Real-time elastography: role in the assessment of hepatic fibrosis in patients with liver iron-overload

Poster No.: B-0413
Congress: ECR 2014
Type: Scientific Paper
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Keywords: Abdomen, Liver, Ultrasound, Diagnostic procedure, Hematologic diseases
DOI: 10.1594/ecr2014/B-0413

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Purpose

In patients with liver iron overload related to primary (genetic) or secondary hemochromatosis [1,2], the risk for developing fibrosis and cirrhosis has been associated to the liver iron content (liver iron concentration [LIC]), the duration of iron exposure by the liver, and the presence of co-factors of hepatotoxicity, such as viruses and alcohol [3,4]. Liver biopsy is still considered the gold-standard method for evaluating the stage of hepatic fibrosis and to measure LIC in patients with hemochromatosis [5]. However, this invasive technique has many procedure-related complications, as well as wide variations in the results [6]. Sampling error studies have shown that a single biopsy may miss cirrhosis in 10-30% of patients and incorrectly classify fibrosis by at least one stage in 20-30% [7]. Currently, magnetic resonance imaging (MRI) with T2*-weighted sequences is considered a reliable method for detecting iron deposits in the liver, showing high correlation to the values found in specimens from biopsy, which are expressed as milligrams of iron per gram of dried tissue (mg Fe/g dry weight) [8]. Beside LIC quantification, a correct estimation of liver fibrosis has important implications regarding patient's management, prognosis assessment and long term follow-up. In recent years, non-invasive methods were developed in order to replace liver biopsy. Non-invasive methods (Fibrotest, aspartate transaminase-to-platelet ratio index) using biological parameters such as bilirubin, haptoglobin, platelet count cannot be applied to hematological diseases requiring blood transfusion [9]. Ultrasound-based elastographic methods include transient elastography (TE), acoustic radiation force impulse (ARFI) elastography, shear wave elastography (SWE), and real-time elastography (RTE) [10]. While TE has already been validated in patients with liver iron overload (i.e. primary hemochromatosis [11], multi-transfused adult #thalassemia major and thalassemia intermedia patients [12], sickle cell disease and myelodysplastic syndrome [13]), no study has investigated the diagnostic performance of RTE in staging liver fibrosis in such patients. The main objective of the present work was to determine the diagnostic value of RTE in assessing hepatic fibrosis stage according to the METAVIR classification [13] in a heterogeneous cohort of patients with liver iron overload using TE as reference standard.

Methods and materials

Patient inclusion:

This was a prospective monocentric study and patient's enrollment was performed at the Unit of Micocitemia and Hereditary Anaemias of our Institution, where subjects with different diseases leading to liver iron overload are routinely evaluated with MRI. Consecutive patients with MRI T2* detectable hepatic iron (liver T2*value#6.3 ms) were enrolled into the study. The study protocol was approved by the institutional review board.
Written informed consent was obtained from all patients before the MRI study. Exclusion criteria were general contraindications to 1.5 T MRI [14], decompensated liver cirrhosis with ascites (which can influence both TE and RTE results [10]), and the presence of an inhomogeneous patchy pattern of iron deposition detected by MRI. This latter criterion was introduced to avoid sampling bias when performing TE, since it is likely that a patchy iron deposition may lead to inhomogeneous fibrosis throughout the liver parenchyma [8]. Serum ferritin levels were determined using a standard laboratory method performed within 1 month from the imaging examinations (normal adult blood levels are 12-300 ng/mL for males and 10-150 ng/mL for females [5]). Eligible patients were also screened for the presence of hepatitis C virus (HCV) antibodies and hepatitis B surface antigen (HBsAg) in serum.

