Detection of steatosis in chronic hepatitis C, based on the evaluation of the attenuation coefficient computed on the ultrasound image

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Abstract

Background and aims. The current study aims to evaluate the performance of the attenuation coefficient (AC) in quantifying liver steatosis in a cohort of consecutive patients with chronic hepatitis C.

Methods. 189 consecutive patients with chronic HCV infection were prospectively included in this study. They were referred to an ultrasound exam 1 day prior to liver biopsy, using the same device setting. The attenuation coefficient (AC) was calculated on each image.

Results. AC values were significantly correlated with steatosis (r= -0.444, p<0.005), but there was no significant correlation with activity (r= -0.135, p=0.076) or fibrosis (r= -0.066, p=0.367). The mean values of AC for each steatosis grade were 0.0284 (no steatosis), -0.0284 (insignificant steatosis <33%) and -0.1140 (significant steatosis ≥33%) (p<0.001). A cutoff value of -0.0024 of AC could distinguish the fatty load <33% from no steatosis (Sn 75%, Sp 61.76%, PPV 60.6%, NPV 75.9%, AUROC 0.734), and a cutoff value of -0.0546 could distinguish the significant steatosis from the absent/insignificant one (Sn 84.21%, Sp 78.53%, PPV 31.4 %, NPV 97.7%, AUROC 0.842).

Conclusions. AC could be used to develop an imaging steatosis detection method in HCV infection. The extra use of certain classifiers might increase the diagnostic performance of the method.

Key words: chronic hepatitis C, steatosis, ultrasonography, computerized analysis, attenuation coefficient

Introduction

Hepatic steatosis is a frequent histological finding in patients with chronic hepatitis C virus (VHC) infection, occurring in more than 50% of cases [1]. The underlying mechanism of steatosis in hepatitis C is not completely understood, and is most likely multifactorial. Even when the most common causes of steatosis are carefully excluded, a significant proportion of patients with chronic HCV infection have steatosis. Recent studies suggest that liver steatosis in HCV infection may be the expression of a direct cytopathic effect of the virus itself, particularly in patients infected with genotype 3 [2]. Evidence has
emerged from findings of a close relationship between intrahepatic HCV-RNA levels and the occurrence of steatosis [3]. These observations are of clinical relevance from both a prognostic and therapeutic point of view. There is increasing evidence that steatosis is an independent risk factor associated with liver necroinflammatory activity and progression of fibrosis in patients with chronic HCV infection [4-6].

Therefore, reliable and early diagnosis of hepatic steatosis is crucial to monitor disease progression and therapeutic intervention. The gold standard for assessing diffuse liver disease, including steatosis, is liver histology. However, liver biopsy is an invasive procedure and associated with potential complications, as well as sampling error and interobserver variability [7].

Hence, reliable non-invasive methods to assess steatosis in patients with chronic HCV infection are needed. Among these, imaging methods have an important role, and of them, ultrasonography (US) is the best choice from the point of view of cost, accessibility and lack of side effects.

The ultrasonic alterations of fatty liver appear when the fatty load of the hepatocytes is more than 15 – 20%. These alterations are represented by hepatomegaly, increased parenchymal echogenicity (“bright liver”), attenuation of the ultrasounds in the sub–capsular strata, difficult visualization of the walls of the portal vein, of the wall of the gallbladder and of the hepatic capsule, the apparent dilatation of the vessels (especially of the suprahepatic ones) and the false transonic aspect of the right kidney parenchyma as opposed to that of the hepatic one [8-10].

However, the performance characteristics of conventional grey-scale US may vary considerably among studies, ranging from good to poor [11-13]. One reason might be that concomitant liver pathology may alter the diagnosis of steatosis with ultrasound [14]. Evaluation of steatosis in patients with hepatitis can be difficult due to the accompanying inflammation and fibrosis [14, 15]. Fibrosis may also appear hyperechoic, but most of the time, fibrosis and fatty infiltration coexist, which is why the term “fatty-fibrotic pattern” is used to define the resulting aspect [16]. Another drawback is the need for subjective interpretation of the images by the investigator, which is accountable for a high inter- and intra-observer variability [17].

Recently, new methods are being evaluated for objective evaluation of steatosis by using artificial neural networks and computerized analysis of liver texture by indices of ultrasonic backscatter [18-20].

