Computed Tomography for noninvasive quantitative assessment of the degree of hepatosteatosis in living donor liver transplantation: comparison of liver attenuation index with histopathological results.

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Aims and objectives

Non-alcoholic fatty liver disease (NAFLD) is described as a micro- and macrovesicular fatty infiltration of hepatocytes, secondary to hepatic damage [1]. Macrovesicular steatosis is the predominant type in adult and pediatric cases. Although the disease affects approximately 20% of the general population, a higher prevalence of hepatic steatosis has been reported with increasing rates of obesity [1, 2]. Hepatic steatosis alone can trigger chronic liver disease, as well as cause functional loss in both cadaveric and living-donor split liver transplants. In cadaveric liver transplantation, cold preservation of the transplant graft leads to the fusion and expansion of fats, causing a compression of the sinusoids and hepatocytes, resulting in a circulation disturbance in the sinusoids and graft injury [3]. The exposure of the graft to cold is minimized; thus, organs with some degree of steatosis can be used in living donor liver transplants (LDLT). A moderate degree of steatosis in the liver increases both perioperative morbidity and mortality rates [4]. Marcos et al. reported that the functional liver mass decreased by 1% for each percentage of either micro- or macrovesicular hepatic steatosis [5]. The use of fatty liver for transplantation should be avoided when there is a risk of small-for-size syndrome (SFSS).

Liver biopsy is currently considered the "gold standard" method for the assessment of hepatic steatosis. However not every potential donor accept that invasive procedure. Evaluation protocols vary and are dependent on the philosophy of the transplantation centers, differing with anthropometric factors. For example, in determining the need for a liver biopsy, Sugawara et al. [6] and Miller et al. [7] preferred body mass indices of over 25 and 28, respectively. However, Ryan et al. [8] specified that biopsies should be performed on all potential living donors, regardless of body mass index.

Computed tomography (CT) is commonly used in the qualitative and quantitative evaluation of hepatic steatosis. In the qualitative assessment, hepatic steatosis is estimated based on the brightness of the liver and the ability to distinguish vascular structures. Lee et al. [9] used a visual grading system to compare the density of vascular structures in the liver and the hepatic parenchymal density with the hepatic attenuation index; they discovered that the visual grading system (qualitative) and the hepatic steatosis index (quantitative) were comparable, with high reliability and similar accuracy levels in terms of detecting a hepatic steatosis rate of # 30%.

A quantitative evaluation can be performed based on the hepatic attenuation measurement. The density levels measured in Hounsfeld units (HU) on non-contrast CT cross-sections had a reverse linear correlation with the degree of steatosis [10]. However, due to patient-to-patient and CT device variations, as well as variations in cardiac and renal functions in the same patient, the absolute hepatic HU value is thought to be inadequate in its ability to diagnose steatosis and its degree of progression. In more recent studies, the density levels of the liver measured using CT were compared with
the values measured from structures, such as the spleen and muscle [11, 12]. Using current methods, the purpose of this study was to determine the association between liver density and the degree of histopathological steatosis, so that the need for liver biopsies could be minimized. By carefully controlling variability, integration of radiological and histopathological studies conducted in a large series should reveal the specified association. We investigated the correlation between the hepatic parenchymal density measured by CT in donor candidates during the preoperative period to determine the degree of histopathologically detected steatosis in the "wedge" biopsy material obtained from the graft during surgery. Based on these results, a mathematical model was developed to determine the degree of steatosis.
Methods and materials

Cases

One hundred and three cases underwent 106 LDLT procedures in the Organ Transplantation Center of Inonu University Turgut Ozal Medical Center, Malatya, Turkey between September 2, 2005 and June 29, 2008. A total of 116 donor candidates were examined for liver transplantations. This study was conducted on a total of 51 LDLT donors (19 females and 32 males) who were operated on at this center between December 2008 and May 2008. The age of the donors ranged from 19-52 years (mean: 34.2 years, SD: 10.2 years).