Magnetic resonance imaging:

MRI is considered a reliable method for detecting iron deposits in the liver [15], and gradient-echo sequences are used to quantify the proton-transverse relaxation through transverse relaxation time (T2*) measurements. The reciprocal of T2*, known as transverse relaxation rate (R2*), increases in the presence of iron and is proportional to LIC over the clinically relevant range [16]. Breath-hold R2*-MRI measurements were performed using an eight-element cardiac/torso coil in a 1.5T Signa HDx scanner (General Electric Medical Systems, Milwaukee, WI, USA) scanning the whole liver of the patients. To obtain quantitative R2* maps, a multigradient echo sequence with the following parameters was used: eight echoes and minimum echo spacing, echo times (TE) 1.1-6.7 ms, repetition time (TR) 200 ms, flip angle 20#, matrix 128×96 pixels, bandwidth 125 kHz, number of excitations 1, slicethickness 10 mm, and spacing 0 mm. The duration of each sequence was 20-30 s. Measurements of R2* were performed using a publicly available software (C-Iron, Camelot Biomedical Systems, Genoa, Italy; website: http://c-iron.camelotbio.com) and the signal decay was fitted to every pixel in the image to an exponential plus a constant function. A region of interest (ROI) comprising the whole liver and excluding blood vessels and biliary ducts was drawn from a transverse midhepatic slice. Hepatic iron overload was defined by MRI T2* values less than 6.3 ms, corresponding to a liver iron concentration in dry tissue (LIC dry weight) of 4.2 mg/g. Hepatic iron overload was categorized as mild (6.3-2.7 ms), moderate (2.6-1.4 ms) or severe (<1.4 ms) [17].

Transient elastography:

TE is a corroborate method for the assessment of liver fibrosis in patients with hemochromatosis [11-13], since it has been shown that iron overload does not influence the diagnostic accuracy of this technique [18]. TE was performed with FibroScan (Echosens, Paris, France). In this device an ultrasound probe, mounted on the axis of a vibrator, transmit slow-frequency vibrations from the right intercostal space, creating an elastic shear wave that propagates into the liver. A pulse-echoultrasound acquisition is
then used to detect the wave propagation velocity, which is proportional to tissue stiffness; faster wave progression occurs through stiffer material. Liver stiffness measurement is then performed and measured in kiloPascals (kPa)(values between 2.5 kPa and 75 kPa are expected) [19]. Measurements of liver stiffness were performed on the right lobe of the liver through intercostal spaces in correspondence to the mid-axillary line, while patients are lying in the supine position with the right arm in maximal abduction. Only patients with 10 correct measurements with an interquartile range (IQR) of less than 30% of the median liver stiffness value were included [20]. TE values were expressed in kilopascals (kPa); further they were converted in the corresponding semi-quantitative fibrosis score of METAVIR. It is based on a semi-quantitative 5-point scale from 0 to 4: F0, the absence of parenchymal fibrosis; F1, enlarged fibrotic portal tract; F2, periportal or initial portal-portal septa but intact architecture; F3, architectural distortion but no obvious cirrhosis; and F4, cirrhosis. The conversion of TE values into the corresponding METAVIR stage was made by means of validated cut-off values (i.e. F0/F1 vs F2-F4 = 8.8 kPa; F0/F1-F2 vs F3-F4 = 9.6 kPa; F0/F1-F3 vs F4 = 14.6 kPa), which were obtained in a previous study by Ziol et al. using biopsy as reference standard [21].

Real-time sonoelastography:

