TR/TE = 5.7/0.9–4.8 ms; FOV = 40 cm; flip angle = 3°; phase FOV = 0.8; matrix = 160×133; slice thickness = 5 mm; acquisition time = 15 s; bandwidth = 252.02 kHz. Images were processed using an interactive post-processing platform (Intelli-space Portal, ISP) provided by the manufacturer. Semi-automatic segmentation of the whole liver was performed, and the hepatic fat fraction was automatically calculated. A hepatic fat fraction of less than 5% was considered normal. The cut-off values for the diagnosis of mild, moderate, and severe steatosis were 5.2%, 11.3%, and 17.1%, respectively [15].

**Liver biopsy and histopathologic examination**

Liver biopsies of segment IV of the gallbladder fossa were performed using laparoscopic scissors. The incised liver surface was then coagulated using a monopolar device. A single expert pathologist reviewed the tissue slides. The severity of steatosis was assessed using a 4-point scale based on the percentage of hepatocytes with macrovesicular steatosis: 0 (<5%), 1 (5–33%), 2 (33–66%), and 3 (>66%) [16].

**Statistical analysis**

All statistical analyses were conducted using R software (v.4.0.4). Continuous variables were expressed as a mean ± standard deviation or median (IQR) according to the data distribution. Categorical variables were expressed as frequencies (percentages). The significance between the two groups (NAFLD vs. non-NAFLD) was assessed using the two-sample t-test or Wilcoxon rank-sum test for continuous variables, and chi-squared test or Fisher’s exact test for categorical variables. The association between UGAP and clinical and imaging parameters was assessed using Pearson’s or Spearman’s correlation according to normal distribution. A correlation coefficient () below 0.2 was considered to be minimal (<0.2), 0.2–0.4, weak; 0.4–0.7, moderate; and >0.7, strong [17]. We also used one-way ANOVA to compare the UGAP values for the different steatosis grades. For pair-wise comparisons, post-hoc analysis with Bonferroni correction was used after ANOVA. We set optimal cut-off values where the sum of the sensitivity and specificity was maximized for the diagnosis of NAFLD. We compared the sensitivity, specificity, positive predictive value, and negative predictive value between the imaging modalities using cut-offs obtained from the receiver operating curves. Statistical significance was set at p<0.05.

**Results**

**Baseline characteristics**

The characteristics of the 87 participants according to the presence of NAFLD are summarized in Table I. Of the 38 patients with NAFLD, 28, 8, and 2 had histological steatosis grades 1, 2, and 3, respectively. Patients with NAFLD had a higher BMI, waist circumference, and liver enzyme levels. The mean and median values of CAP, UGAP, and MRI-PDFF were significantly higher in the NAFLD group than in the non-NAFLD group (all p<0.0001). The mean UGAP values for the non-NAFLD and NAFLD group were 0.57 ± 0.08 dB/cm/MHz and 0.68 ± 0.09 dB/cm/MHz, respectively. The median MRI-PDFF values for the non-NAFLD and NAFLD groups were 4.1% (IQR, 2.9–4.8%) and 8.8% (IQR, 5.0–11.1%), respectively. Of the 38 patients with NAFLD, 8, 28, 1, 1 had histological fibrosis grades 0, 1, 2, and 3, respectively. The mean values of UGAP were 0.64±0.54 dB/cm/MHz and 0.69±0.98 dB/cm/MHz for fibrosis grade 0 and 1. UGAP values were 0.72 dB/cm/MHz and 0.79 dB/cm/MHz for grade 2 and 3.
Table II shows the correlation between UGAP and clinical and imaging parameters. The MRI-PDFF values were log-transformed because the data showed a skewed distribution. There was a strong positive relationship between UGAP and MRI-logPDFF ($r=0.704$, $p<0.0001$) (fig 2). CAP values were positively associated with UGAP values ($r=0.623$, $p<0.0001$). There was no significant association between liver stiffness measurements by transient elastography and UGAP values ($r=0.013$, $p=0.91$).

**Correlation between UGAP and steatosis grade**

Figure 3 shows the step-wise increase in UGAP values corresponding with the severity of steatosis ($p<0.0001$). The mean UGAP values were 0.57, 0.67, 0.69, and 0.84 dB/cm/MHz for steatosis grades 0, 1, 2, and 3, respectively. In pair-wise comparisons, there were significant differences between subjects with S0 versus S1 ($p<0.0001$), S0 versus S2 ($p=0.002$), S0 versus S3 ($p<0.0001$), and S1 versus S3 ($p=0.033$).

