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Controlled Attenuation Parameter by Transient Elastography for Noninvasive Assessment of Macrovesicular Steatosis in Potential Living Liver Donors

Abbreviated Title: CAP for macrovesicular steatosis

Sunyoung Lee, MD, PhD¹, Kyoung Won Kim, MD, PhD², So Yeon Kim, MD, PhD², Nieun Seo, MD, PhD¹, Gi-Won Song, MD, PhD³, Sung-Gyu Lee, MD, PhD³

¹Department of Radiology and Research Institute of Radiological Science, Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea
²Department of Radiology and Research Institute of Radiology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea
³Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

Corresponding author: Kyoung Won Kim, MD, PhD

Department of Radiology and Research Institute of Radiology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Republic of Korea
Tel: 82-2-3010-4400; Fax: 82-2-476-4719; Email: kimkw@amc.seoul.kr
ORCID

Sunyoung Lee: https://orcid.org/0000-0002-6893-3136
Kyoung Won Kim: https://orcid.org/0000-0001-6471-6727
So Yeon Kim: https://orcid.org/0000-0001-6853-8577
Nieun Seo: https://orcid.org/0000-0001-8745-6454
Gi-Won Song: https://orcid.org/0000-0002-1581-7051
Sung-Gyu Lee: https://orcid.org/0000-0002-8209-3540

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Controlled Attenuation Parameter by Transient Elastography for Noninvasive Assessment of Macrovesicular Steatosis in Potential Living Liver Donors
ABSTRACT

Purpose: This study aimed to determine the diagnostic performance of controlled attenuation parameter (CAP) measured by transient elastography (TE) for assessing macrovesicular steatosis (MaS) in potential living liver donors using same-day biopsy as a reference standard.

Methods: This retrospective study included 204 living liver donor candidates who underwent TE and liver biopsy on the same day between July 2013 and June 2014. The histologic degree of MaS was determined. The area under the receiver operating characteristic curve (AUROC) was used to evaluate the performance of CAP for diagnosing MaS of > 10%, and the optimal cutoff value was identified using the maximal Youden index.

Results: Based on liver biopsy, 185 subjects had MaS of ≤ 10% and 19 had MaS of > 10%. The CAP value was significantly correlated with the percentage of MaS on liver biopsy ($r = 0.635$, $P < 0.001$), and the median CAP value was significantly higher in subjects with MaS of > 10% than in those with MaS of ≤ 10% (300 vs. 209 dB/m, $P < 0.001$). The AUROC for diagnosing MaS of > 10% by CAP was 0.938 (95% confidence interval, 0.896–0.967), with a CAP of > 259 dB/m yielding a sensitivity of 84.2% and a specificity of 92.4%.

Conclusion: CAP by TE significantly correlates with MaS and accurately detects substantial MaS in potential living liver donors. The CAP is a promising tool for the noninvasive diagnosis of MaS and may be used to screen unsuitable living liver donor candidates.

Keywords: Controlled attenuation parameter; Transient elastography; Macrovesicular steatosis; Liver biopsy; Living donors
INTRODUCTION

Liver transplantation (LT) is the most effective treatment for end-stage liver disease [1]. Most LT centers in Asia predominantly perform living donor LT (LDLT) due to a shortage of deceased organ donations [2]. However, a rising incidence of nonalcoholic fatty liver disease (NAFLD) in the general population has been reflected in an increasing prevalence of living liver donors with NAFLD [3]. The degree of macrovesicular steatosis (MaS) in donated livers is known to be a critical factor determining outcomes after transplantation. MaS affects the recovery of remnant liver in living donors as well as the graft function in recipients. Therefore, the evaluation of MaS in donated liver is crucial, particularly for LDLT, during which donor safety is a primary concern. Many LDLT centers prefer to accept right liver donors with MaS of up to 10% [4-6]. According to the International Liver Transplant Society guideline on living liver donation, MaS of > 10% is considered substantial [7].

Liver biopsy is considered the gold standard for assessing the degree of hepatic steatosis [8]. Although this procedure has a relatively low complication rate in healthy living donors [9], it is an invasive procedure that can lead to morbidity [10]. Therefore, noninvasive methods have been investigated for the evaluation of steatosis. Recently, the controlled attenuation parameter (CAP) measured by transient elastography (TE) has been proposed as a promising noninvasive method for the detection and quantification of hepatic steatosis [11]. The CAP by TE has been validated for the assessment of steatosis in patients with chronic liver disease of various etiologies [12], and meta-analyses have shown good diagnostic accuracy of CAP for identifying hepatic steatosis [13-16]. However, only few studies have investigated the usefulness of CAP for assessing steatosis in potential living liver donors [17,18]. In addition, to our knowledge, the potential role of CAP in identifying substantial MaS in living liver donor candidates has not yet been evaluated.
Therefore, our study aimed to determine the diagnostic performance of CAP for the assessment of MaS in potential living liver donors using biopsy as a reference standard.