A radiologist (FP) with more than 5 years of experience in conventional ultrasound examinations and 1 year of experience in RTE, blinded to TE results, consecutively performed all RTE examinations. RTE measures mechanically probe-induced deformation (strain) of structures examined in the B-mode ultrasound image, generating color-coded maps of the strain distribution (i.e. elastograms), which reflect tissue elasticity [22,23]. The RTE module displays two images simultaneously: the conventional B-mode image and the color-coded elastography region of interest (ROI), overlaid on the B-mode image (Fig. 1). The system generates a color map where hard tissue areas are marked with blue, intermediate tissue areas with green, and soft tissue areas with red. In the Esaote elastographic module (Elaxto) numerical values of pixels are from 0 to 100 (100 stepwise grading) according to color mapping from blue (0) to red (100). It is possible to generate a histogram of pixel distribution derived from the color image by 100 stepwise grading. The examinations were performed on the right lobe of the liver through the intercostal spaces in correspondence to the mid-axillary line, applying gentle pulsed compression on the skin. We used an original multi-frequency linear probe with a range of 3-11 MHz (LA332 apple probe, Esaote, Genoa, Italy). The small array (3 cm) of the transducer guarantees a perfect coupling with the patient’s intercostal space, while its trapezoidal (“convex-like”) view is more panoramic that the conventional view of a linear probe. The elastogram ROI was positioned in the most superficial portion of the image, which is not affected by degradation or distortion. Patients were instructed to continue breathing as usual, because each elastography image is obtained in a few milliseconds and breathing did not cause any motion artifacts. The acquisitions were considered reliable only if a pressure of 3-4 at least on a scale of 0-6 arbitrary units was recorded. Such indicator is a simple feedback for the operator to indicate that the movement of tissues subjected to compression is more or less suitable to the rate of
acquisition of the system. The elastography ROI positioning was made according to a method formerly proposed by Saftoiu et al. [24,28], where the elastogram includes the perihepatic soft-tissues, such as the diaphragm and intercostal muscles, in order to clearly compare and distinguish the strain between the liver and these structures. The abdominal wall layers visualized through the right intercostal spaces include the skin, subcutaneous fat tissue, intercostal muscles (external and internal), diaphragm, and liver parenchyma. In some patients, even the thin adipose tissue between the Glisson’s capsule and visceral peritoneum (tela subserosa) can be depicted by the elastography software [24]. To standardize measurements, a rectangular ROI of 25 mm×20 mm was positioned to visualize the strain map of the inner perihepatic soft tissues (internal intercostal muscle, diaphragm, perihepatic fat tissue) and a superficial portion of liver parenchyma free of large vessels. Ten elastograms were acquired for each patient. The qualitative information provided by the elastograms was converted in quantitative data by calculating the elastic ratio, which is the ratio of strain distribution in two selected regions of interest (ROIs). A large elliptical ROI of 1 cm2(Z1) was positioned in the liver parenchyma near the center of the image and a smaller elliptical ROI of 2 mm2(Z2) was positioned in a homogeneously soft region of the diaphragm, which was considered as internal control to calculate the elastic ratioZ2/Z1. The diaphragm was chosen as internal control since it appeared quite homogeneously soft (when compared to the liver parenchyma) in all patients. A higher elastic ratio indicates harder hepatic elasticity, corresponding to a higher stage of fibrosis (Fig. 2). Our method to calculate the elastic ratio was similar to that of Xie et al. [29], who reported excellent values of intra- and inter-observer agreement (intra-class correlation coefficient = 0.906, P< 0.001) using a free-hand compression approach. ROI positioning and calculation of the elastic ratios were performed on a personal computer by a trainee in radiology, blinded to TE results, using the original software MyLabDesk (Esaote, Genoa, Italy). Elastic ratios were calculated on the 10 color-coded images obtained from each patient, and the mean value was used for further statistical analysis. Each RTE examination lasted about 5 min with other 10 min to calculate the mean elastic ratio. The mean time necessary to complete a RTE examination was almost the same across the duration of the study. On the other hand, the time needed for the off-line analysis of the elastograms was seen to progressively shorten according to the increasing confidence of the operator with the software interface.

Statistical analysis:

Descriptive statistics were produced for demographic, clinical, and laboratory characteristics of patients. The Shapiro-Wilk test was used to test the normality (i.e. Gaussian distribution) of T2*, TE and RTE values. Box plots were used to study the distribution of RTE elastic ratios according to the patient’s stage of fibrosis, and the presence of significant differences in the mean elastic ratio among the four METAVIR stages was assessed by the one-way analysis of variance (ANOVA). The correlation between fibrosis stage and elastic ratio was calculated via the Spearman’s rank order correlation coefficient. The Pearson correlation coefficient was used to test associations between variables with a normal distribution. Nominal statistical significance was defined
with aPof 0.05. The diagnostic performance of RTE was assessed by using receiver operating characteristic (ROC) curves. For the ROC curve analysis, the area under curve (AUC), optimal cut-off values, sensitivity, specificity, positive and negative predictive values were calculated. Optimal cut-off values of RTE elastic ratio were chosen to maximize the sum of sensitivity and specificity for different fibrosis thresholds: F0-F1 vs F2-F4 (F#2), F0-F2 vs F3-F4 (F#3), and F0-F3 vs F4 (F= 4).