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CT densitometry

CT images were obtained using a multi-slice CT device with 64 detectors (Aquilion 64, model TSX-101A; Toshiba Medical Corporation, Tochigi, Japan). After obtaining non-contrast images of the liver in a single breath-hold, multiphasic CT angiography images were obtained by administering 81.65-g iomeprol (Iomeron 400, Bracco s. p. a. Milano, Italy), equivalent to 40 g of iodine in 100 mL of iomeprol, using an automatic injector (Missouri, Ulrich Medical, Netherlands). Scanning and reconstruction of the non-contrast sections were performed using the following parameters: collimation 0.5 × 64 mm, pitch 0.828, gantry rotation time of 0.5 s, 120 kVp, 31 mAs, section thickness of 5 mm, and a reconstruction interval of 5 mm.

In the non-contrast CT images, (Hounsfield unit) HU values were measured using the Aquilion VB.10ER004 software on sample areas with a 10-mm diameter ROI in four sections. For the liver, 20 separate ROIs were placed in regions without vascular structures; 12 were placed in the right lobe and 8 in the left lobe. During ROI placement, particularly in cases with hepatosteatosis, the selection of problematic areas, free of main vascular structures, was performed by referencing contrast-enhanced images corresponding to the section in question (Fig. 1). The mean hepatic attenuation (MHA) was determined by calculating the mean of the HU values measured from these regions. In the same sections, ten separate ROIs (diameter: 10 mm) were placed in the region of the spleen free of the main vascular structures. Mean splenic attenuation (MSA) was determined by calculating the mean of the HU values measured from the ROIs positioned in the spleen. The liver attenuation index (LAI) was calculated by subtracting the MSA value from the MHA value (LAI = MHA # MSA).

Intraoperative "wedge" biopsy
Before the LDLT operation, a right or left lobe resection was performed to obtain the graft, whose size was calculated based on the weight and metabolic requirements of the recipient, following a series of laboratory and radiological procedures (Fig. 2). After the liver graft from the donor was removed, a hepatic parenchymal sample was taken from a region on the resected surface, far from the subcapsular region. Because the manipulations performed during the period from resection to placement in formalin may negatively affect the evaluation of the degree of histopathological steatosis, we were careful to avoid contact between the specimen and the sponge. The specimen was stored in 10% formaldehyde solution until histopathological evaluation.

**Histopathological evaluation**

All specimens were evaluated by an experienced pathologist, with no knowledge of the clinically and/or radiologically estimated degree of steatosis. Paraffin-fixed liver tissue sections were stained with hematoxylin-eosin (H&E), Masson’s trichrome, Gomori’s reticulin, and Perl’s Prussian blue stains. The percentage of hepatocytes with macrovesicular steatosis was determined during the evaluation of steatosis (Figs. 3 and 4). When the percentage of steatosis was < 5%, 1000 hepatocytes were counted in five regions; note that an exact percentage could not be determined. The degree of steatosis was determined by calculating the mean of the number of hepatocytes (n1, n2, ..., n5) with steatosis (n/1000) [where the degree of steatosis (n/1000) = (n1 + n2 +...+ n5) / 5 / 1000]. The calculated value was again converted into a percentage. The zonal distribution of macrosteatosis was recorded, if present. The presence of microvesicular steatosis was not included in the study, because it is clinically insignificant and almost always associated with macrosteatosis. Lobular inflammation, fibrosis, the presence of Mallory’s hyaline bodies portal area alterations, hepatocellular ballooning degenerations consistent with steatohepatitis, and iron accumulation were investigated in the enrolled cases. No pathological findings were detected, other than macrosteatosis, in any of the cases.

**Statistical analysis**

Cases were grouped according to their degree of steatosis: < 2%, # 2%, < 5%, or # 5%. The Kolmogorov-Smirnov test was used to compare the distribution of the groups, and the Kruskal-Wallis test was used to compare multiple independent variables among groups. The specificity and sensitivity values among paired groups were determined using receiver operating characteristic (ROC) curve coordinates. In all statistical tests, p < 0.05 was accepted as the level of significance.

LAI values were accepted as x values and histopathological steatosis degrees were accepted as y. The mathematical relationship between these values was expressed as a second-order polynomial and an exponential function, using the least squares method.

Statistical analysis was performed using the Statistical Package for the Social Sciences (version 15.0 software, SPSS Inc., USA).
Fig. 1: Placement of ROIs in the liver and spleen without overlapping with venous structures.