MATERIALS and METHODS
The study was approved by the institutional review board of our institution (IRB approval number: AMC 2021-0378). The requirement for written informed consent was waived due to the retrospective nature of the analysis.

Study Population
Between July 2013 and June 2014, 299 potential living liver donors underwent ultrasound (US)-guided liver biopsy as part of a routine predonation work-up. Liver biopsy was performed if candidates met the following conditions: (1) no more than 20 g of alcohol consumption per day, (2) no regular drug use including herbal medications, and (3) no serological evidence of hepatitis B, hepatitis C, or human immunodeficiency virus. Subjects were considered eligible for inclusion in our study if they were living liver donor candidates who had undergone TE with CAP and liver biopsy on the same day. Donor candidates who had undergone TE and liver biopsy on different days (n = 70) or who had unreliable CAP measurements (n = 25) were excluded (Fig. 1).

Clinical and Laboratory Data
Demographics (age and sex), anthropometric measurements (body weight and height), and laboratory data (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], gamma-glutamyl transpeptidase [GGT], total cholesterol, triglyceride, high-density lipoprotein [HDL], low-density lipoprotein [LDL], and fasting glucose) were
collected. Body mass index (BMI) was calculated as body weight (kg) divided by the square of height (m$^2$).

**TE and CAP Measurement**

CAP measurements were obtained using TE (FibroScan® 502 touch, Echosens, Paris, France) by trained technicians blinded to clinical and histologic data. All measurements were performed on the right hepatic lobe through the intercostal spaces of the donor candidates lying in the dorsal position with the right arm in abduction. CAP was calculated as the attenuation of the ultrasonic signal at 3.5 MHz acquired by TE. The principles of CAP measurement have been previously described in detail [19]. CAP values were expressed in dB/m, and the median of successful measurements was selected as the representative value. The success rate was calculated as the ratio of the number of successful acquisitions to the total number of measurements. As an indicator of variability, the interquartile range (IQR)-to-median ratio was calculated. In this study, CAP measurements with 10 valid shots, a success rate of at least 60%, and an IQR-to-median ratio of less than 30% were considered reliable and used for statistical analysis [20,21].

**Liver Biopsy**

US-guided percutaneous biopsy was performed during the living liver donor work-up on the right hepatic lobe using an 18-gauge needle (Stericut 18G coaxial, TSK Laboratory, Tochigi, Japan). Two or more biopsy specimens, each approximately 1.5 cm in length, were obtained and stained with hematoxylin and eosin. The degree of MaS, which was the percentage of hepatocytes that contained intracellular macrovesicular fat droplets, was assessed quantitatively. MaS of > 10% was considered to be substantial MaS [7].
**Statistical Analysis**

Continuous variables were expressed as means with standard deviations or medians with IQRs, as appropriate. Pearson’s correlation coefficients ($r$) were calculated to evaluate correlations between variables. The difference between two continuous variables was analyzed using the Mann–Whitney $U$ test. The normality of the CAP values in the reference population with histologic MaS of $\leq 10\%$ was checked by using the Shapiro-Wilks test. The lower and upper limits of the reference range were estimated to be the 2.5 and 97.5 percentiles, respectively, of the distribution of CAP values in the reference population. The performance of CAP for diagnosing substantial MaS ($> 10\%$) was estimated as the area under the receiver operating characteristic curve (AUROC) with the 95% confidence interval (CI). The cutoff value for CAP was chosen to maximize the sum of the sensitivity and specificity on the Youden index. At the optimal cutoff value, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with their 95% CIs were determined. A $P$-value of $< 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS version 23.0 (IBM, Armonk, NY) and MedCalc version 16.2.1 (MedCalc Software, Ostend, Belgium).

**RESULTS**

**Characteristics of the Study Subjects**

A total of 204 potential living liver donors (mean age, $30.5 \pm 9.1$ years; 133 male and 71 female) were included in the analysis. The characteristics of the included subjects are summarized in Table 1. Of these donor candidates, 185 (90.7%) had MaS of 10% or less and 19 (9.3%) had MaS of greater than 10%. The median CAP value in our study subjects was 214 dB/m (IQR, 190–241).
Correlations with CAP
CAP was significantly correlated with BMI ($r = 0.561, P < 0.001$), AST ($r = 0.302, P < 0.001$), ALT ($r = 0.448, P < 0.001$), GGT ($r = 0.231, P = 0.001$), total cholesterol ($r = 0.283, P < 0.001$), triglyceride ($r = 0.364, P < 0.001$), HDL ($r = -0.270, P < 0.001$), LDL ($r = 0.309, P < 0.001$), fasting glucose ($r = 0.195, P = 0.005$), and the percentage of MaS on liver biopsy ($r = 0.635, P < 0.001$; Fig. 2) but not with ALP (Table 2).