The current study aims to evaluate the performance of the attenuation coefficient (AC) in quantifying liver steatosis in a cohort of consecutive patients with chronic hepatitis C.

**Patients and methods**

**Patients**

189 consecutive patients with chronic HCV infection examined at the 3rd Medical Clinic, University of Medicine and Pharmacy Cluj-Napoca, between January 2007 and December 2007, were prospectively included in this study. All of them had positive HCV-RNA in their serum and underwent percutaneous liver biopsy (LB) for grading and staging the diseases. All patients were referred to an ultrasound exam 1 day prior to LB. Besides the epidemiological data, the following biological parameters were determined for all patients on the same day as LB: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transpeptidase (GGT), total bilirubin, alkaline phosphatases, fasting blood glucose, fasting serum cholesterol and triglycerides.

The study was approved by a local ethical committee of the University of Medicine and Pharmacy Cluj-Napoca. The nature of the study was explained to the patients, each of whom provided written informed consent before the beginning of the study, in accordance with the principles of the Declaration of Helsinki (revision of Edinburgh, 2000).

**Ultrasound exam**

Each studied patient was submitted to an abdominal ultrasound exam by means of a GE Logiq 7 device, using a 5.5 MHz convex probe, one day before the LB. The examination protocol was built so as to acquire the maximum amount of information from the tissue level, with as little “noise” as possible overadded to this process. In order not to change the textural elements, the amount of digital post-processing must be as small as possible, and thus, all the post-processing parameters were set at minimum, and in order to exclude movement artefacts, the tissue image „Freeze” took place as quick as possible (by using a „Frame rate” which must be as high as possible). We worked with harmonic (because it increases the quantity of information coming from tissues). The “Time Gain Compensation” curve was adjusted to a neutral position.

The device was set so as to stand on all these principles, and once the setting took place, it was used for all the examined patients. For each patient, US images were acquired from the right lobe through intercostal spaces. Depth was set at 16 cm. The images were saved on the ultrasound machine hard disk in DICOM format and further processed using a special soft designed by the Technical University of Cluj-Napoca.

**Computing the image coefficients**

On each US image, a straight line was fitted so as to avoid artefacts. This line represents the ultrasound beam path into the liver tissue and it has to be as parallel as possible to the US rays, preferably vertical. The fitted line is the region of interest (fig.1).
The grey level values for each point along this line are calculated by averaging 7 horizontal pixels (the pixel under the line and three more pixels from each side) [20]. For each point of the line, two values were stored: the average grey level computed as above and the depth (in millimeter units) (fig.2). As a measure of ultrasonic attenuation, linear regression by least-squares approximation was applied to this dataset. The slope of this line (in grey-level units per mm) represents the attenuation coefficient.

Histological study

A liver biopsy examination was performed by using the TruCut technique with a 1.8 mm (14G) diameter automatic needle device - Biopty Gun (Bard GMBH, Karlsruhe, Germany).

Liver biopsy specimens were fixed in formalin and embedded in paraffin. Unaware of the clinical data, a single expert pathologist evaluated the slides. Only biopsy specimens with more than 6 intact portal tracts were eligible for evaluation [21]. Liver fibrosis and necroinflammatory activity were evaluated semiquantitatively according to the METAVIR scoring system [21, 22]. Fibrosis was staged 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; F4 – cirrhosis. Necroinflammatory activity was graded as follows: A0 – none; A1 – mild; A2 – moderate; A3 – severe.

Steatosis was categorized by visual assessment as: 0 - none; 1- steatosis in <33% of hepatocytes; 2- steatosis in 33% to 66% of hepatocytes; and 3- steatosis in > 66% of hepatocytes.

Statistical analysis

Statistical analysis was performed using the SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA).

Attenuation coefficient (AC) data were expressed as median values. The distribution of attenuation coefficient values in the various classes of the three histological parameters was visually inspected through box plots. Differences in mean values were tested by one-way analysis of variance (ANOVA) and Kruskal-Wallis test; relationships between the parameters were characterized using the Spearman correlation coefficients.

The diagnostic performance of AC was assessed using sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), accuracy, likelihood ratios (LR) and receiver operating characteristic (ROC) curves. The ROC curve is a plot of sensitivity versus 1-specificity for all possible cut-off values. The most commonly used index of accuracy is the area under the ROC curve (AUROC), with values close to 1.0 indicating higher diagnostic accuracy. Optimal cut-off values for AC were chosen to maximize the sum of sensitivity and specificity, and positive and negative predictive values were computed for these cut-off values.