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Fig. 2: Right lobe resection performed during the operation to obtain the graft

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Fig. 3: Histopathological appearance of normal liver parenchyma; (H&E)x200

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Fig. 4: Histopathological appearance of A. 2%, B. 5%, C. 10% and D. 20% steatosis; (H&E)x100

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Results

Based on the histopathological results, the degree of macrosteatosis of the 51 donor candidates ranged from 0-8% (mean: 1.1%, SD: 2). Seven cases (13.7%) had # 5% and 12 cases (23.5%) had # 2% macrosteatosis. The presence of microvesicular steatosis was not taken into consideration, because it is clinically insignificant and almost always associated with macrosteatosis.

Steatosis was not found in 29 cases during histopathological evaluation. In the evaluation of the results of CT densitometry of these patients, performed before LDLT, it was estimated that 27 cases had a steatosis degree of < 5% and two cases had a steatosis degree of 5-30% (Table 1).

The cases were initially grouped as Group I and Group II; Group I corresponded to a steatosis degree of < 2% and Group II to a steatosis degree of # 2%. The groups exhibited a normal distribution based on the Kolmogorov-Smirnov test. The Kruskal-Wallis test was used to compare the multiple independent variables of Groups I and II (Table 2), with \( p < 0.05 \) considered as statistically significant. Box plots showing the mean LAI values and standard deviations for Groups I and II were generated (Fig. 5).

In the second step, the cases were grouped as Group I or Group II, with a percentage of steatosis of < 5% and # 5%, respectively. The groups exhibited a normal distribution based on the Kolmogorov-Smirnov test. The Kruskal-Wallis test was used to compare the multiple independent variables of Groups I and II (Table 3); \( p < 0.05 \) was considered statistically significant. Box plots showing the mean LAI values and standard deviations for Groups I and II were generated (Fig. 6).

LAI had the highest level of significance among the assessed parameters in the combined evaluation of Tables 2 and 3. Therefore, ROC curves were created for this parameter.

Figure 7 shows the ROC curve for determining < 2% steatosis using LAI. Based on these curve coordinates, when the cut-off value for LAI was 9.1, the sensitivity and specificity of this parameter for identifying < 2% steatosis were 77% and 75%, respectively. When the cut-off value considered was 7.2, the sensitivity and specificity of this parameter for identifying < 2% steatosis were 87% and 60%, respectively.

The ROC curve created to determine < 5% steatosis using LAI is presented in Fig. 8. For a cut-off value of 9.1, the sensitivity and specificity of this parameter for identifying < 5% steatosis were 73% and 86%, respectively. For a cut-off value of 7.2, the sensitivity and specificity of this parameter were 82% and 67%, respectively.

The LAI values were denoted as \( x \) values and the histopathological steatosis degrees corresponded to the \( y \) values. The mathematical relationship between these values is expressed using the following functions:
i) The function created by including all values can be expressed as a second-order polynomial:

[1]

or

as an exponential function:

[2]

ii) The values remaining after removal of the four highest LAI values and one histopathological steatosis value can be expressed as a second-order polynomial:

[3]

or

as an exponential function:

[4]
Fig. 5: Box plot showing the LAI values and standard deviations for Group I (< 2%) and Group II (# 2%)

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Fig. 6: Box plot showing the LAI values and standard deviations for Group I (< 5%) and Group II (# 5%)

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Fig. 7: ROC curve created for significant steatosis degree # %2

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Fig. 8: ROC curve created for significant steatosis degree #5%

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<table>
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<th>CT densitometric steatosis value</th>
<th>Percentages of histopathological steatosis</th>
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</thead>
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<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>≤5%</td>
<td>27</td>
</tr>
<tr>
<td>6%-30%</td>
<td>2</td>
</tr>
<tr>
<td>≥30%</td>
<td>0</td>
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</table>
**Fig. 9:** Table 1. Comparison of CT densitometric and histopathological results

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<table>
<thead>
<tr>
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<th>Group I (n=39)</th>
<th>Group II (n=12)</th>
<th>p value</th>
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<tr>
<td>MHA</td>
<td>59.90±5.25</td>
<td>54.15±5.44</td>
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<td>MSA</td>
<td>47.90±3.57</td>
<td>46.98±2.98</td>
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<tr>
<td>MHA-MSA (LAI)</td>
<td>12.06±4.50</td>
<td>7.18±4.17</td>
<td>0.003 *</td>
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<tr>
<td>MHA/MSA</td>
<td>1.25±0.10</td>
<td>1.15±0.09</td>
<td>0.006 *</td>
</tr>
</tbody>
</table>