Distribution of CAP Based on MaS
The median CAP values for subjects with MaS of $\leq 10\%$ and MaS of $> 10\%$ were 209 dB/m (IQR, 188–233) and 300 dB/m (IQR, 261–313), respectively. CAP values were significantly higher in subjects with MaS of $> 10\%$ than in those with MaS of $\leq 10\%$ ($P < 0.001$, Fig. 3).

Reference Range for CAP on MaS of $\leq 10\%$
CAP values in the reference population with histologic macrovesicular steatosis of $\leq 10\%$ ranged from 149 to 276 and followed a Gaussian distribution ($P = 0.170$, Shapiro-Wilks test) (Supplementary Fig. 1).

Diagnostic Performance of CAP for MaS
For diagnosing substantial MaS ($> 10\%$), the AUROC of CAP was 0.938 (95% CI, 0.896–0.967), and the optimal cutoff value was determined to be 259 dB/m (Fig. 4). Using this cutoff value, the sensitivity, specificity, PPV, and NPV for diagnosing substantial MaS ($> 10\%$) were 84.2% (95% CI, 60.4–96.6%), 92.4% (95% CI, 87.6–95.8%), 53.3% (95% CI, 34.3–71.7%), and 98.3% (95% CI, 95.0–99.6%), respectively (Table 3).

DISCUSSION
This study demonstrated that the CAP obtained by TE was well correlated with the degree of MaS on histologic analysis in potential living liver donors. Our results also showed that CAP had excellent diagnostic accuracy for detecting substantial MaS, with an AUROC of 0.938. Therefore, CAP is potentially useful for identifying substantial MaS (> 10%), which may hinder the success of LT.

MaS in donated livers has been reported to be associated with poor graft function, including early graft dysfunction and primary nonfunction in recipients, which can result in decreased graft survival [22]. MaS also impairs the recovery and regeneration of remnant liver in living donors. The large fat vacuoles of MaS are believed to reduce sinusoidal blood flow and disturb the hepatic microcirculation, making liver grafts more vulnerable to ischemia-reperfusion injury [23]. In addition, cellular, biochemical, and signaling processes are interrupted in hepatic steatosis, leading to mitochondrial dysfunction and endoplasmic reticulum stress, which can affect the process of liver regeneration [24]. Although the acceptable degree of MaS for living liver donation remains controversial, MaS of less than 10% has been commonly used as a threshold for LDLT to ensure recipient outcome and donor safety [4-6].

Limited studies have evaluated hepatic steatosis in potential living liver donors using the CAP measured by TE [17,18]. Hong et al. evaluated the usefulness of CAP for detecting steatosis in 55 living liver donor candidates [17]. The authors found that the AUROC of CAP for identifying total steatosis (> 33%) was 0.88 with a cutoff value of 276 dB/m using US-guided liver biopsy as the reference standard [17]. In another study by Yen et al. [18], 54 living liver donors were assessed by CAP, and the results were compared with intraoperative biopsy. In that study, CAP had an AUROC of 0.96 for detecting total steatosis (> 5%) with a cutoff value of 257 dB/m [18]. Though these studies were limited by relatively small sample sizes, their findings suggest the utility of CAP for detecting total steatosis.
Unlike these previous studies, we focused on MaS rather than total steatosis, since MaS is known to be independently associated with graft dysfunction [25,26]. This study demonstrated the excellent diagnostic accuracy of CAP for detecting substantial MaS (> 10%) in potential living liver donors, with an AUROC of 0.938. We found that the optimal cutoff value of CAP for identifying substantial MaS was 259 dB/m, with a sensitivity of 84.2% and a specificity of 92.4%. CAP measured by TE has many advantages, including its noninvasive, nonionizing, quantitative, rapid, and reproducible features, suggesting that this parameter may be a promising tool for screening potential living liver donors. In living liver donor candidates, a CAP cutoff value of 259 dB/m may potentially be used to detect those with substantial MaS.