Results

Baseline characteristics of patients

Clinical and biochemical characteristics of the study patients are summarized in table 1.

The median length of the LB samples was 11.34 mm, and the mean number of portal spaces was 12.51. The fibrosis stage distribution in our patients was as follows: F0 – 12 (6.3%), F1 – 67 (35.4%), F2 – 67 (35.4 %), F3 – 28 (14.8%), F4 – 15 (7.9%). The steatosis grade distribution was as follows: S0 – 109 (57.7%), S1 – 61 (32.3%), S2 – 12 (6.3%) and S3 – 7 (3.7%). Patients’ distribution according to fibrosis stages, activity grades and degrees of steatosis is illustrated in tables 2 and 3.
Table 1. Baseline characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristics of patients</th>
<th>Mean ± SD or number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female)</td>
<td>122 (64.6 %)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.26 ± 10.39 (21-66)</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>27.07 ± 5.14</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>59.17 ± 33.22</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>88.94 ± 55.42</td>
</tr>
<tr>
<td>GGT (U/l)</td>
<td>80.58 ± 119.84</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.803 ± 1.39</td>
</tr>
<tr>
<td>Alkaline phosphatases (U/l)</td>
<td>269.62 ± 308.00</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>112.05 ± 36.02</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>199.26 ± 45.91</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>125.71 ± 69.05</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>55.28 ± 18.65</td>
</tr>
</tbody>
</table>

Abbreviation: body mass index (BMI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transpeptidase (GGT)

Due to the small number of patients with severe steatosis (>66%), we decided to introduce the 7 patients with severe steatosis in the group of those with moderate steatosis, which resulted in: S0 (no steatosis), S1 (insignificant steatosis <33%) and S2 (significant steatosis ≥33%).

Table 2. The distribution of patients in relation to the fibrosis stage (META VIR), necroinflammatory activity and steatosis grade.

<table>
<thead>
<tr>
<th>Fibrosis stage (META VIR)</th>
<th>No steatosis</th>
<th>Steatosis &lt; 33%</th>
<th>Steatosis 33-66%</th>
<th>Steatosis &gt; 66%</th>
<th>Total patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>15 patients</td>
<td>109 patients</td>
<td>36 patients</td>
<td>1 patients</td>
<td>189 patients</td>
</tr>
<tr>
<td>F1</td>
<td>3 patients</td>
<td>21 patients</td>
<td>5 patients</td>
<td>2 patients</td>
<td>36 patients</td>
</tr>
<tr>
<td>F2</td>
<td>0 patients</td>
<td>5 patients</td>
<td>1 patient</td>
<td>0 patients</td>
<td>6 patients</td>
</tr>
<tr>
<td>F3</td>
<td>0 patients</td>
<td>2 patients</td>
<td>1 patient</td>
<td>0 patients</td>
<td>3 patients</td>
</tr>
</tbody>
</table>

Histological activity (META VIR)

A0 30p (15.8 %)
A1 28p (14.9 %)
A2 99p (52.4 %)
A3 32p (16.9 %)

Steatosis grade

S0 109 p (57.7 %)
S1 61p (32.3 %)
S2 12p (6.3 %)
S3 7p (3.7 %)

Correlation between the attenuation coefficient and different histological parameters

Figure 3 shows box-plots of AC value variability according to different grades of steatosis and necroinflammatory activity and different stages of fibrosis. AC values were significantly correlated with steatosis (r=-0.444, p<0.005), but there was no significant correlation with activity (r=-0.135, p=0.076) or fibrosis (r=-0.066, p=0.367) (table 4).

Then, we analyzed how the steatosis grade influenced the AC value. The mean values of AC for each steatosis...
grade were 0.0284 (for patients with no steatosis – S0), -0.0284 (for patients with insignificant steatosis – S1) and -0.1140 (for patients with significant steatosis – S2) (table 5). The differences between the groups were as follows: S0 vs S1 (p<0.005) and S2 (p<0.005); S1 vs S2 (p=0.001).