**Fig. 10:** Table 2. Comparison of multiple independent variables with the Kruskal-Wallis test between the groups (significant degree of steatosis ≥ 2%)

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<table>
<thead>
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<th>Group I (n=44)</th>
<th>Group II (n=7)</th>
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<tr>
<td>MHA</td>
<td>59.30±5.41</td>
<td>54.10±6.54</td>
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</tr>
<tr>
<td>MSA</td>
<td>47.75±3.43</td>
<td>47.30±3.75</td>
<td>0.46</td>
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<tr>
<td>MHA-MSA (LAI)</td>
<td>11.55±4.69</td>
<td>6.80±4.04</td>
<td>0.016 *</td>
</tr>
<tr>
<td>MHA/MSA</td>
<td>1.24±0.10</td>
<td>1.14±0.08</td>
<td>0.025 *</td>
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</tbody>
</table>

**Fig. 11:** Table 3. Comparison of multiple independent variables with the Kruskal-Wallis test between the groups (significant degree of steatosis ≥ 5%)

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Conclusion

In this study, the advantages of CT for identifying the degree of hepatic steatosis in LDLT donor candidates were evaluated. For this purpose, the hepatic steatosis index values, obtained using non-contrast CT sections in the study group of potential donor candidates, were compared with the histopathological evaluation results of hepatic "wedge" biopsy material removed during the operation. Unlike many studies that compared preoperative needle biopsy results and the LAI calculated using non-contrast CT, instead of fine-needle aspiration biopsy or tru-cut biopsy, this study used liver tissue taken during the operation. Thereby, hepatic parenchyma specimens were obtained in the desired amount (in an amount greater than that obtained with a needle biopsy) and in a single piece from the region preferred by the pathologist (far from the subcapsular region).

In cadaveric liver transplantation, organs with a macrovesicular steatosis degree of > 60% were strictly excluded. However, the presence of microsteatosis does not affect short- or long-term survival [13]. In many centers, moderate-to-severe (# 30%) macrovesicular steatosis represents an absolute contraindication for LDLT, while a mild degree (< 30%) of steatosis is considered to be safe [9]. Fan et al. [14] considered a macrovesicular steatosis level of # 20% as inappropriate for transplantation, while some centers used grafts with a steatosis level of < 50%, provided that the residual volume to total liver volume percentage was # 40% [15]. However, in LDLT, grafts with moderate-to-severe steatosis may be used in some cases, due to the limited number of donor candidates available per recipient [16]. It is well known that the use of grafts with severe steatosis represents a risk for LDLT [17]. However, high steatosis degrees, found in a limited number of donor candidates by imaging techniques as false-positive results, and their subsequent exclusion (though they are actually suitable for transplantation), is an undesirable event for transplantation centers. It is, therefore, of utmost importance to improve the radiological methods used in the determination of steatosis and to establish their validity.

In some centers, percutaneous liver biopsies are performed in all donor candidates to determine the hepatic steatosis level before LDLT [5]. Liver biopsies can also determine the degree of steatosis and investigate parenchymal pathologies with a subclinical course, such as fibrosis and hepatitis, which represent risk factors for the recipient and the donor. However, this is a highly invasive method. Additionally, a liver biopsy is a painful procedure, which requires bed rest for 6 h and has a bleeding and mortality risk, although the mortality rate is low [8,18]. Because the approach is easier, a biopsy is usually performed from the right lobe and by taking one or several specimens. In heterogeneous hepatic steatosis cases, a biopsy may not show the actual steatosis level due to the limited sample amount [1]. Another disadvantage of a percutaneous liver biopsy is that it is not possible to know the hepatic steatosis level in the residual liver of the donor to avoid complications which can adversely affect the health of the donor. In adults, the right lobe is usually used for LDLT, and the left lobe remains in the donor. Liver biopsies performed
for histopathological evaluation are usually taken from the right lobe; thus, the biopsy results may not reflect the steatosis level of the residual liver in donor candidates with non-homogeneous steatosis. Due to the above-mentioned limitations, noninvasive diagnostic methods, involving the utilization of clinical, imaging and/or biochemical parameters, were developed [19,20]. CT examination is a noninvasive screening technique used for the preoperative evaluation of hepatic steatosis in potential donor candidates. The use of this method avoids unnecessary biopsies. The opportunity to evaluate whole liver tissue, including both the graft and the residual part, offers an advantage over biopsy procedures involving sampling from only one region (usually the right lobe).