Previous studies have shown that rates of unreliable CAP measurements ranged from 28% to 57% in the population with chronic liver disease or NAFLD [27-29]. Although the rate of unreliable CAP measurement in our study was lower than in previous studies using patients with chronic liver disease or NAFLD, the rate of unreliable CAP measurements in our study (8.4%) was somewhat disappointing because living liver donor candidates are usually young and healthy. Unreliable measurements rates may be a limitation of the CAP. This study had several limitations. First, it was a single-center, retrospective study with an unavoidable selection bias. Second, the number of subjects with MaS of > 10% was small. Third, this study was conducted in an East Asian population with a mean BMI of 22.7 kg/m², potentially limiting its applicability to a Western population. Due to these limitations, our results need to be validated in prospective, large-scale, multi-center studies.

In conclusion, CAP measured by TE significantly correlates with MaS and accurately detects substantial MaS in potential living liver donors. Thus, CAP is a promising tool for the noninvasive diagnosis of MaS and may be used to screen unsuitable living liver donor candidates.
REFERENCES


### Table 1. Characteristics of the study subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n = 204)</th>
<th>Macrovesicular steatosis ≤ 10% (n = 185)</th>
<th>Macrovesicular steatosis &gt; 10% (n = 19)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>30.5 ± 9.1</td>
<td>30.2 ± 8.9</td>
<td>33.7 ± 10.7</td>
<td>0.111</td>
</tr>
<tr>
<td>Men *</td>
<td>133 (65.2)</td>
<td>117 (63.2)</td>
<td>16 (84.2)</td>
<td>0.079</td>
</tr>
<tr>
<td><strong>Anthropometric measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>65.0 ± 11.0</td>
<td>64.2 ± 10.7</td>
<td>73.3 ± 10.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.7 ± 2.9</td>
<td>22.5 ± 2.8</td>
<td>25.0 ± 2.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Laboratory data</strong></td>
<td></td>
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</tr>
<tr>
<td>AST, IU/L</td>
<td>18.5 ± 5.9</td>
<td>17.8 ± 5.2</td>
<td>25.6 ± 8.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>17.9 ± 12.4</td>
<td>16.0 ± 10.1</td>
<td>36.7 ± 16.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ALP, IU/L</td>
<td>63.3 ± 18.0</td>
<td>63.1 ± 18.2</td>
<td>65.3 ± 16.7</td>
<td>0.618</td>
</tr>
<tr>
<td>GGT, IU/L</td>
<td>21.3 ± 18.7</td>
<td>19.9 ± 18.3</td>
<td>35.2 ± 17.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>173.1 ± 28.7</td>
<td>171.2 ± 28.1</td>
<td>192.1 ± 28.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>95.7 ± 50.2</td>
<td>90.9 ± 46.0</td>
<td>142.0 ± 65.4</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>56.1 ± 14.9</td>
<td>57.0 ± 14.8</td>
<td>47.2 ± 13.2</td>
<td>0.006</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>97.9 ± 27.4</td>
<td>96.0 ± 27.0</td>
<td>116.5 ± 25.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>90.0 ± 9.3</td>
<td>89.6 ± 9.0</td>
<td>93.2 ± 11.4</td>
<td>0.115</td>
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<tr>
<td><strong>Liver histology</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Total hepatic steatosis grade *</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Stage</td>
<td>% Steatosis</td>
<td>N1 (59.8%)</td>
<td>N2 (65.9%)</td>
<td>N3</td>
</tr>
<tr>
<td>-------</td>
<td>------------</td>
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</tr>
<tr>
<td>S0 (&lt; 5%)</td>
<td>122</td>
<td>122</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S1 (5–33%)</td>
<td>75 (36.8)</td>
<td>63 (34.1)</td>
<td>12 (63.2)</td>
<td></td>
</tr>
<tr>
<td>S2 (34–66%)</td>
<td>7 (3.4)</td>
<td>0</td>
<td>7 (36.8)</td>
<td></td>
</tr>
<tr>
<td>S3 (&gt; 66%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

**Macrosesicular steatosis, %**
- S0: 4.0 ± 7.5
- S1: 1.9 ± 2.4
- S2: 24.4 ± 10.1

**Transient elastography†**
- **Controlled attenuation parameter**, dB/m
  - S0: 214 (190–241)
  - S1: 209 (188–233)
  - S2: 300 (261–313)
- **Liver stiffness measurement**, kPa
  - S0: 3.8 (3.3–4.6)
  - S1: 3.8 (3.3–4.6)
  - S2: 4.1 (3.7–5.1)

Unless otherwise indicated, data are presented as mean ± standard deviation.