The most discriminate cut-off values were determined from the distribution of AC values according to steatosis grades. Fig.4 shows the ROC curves according to different steatosis grade thresholds: S0 versus S1 and S2 patients (S≥1), S0 and S1 versus S2 patients (S=2). The areas under the ROC curves (95% CI) were 0.734 (0.663 – 0.796) for S≥1, 0.842 (0.781 - 0.892) for S=2. Table 6 shows the optimal cut-off values as well as the corresponding sensitivity, specificity, positive and negative predictive values.

### Discussion

The surest method for the detection of steatosis is hepatic needle biopsy [23]. As opposed to this method, US is the most commonly used modality for evaluating hepatic steatosis [25]. Hyperechogenic liver tissue with fine, tightly packed echoes on ultrasound examination (“bright liver”) is considered characteristic of liver steatosis [26]. The decreased ability of the ultrasound beam to penetrate the liver tissue causing posterior darkness and loss of definition of the diaphragm (posterior beam attenuation) is another important ultrasonographic finding in steatosis. The attenuation of the ultrasound beam by the fat often results in a relatively hypoechoic kidney. This is not a reliable sign because it assumes normal echogenicity of the kidney [26]. Other detectable features include hepatomegaly and decreased sonographic visualization of portal and hepatic veins giving rise to a featureless or bland appearance of the liver (because the increase of hepatic echogenicity determines the decrease of acoustic impedance between the parenchyma and the walls of the veins).

In the diagnosis of liver steatosis, US is a simple method and it provides useful information, though it has some disadvantages. It cannot appreciate the etiology of the process; it cannot formulate an accurate quantification of the volume of fat content and cannot accurately dif-
Differentiate steatosis from fibrosis [27]. In clinical practice, fibrosis in the absence of fat may not be associated with increased attenuation. Certainly, one study concluded that fat alone accounted for the increased attenuation in patients with cirrhosis [28], although another study did suggest that, in vitro, fibrosis caused some attenuation but only half that of fat [29]. Fibrosis may also be distinguished from fat by the coarser echo pattern produced and the increased definition of portal veins [23].

Many times steatosis and fibrosis coexist, which is why the term “fatty-fibrotic pattern” is used to define the resulting aspect [16]. Though these pathological conditions are different (as substrata) the main obstacle in the differentiation is the extremely subtle “visual” differences that they generate on the ultrasonic image [30]. The visual criteria of discrimination depend on the subjective interpretation of the examiner, which can lead to the limitation of the reproducibility of the method, and not least to diagnostic errors.

Under these circumstances, needle biopsy of the liver is often necessary. However, liver biopsy is invasive and can cause severe complications.

The need for non-invasive tests for the quantitative estimation of fat infiltration, detection of response to treatment or evaluation of disease progression is, therefore, extremely important, especially in chronic hepatitis C because there is increasing evidence that steatosis is an independent risk factor associated with liver necroinflammatory activity and progression of fibrosis [4-6].

A possible approach might be the computerized processing of data that comprises the ultrasonic image, taking into consideration that all the information concerning the characteristics of the tissue already exists in the echoes returned by the transducer. This is based on the principle according to which the pathological tissue modifications due to a specific disease (such as steatosis or fibrosis) determine alterations of the physical and microarchitectural features (density, thickness, elasticity, homogeneity, etc.). These are very difficult to visualize, but because they affect the propagation of the ultrasounds they can be perceived through the complex analysis of the image (the ultrasonic tissue characterization) as a different textural pattern as opposed to the healthy one [31].

The ultrasonic tissue characterization can be achieved either by methods based on the study of the echogenicity of the parenchyma and the attenuation of the ultrasounds, or by methods based on the quantification of some textural parameters [19, 20, 32-35].

Gaitini et al. showed that attenuation/backscatter based indices have better potential than the textural based indices for serving as an ultrasonic fatty liver biopsy tool [20].

In this context, we prospectively assessed the performance of the attenuation coefficient in quantifying liver steatosis in a cohort of consecutive patients with chronic hepatitis C, with different fibrosis grades. We used LB examination as the reference standard because it is recommended for pre-treatment assessment of CHC in the latest consensus statements [36]. However, its reproducibility is poor, owing to heterogeneity in liver fibrosis and sample size, and also to inter and intra-observer variability. Ragev et al. reported a difference of at least 1 fibrosis stage between the right and left lobes in 33% of 124 patients [7], and Siddique et al. showed in 45% of patients a difference of at least 1 fibrosis stage between 2 specimens (at least 15 mm long) obtained from the same puncture site [37]. This might be one of the limitations of our study, the fact that we submit to a golden standard which is unfortunately not perfect.