In CT, further examination is required to confirm that the remaining part in the donor after the transplantation will gain proper and adequate function, if the steatosis level of the residual liver is higher than that of the graft that will be transplanted. A histopathological examination, considered as the "gold standard" method, may not reflect exact rates of steatosis in donor candidates with non-homogenous steatosis, because an only limited sample quantity can be obtained. Despite these limitations, histopathological evaluation remains the most widely accepted and practical method for objective evaluation of the degree of steatosis. This "gold standard" evaluation is invasive and time-consuming and may cause complications; thus, attempts have been made to use imaging techniques such as ultrasound (US), CT, and magnetic resonance imaging (MRI) to evaluate hepatic steatosis; these have different success rates [11, 16, 21]. The sensitivity of the imaging techniques used to determine the hepatic steatosis level is higher for the detection of mild hepatic steatosis than more severe steatosis. A significant correlation was found between US evaluation and histopathological assessment in cases with a high degree of steatosis [1]. Lang et al. [22] showed that US had a specificity of 100% in demonstrating hepatic steatosis in multi-organ donor candidates before organ provision. However, determining the degree of steatosis using this method can be done only qualitatively, based on the image, rather than quantitatively. The results of image-based evaluation may vary with differences between assessors and devices. The threshold value for the detection of hepatic fat percentage by US was reported to be 30% [23]. In slice-imaging studies, MRI examination using a gradient echo sequence was reported to be superior to CT in characterizing hepatic steatosis [24]; this was the case, in particular, for cases with minimal hepatic steatosis [25]. Schuchmann et al. [1] determined that the degree of steatosis could be estimated numerically using the chemical shift technique on fast spin-echo images; this technique provided acceptable results compared with biopsies, even in cases with a steatosis percentage < 20%. CT imaging remains a more sensitive technique compared with other methods, due to the introduction of high-resolution helical techniques [26]. Ryan et al. [27] used a combination of US and CT, and reported that the detection of a steatosis level of # 10% had an accuracy rate of 65%, while the rate of accuracy was 80% in the determination of a steatosis level # 30%. According to Lee et al., visual grading (qualitative) and LAI (quantitative) with non-contrast CT have a high rate of accuracy in the diagnostic evaluation of # 30% macrosteatosis in the liver [9]. In another study, Raptopoulas et al. [28] used a dual-energy CT technique for the calculation of the degree of steatosis; CT images were obtained at 80 and 140 kV. When compared
to a normal liver, the fatty liver showed a marked decrease in density on the 140-kV images, compared with the 80-kV images [28]. Panicek et al. [12] compared the liver density measured from a contrast-enhanced CT scan with the densities of the spleen and intercostal or paraspinal muscle groups. They concluded that the method used to compare the densities of the liver and spleen on non-contrast CT was not appropriate for contrast-enhanced CT; only cases with advanced hepatic steatosis can be identified in a comparison with the intercostal or paraspinal muscle group.

The measurable density of a normal liver in HU on non-contrast CT is 50-60. This value varies from person to person and with the device used for the examination. However, there is a constant relationship between the densities of the liver and spleen. The parenchymal density of a normal liver is approximately 5-10 HU higher than the spleen. The spleen is an ideal organ for this comparison, because it can be evaluated in the same section as the liver, and it is not affected by most metabolic conditions, in contrast to the liver. However, it should be noted that in rare cases, splenic density may change under metastasis, iron accumulation, splenic vascular damage, or post-traumatic hypotension conditions. Iwasaki et al. [16] stated that the degree of steatosis could be estimated by calculating the ratio of the liver attenuation value (L) to the spleen attenuation value measured on non-contrast images (S); a L/S ratio of # 1.1 had 83% sensitivity and 82% specificity in the determination of the steatosis degree of # 30%. Duman et al. [29] determined that the difference between the MSA and MHA on non-contrast CT images was correlated to the degree of histopathological steatosis in all cases, with or without iron accumulation, in non-alcoholic fatty liver disease. Kodama et al. [25] compared the mean attenuation value with the degree of steatosis determined in the histopathological evaluation in cases that underwent liver resection due to metastatic disease, and concluded that the measurement of the hepatic attenuation value on non-contrast CT images is the optimum method of estimating the degree of steatosis. Park et al. [30] used three indices, including the ratio of the liver attenuation value (L) to splenic attenuation value (S) (= L / S), the difference between the mean hepatic attenuation (L) and mean splenic attenuation (S) (= L # S), and hepatic parenchymal attenuation, and reported that all three indices were correlated to the histopathologically measured rate of macrovesicular steatosis.