* Data are presented as the number of subjects with the percentage in parentheses.
† Data are presented as median with interquartile range in parentheses.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, \( \gamma \)-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
### Table 2. Correlation between CAP and clinical parameters

<table>
<thead>
<tr>
<th>Correlation coefficient (r)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td>0.561</td>
</tr>
<tr>
<td><strong>AST, IU/L</strong></td>
<td>0.302</td>
</tr>
<tr>
<td><strong>ALT, IU/L</strong></td>
<td>0.448</td>
</tr>
<tr>
<td><strong>ALP, IU/L</strong></td>
<td>0.000</td>
</tr>
<tr>
<td><strong>GGT, IU/L</strong></td>
<td>0.231</td>
</tr>
<tr>
<td><strong>Total cholesterol, mg/dL</strong></td>
<td>0.283</td>
</tr>
<tr>
<td><strong>Triglyceride, mg/dL</strong></td>
<td>0.364</td>
</tr>
<tr>
<td><strong>HDL, mg/dL</strong></td>
<td>-0.270</td>
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<tr>
<td><strong>LDL, mg/dL</strong></td>
<td>0.309</td>
</tr>
<tr>
<td><strong>Fasting glucose, mg/dL</strong></td>
<td>0.195</td>
</tr>
<tr>
<td><strong>Macrovesicular steatosis, %</strong></td>
<td>0.635</td>
</tr>
</tbody>
</table>

*Statistically significant results from the Pearson correlation coefficient analysis.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Table 3. Diagnostic performance of CAP for identifying macrovesicular steatosis of > 10%

<table>
<thead>
<tr>
<th></th>
<th>Macrovesicular steatosis &gt; 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUROC (95% CI)</td>
<td>0.938 (0.896–0.967)</td>
</tr>
<tr>
<td><strong>Optimal CAP cutoff value, dB/m</strong></td>
<td>259</td>
</tr>
<tr>
<td>Sensitivity (95% CI), %</td>
<td>84.2 (60.4–96.6)</td>
</tr>
<tr>
<td>Specificity (95% CI), %</td>
<td>92.4 (87.6–95.8)</td>
</tr>
<tr>
<td>PPV (95% CI), %</td>
<td>53.3 (34.3–71.7)</td>
</tr>
<tr>
<td>NPV (95% CI), %</td>
<td>98.3 (95.0–99.6)</td>
</tr>
</tbody>
</table>

*Optimal CAP cutoff value defined by the maximal sum of sensitivity and specificity. AUROC, area under the receiver operating characteristics curve; CAP, controlled attenuation parameter; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
FIGURE LEGENDS

Figure 1. Flowchart of the study subjects.
CAP, controlled attenuation parameter; TE, transient elastography; US, ultrasound.

Figure 2. Correlation between CAP and the percentage of macrovesicular steatosis in potential living liver donors ($r = 0.635$, $P < 0.001$).
CAP, controlled attenuation parameter.

Figure 3. Distribution of CAP based on histologic macrovesicular steatosis of $\leq 10\%$ or $> 10\%$. CAP values were significantly higher in subjects with macrovesicular steatosis of $> 10\%$ than in those with macrovesicular steatosis of $\leq 10\%$ ($P < 0.001$).
CAP, controlled attenuation parameter.

Figure 4. Receiver operating characteristic curve of CAP for the diagnosis of macrovesicular steatosis of $> 10\%$.
AUROC, area under the receiver operating characteristic curve; CAP, controlled attenuation parameter; CI, confidence interval.
Potential living liver donors with US-guided liver biopsy (n = 299)

Inclusion:
- Same-day TE and liver biopsy

Excluded:
- Different-day TE and liver biopsy (n = 70)
- Unreliable CAP measurement (n = 25)

Final subjects (n = 204)

Figure 1

Figure 1. Flowchart of the study subjects. CAP, controlled attenuation parameter; TE, transient elastography; US, ultrasound.
Figure 2

Figure 2. Correlation between CAP and the percentage of macrovesicular steatosis in potential living liver donors ($r = 0.635, P < 0.001$). CAP, controlled attenuation parameter.
Figure 3

Figure 3. Distribution of CAP based on histologic macrovesicular steatosis of ≤ 10% or > 10%. CAP values were significantly higher in subjects with macrovesicular steatosis of > 10% than in those with macrovesicular steatosis of ≤ 10% (P < 0.001). CAP, controlled attenuation parameter.
Figure 4

Figure 4. Receiver operating characteristic curve of CAP for the diagnosis of macrovesicular steatosis of > 10%. AUROC, area under the receiver operating characteristic curve; CAP, controlled attenuation parameter; CI, confidence interval.

AUROC = 0.938 (95% CI, 0.896–0.967)