In the context of simultaneous steatosis and fibrosis, first of all we wanted to see how much the attenuation coefficient was influenced by the histopathological aspects found in patients with VHC infections. First, the attenuation coefficient in patients with VHC was found to be significantly correlated with steatosis (r = -0.444, p<0.005), but there was no significant correlation with activity (r = -0.135, p = 0.076) or fibrosis (r = -0.066, p = 0.367). This ascertainment might be the first step in the thorough study of this coefficient when differentiating between steatosis and fibrosis on the ultrasonic image.

The mean values of AC for each steatosis grade were 0.0284 (for patients with no steatosis), -0.0284 (for patients with insignificant steatosis) and -0.1140 (for patients with significant steatosis), proving that attenuation increases with the steatosis grade. Because of the way in which this coefficient is computed, its numerical value decreases. A lower numerical value means a stiffer slope and a higher attenuation rate of the ultrasounds into the liver tissue.

Still, when we focused on the diagnosis of the steatosis grade, the AC performances were good. The areas under the ROC curve for the diagnosis of insignificant steatosis (<33%) and significant steatosis (≥33%) were 0.734 and 0.842, respectively.

The sensitivity and specificity will vary according to the threshold value selected. Naturally, one could trade off sensitivity for better specificity and vice versa, by setting a different threshold value. In our study, optimal cut-off values for the attenuation coefficient were chosen to maximize the sum of sensitivity and specificity, and positive and negative predictive values were computed for these cut-off values. A cutoff value of -0.0024 of the attenuation coefficient could distinguish the fatty load ≤33% from the absence of it (Sn 75%, Sp 61.76%, PPV
specialized services, this method might facilitate early di-
setting of the device. By using a standard examination in
the cut-off values obtained are valid for a certain predefined
of the device for all the patients. This leads to the idea that
without any change in hardware, but using the same setting
ultrasonic images using clinically available equipment,
dependent. The computerized analysis was performed on
for the detection of liver steatosis that is less operator
coefficient could be used to develop an imaging method
decision.

Fatty grades are eval-
ated using biopsy, but in their study, patients without
severe steatosis were excluded [20]. We think that this
fact has quite a low practical usefulness, because the US
changes in these patients can also be visible by a simple
visual inspection. In this paper we focus on the complex
evaluation of the attenuation coefficient in the presence
of other pathologies that affect hepatic tissue, especially
on tracing out the subtle changes caused by the fatty load
in a low degree, which is hard to differentiate just by a
simple visual inspection.

An explanation for our not very high differentiation
rates between grade 1 and 2 is that we divided the groups
using a fixed threshold (33% fatty). Fatty grades are evalu-
ated using biopsy, but still involve a certain qualitative
evaluation. The pathologist cannot distinguish between
a 32% and a 34% fatty liver. For this reason, a further
development of our work is to numerically quantify the
fatty infiltration and try to predict the fatty percentage
using image processing techniques. Our previous results
[39, 40] indicate that the use of classifiers can sometimes
improve the detection rates, but they work as a “black box”
and do not allow the physician to motivate the medical
decision.

**In conclusion,** our findings suggest that the attenuation
coefficient could be used to develop an imaging method
for the detection of liver steatosis that is less operator
dependent. The computerized analysis was performed on
ultrasonic images using clinically available equipment,
without any change in hardware, but using the same setting
of the device for all the patients. This leads to the idea that
the cut-off values obtained are valid for a certain predefined
setting of the device. By using a standard examination in
specialized services, this method might facilitate early di-
agnosis and provide a better accuracy of certain conditions
which give similar ultrasonic images (steatosis, fibrosis),
and could serve for a more accurate monitoring when it
comes to the evolution of the disease. An important step
might be the finding of certain coefficients which would
ease an “equalization” of the examinations, regardless
of the device that is being used. The extra use of certain
classifiers, which would contain along with the attenua-
tion coefficient other relevant textural parameters, might
increase the diagnostic performance of such a method. The
use of the computerized analysis of ultrasonic image might
represent an important development in the non-invasive
evaluation of diffuse liver diseases, a big step forward to
the goal of “virtual liver biopsy”.

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