**Diagnostic performance of UGAP**

Table III summarizes the results of the comparison of diagnostic performance between the imaging modalities. The areas under the receiver operating curves (AU-ROCs) for the diagnosis of fatty liver were 0.821 (95% confidence interval [CI], 0.729–0.913), 0.829 (95% CI,
0.723–0.936), 0.788 (95% CI, 0.684–0.891), and 0.766 (95% CI, 0.767–0.856) for UGAP, MRI-PDFF, CAP, and BUSG respectively. The best cut-off value of UGAP for diagnosing fatty liver was 0.59 (dB/cm/MHz) according to Youden index. The accuracy of UGAP was comparable to that of MRI-PDFF (75.9% vs. 76.8%); however, the sensitivity of UGAP was superior to that of MRI-PDFF (86.8% vs. 71.0%). For the diagnosis of grade ≥2, the AUROCs were 0.796 (95% CI, 0.616–0.975), 0.971 (95% CI, 0.936–1.000), 0.726 (95% CI, 0.561–0.891), and 0.774 (95% CI, 0.612–0.936) for UGAP, MRI-PDFF, CAP, and BUSG, respectively. The numbers of correctly diagnosed patients with histologic steatosis grade ≥2 were 8 of 10, 9 of 10, 6 of 10, and 6 of 10 when using UGAP, MRI-PDFF, and CAP, respectively.

Discussion

Our study demonstrated a high diagnostic yield of UGAP for detecting fatty liver in patients with NAFLD using histopathology as the gold standard. The AUROC for hepatic steatosis grade ≥1 was higher for UGAP for BUSG, which is the most widely used in routine clinical practice for the detection of fatty liver (0.821 vs. 0.766). Additionally, the AUROC of UGAP was similar to that of MRI-PDFF (0.821 vs. 0.829), and UGAP showed superior sensitivity and similar accuracy than it did in MRI-PDFF (sensitivity, 86.8% vs. 71.0%; accuracy, 75.9% versus 76.8%). UGAP not only had a high diagnostic performance, similar to that of MRI-PDFF for the diagnosis of steatosis, but also had the advantages of conventional US in terms of easy accessibility and cost-saving. Ultimately, UGAP is expected to be a valuable screening tool for first-line assessment of steatosis in patients with NAFLD.

Overall, these findings are in accordance with findings reported by previous studies with AUROCs of 0.83–0.92 [5-8]. However, even when using the same method to define an optimal cut-off for the diagnosis of fatty liver (Youden index), substantial discrepancies were found with a cut-off of 0.53–0.69. Several covariates, such as etiology, BMI, and age, may have contributed to these differences. In a previous study of 163 patients with two different etiologies (hepatitis C virus infection and NAFLD), patient characteristics were significantly different between the two groups [6]. This finding supports the notion that UGAP values are influenced by etiology. Therefore, the study of specific populations is essential to establish optimal cut-off values that appear to be specific for individual etiologies. Previous studies have investigated the accuracy of UGAP including the heterogeneous etiology of chronic liver disease. The strength of this study was that the data were gathered from patients in the pure NAFLD group. In this study, the optimal cut-off value of UGAP for the detection of fatty liver in patients with NAFLD was 0.59 (dB/cm/MHz). Imajo K et al [7] have proposed an optimal cut-off of UGAP for the diagnosis of NAFLD grade 1 of 0.69 dB/cm/MHz in the subgroup analysis. The difference between the cut-off values can be attributed to demographic differences in the enrolled populations. Here, we recruited participants with average-to-low risk of NAFLD; however, they enrolled patients with chronic liver disease. Also, the difference between the cut-off values may be the result of the small number of NAFLD patients with unbalanced steatosis distribution in this study. The majority of NAFLD patients were steatosis grade 1 (74%, 28/38) and only 10

### Table II. Association between UGAP and clinical and imaging parameters

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Correlation coefficient (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.223</td>
<td>0.0375</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.501</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AST</td>
<td>0.256</td>
<td>0.0168</td>
</tr>
<tr>
<td>ALT</td>
<td>0.310</td>
<td>0.0035</td>
</tr>
<tr>
<td>GGT</td>
<td>0.149</td>
<td>0.1686</td>
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<tr>
<td>Fasting glucose</td>
<td>-0.095</td>
<td>0.3828</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.184</td>
<td>0.0882</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.373</td>
<td>0.0004</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>-0.064</td>
<td>0.5548</td>
</tr>
<tr>
<td>CAP (dB/m)*</td>
<td>0.623</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Transient elastography</td>
<td>0.013</td>
<td>0.9051</td>
</tr>
<tr>
<td>MRI-PDFF (%)†</td>
<td>0.660</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MRI-PDFF (%) in log scale†</td>
<td>0.704</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

AST = aspartate transaminase; ALT = alanine transaminase; GGT = gamma-glutamyl transferase; HbA1c, hemoglobin A1c; CAP, controlled attenuation parameter; MRI, magnetic resonance imaging; PDFF, proton density fat fraction. * Data on 82 patients; † Data on 77 patients
The etiology may have contributed to this difference. In addition, the sample size of grade ≥2 was too small here (n=10). In other studies with larger cohorts of NAFLD patients with even severe fatty liver disease was expected to be small. Further studies with larger cohorts of NAFLD patients with even steatosis grades should be performed. Second, NAFLD and gallstone disease often coexist; therefore, gallstone disease may have affected the results. However, the sequential relationship between these two diseases remains controversial, and we applied strict exclusion criteria to minimize the effects of gallstone disease on hepatotoxicity. Finally, our reference standard was the histological examination. Although liver biopsy remains the gold standard for the evaluation of steatosis, the spatial variability in steatosis may result in sampling errors. In conclusion, UGAP may be considered a promising screening tool for first-line assessment of liver steatosis in patients with NAFLD.

Conflict of interest: none

References


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