In predicting pathologic fat content, non-contrast CT is more accurate than contrast-enhanced CT [25]. The hepatic attenuation value is affected by the amount, administration rate, the distribution in circulation, and the time of measurement of the administered contrast medium on contrast-enhanced CT images. In conclusion, the decrease in density caused by steatosis may be masked by the attenuation differences induced by the contrast medium. Therefore, non-contrast slices should definitely be obtained, although this will increase the radiation exposure to the donor.

Because the parenchymal density of the liver increases in rare conditions that affect liver parenchyma, the degree of steatosis may be misevaluated. As a result, the degree of steatosis is underestimated and steatosis may be found to be absent, even though it is present. This in turn limits the sensitivity of the method in the determination of the
degree of steatosis by CT densitometry. However, this can be detected by means of the signal loss caused by the supermagnetic agent iron on the MRI slices obtained during MR cholangiopancreatography (MRCP).

This study had several limitations. First, the degree of steatosis of the patients in this study group was < 10%, because the donor candidates whose degree of steatosis was estimated to be high by clinical evaluation were excluded at baseline. Among the 51 cases included in this study, steatosis was not detected in 29 (approximately 2/3) cases. This affects the creation of functions that show the association between LAI and the percentage of steatosis. Secondly, the measurement may be faulty in cases with non-homogenous steatosis, because only a small portion of the liver can be obtained by liver "wedge" biopsy.

The ROC curve is a plot of the sensitivity as a function of the specificity. The ROC curve analysis is independent of the disease prevalence in the real population. In this study, the sensitivity and specificity of the LAI parameter, in the determination of hepatic steatosis before LDLT, was analyzed using a ROC curve. For this purpose, when a significant degree of steatosis was considered to be 2%, the sensitivity and specificity were 77% and 75%, respectively, for a cut-off value of 9.1. When the significant degree of steatosis was 5%, the sensitivity and specificity were 73% and 86%, respectively, for a cut-off value of 9.1. The success of LAI, which is currently used in this center, in distinguishing the groups, was higher than the other parameters. Iwasaki et al. [21], using ROC analyses performed using the L/S ratio and anthropometric and biochemical parameters, reported that the L/S ratio had a higher sensitivity and specificity than other parameters; the sensitivity and specificity were 83% and 81%, respectively, when the cut-off value for L/S considered was 1.1, in cases with a steatosis degree of # 30%.

Calculation of hepatic steatosis index using CT densitometry is a non-invasive and safe method of determining the degree of hepatic steatosis. Multiphasic CT is routinely used to evaluate the diffuse and focal parenchymal disease, to determine the vascular anatomy, and to perform volumetric measurements in donor candidates before LDLT. Calculation of the hepatic steatosis index during these evaluations is an efficient, simple procedure. However, the use of radiation is the only known disadvantage when compared with the alternative method, MRI. In this context, the decision on which method to use depends on the preferences of the radiologists performing the evaluation, based on their knowledge and experience.

In this study, it was concluded that CT densitometry can be used in the evaluation of the degree of hepatic parenchymal steatosis, which is one of the main steps in the investigation and analysis of donor candidates before LDLT. While there were no cases with a moderate-to-severe degree of hepatic steatosis among the subjects included in the study group, even a mild degree of steatosis, which is more challenging to detect, can be accurately determined using the CT densitometry method. Thereby, the painful
and time-consuming biopsy procedure, which may cause complications, can be avoided in healthy donor candidates.
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