**Images for this section:**

**Fig. 1:** The RTE module displays two images simultaneously: the conventional B-mode image (A) and the color-coded elastography region of interest (B) superimposed to the B-mode image. A standardized rectangular ROI is positioned in order to visualize the strain map of the inner perihepatic soft tissues and a portion of liver parenchyma free of large vessels. The two arrows in the image A indicate the inner and outer border of the diaphragm. The acquisitions are considered reliable only if a pressure of 3-4 at least on a scale of 0-6 arbitrary units was recorded (void arrow in B).
Fig. 2: Calculation of the elastic ratio. A large elliptical ROI of 1 cm² (Z1) is positioned in the liver parenchyma, while a smaller elliptical ROI of 2 mm² (Z2) is positioned in a homogeneously soft region of the diaphragm, which represents the internal control to calculate the elastic ratio Z2/Z1. A higher elastic ratio indicates lower hepatic elasticity, corresponding to a higher stage of fibrosis. (A) 40 year-old man with thalassemia...
intermedia. His TE value was 4.09 kPa corresponding to a F0/1METAVIR grade; the elastic ratio is 1.95, as shown in the figure. (B) 42 year-oldman with -thalassemia major. His TE value was 11.04 kPa corresponding to a F3METAVIR grade; the elastic ratio is 2.34.
Results

Sixty-seven patients met the inclusion criteria. Seven patients were excluded: both TE and RTE were unsuccessful because of narrow intercostal spaces in 2 cases, and BMI > 27.5 kg/m² in other 3 cases; in the remaining 2 patients TE measurements had an IQR > 30%. The resulting 60 patients were 34 males (56.7%), 26 females (43.3%) with a median age of 42 (21-76) years and a mean BMI of 23.83±3.67. They included 37 adult homozygous-β-thalassemic patients (thalassemia major), 13 patients with thalassemia intermedia, 6 patients with primary (genetic or type 1) hemochromatosis, and 4 patients with myelodysplastic syndrome. The overall prevalence of hepatitis C infection in our patient's cohort was 18/60 (30%). Mean serum ferritin level was 1690 (890-5242) ng/mL. Patient's characteristics are reported in Table 1. T2* values did not follow the normal distribution, while both TE values and RTE elastic ratios followed the normal distribution. The mean TE value of each patient was converted in its corresponding METAVIR fibrosis stage, thus resulting in 28 (46.6%) F0/1, 12 (20%) F2, 12 (20%) F3 and 8 (13.4%) F4 patients (Table 2). Neither TE measurements (r=0.0820, 95%CI#0.329-0.176, P= 0.533), nor RTE elastic ratiosr=0.170, 95%CI#0.406-0.0877, P= 0.1942) were significantly associated to MRI T2* values of liver iron overload. A significant increase in elastic ratios was observed with increasing stiffness values measured by TE (r=0.645, 95% CI 0.468-0.772,P< 0.0001). The mean elastic ratios for each METAVIR group were as follows: F0/1 = 1.9±0.4; F2 = 2.2±0.4; F3 = 2.9±0.5; F4 = 3.2±0.4. The one-way analysis of variance (ANOVA) test demonstrated that mean elastic ratios of F0/1 and F2 METAVIR groups were significantly different from those of F3 and F4 groups (P< 0.05). Box-and-Whisker plots for measurements of elastic ratios for each fibrosis stage are shown in Fig. 3. The diagnostic accuracy of RTE for F2 evaluated by AUC-ROC analysis was 0.798 (95%CI 0.674-0.890). At a cut-off of elastic ratio2.01, RTE showed a sensitivity of 80% (95% CI 64.4-90.9), a specificity of 75% (95%CI 50.9-91.3), a positive predictive value (PPV) of 86.5% (95% CI71.2-95.5) and a negative predictive value (NPV) of 65.2% (95% CI42.2-84)(Fig. 4A). The diagnostic accuracy of RTE for F3 evaluated by AUC-ROC analysis was 0.909 (95% CI 0.806-0.968). At a cut-off of elastic ratio2.75, RTE showed a sensitivity of 70% (95% CI 45.7-88.1), a specificity of 97.5% (95% CI 86.8-99.9), a PPV of93.3% (95% CI 66.8-99.9) and a NPV of 86.7% (95% CI 73.2-94.9)(Fig. 4B). Despite the small sample size of F4 patients, the diagnostic accuracy of RTE for F= 4 evaluated by AUC-ROC analysis was 0.906 (95% CI 0.803-0.966). The best elastic ratio cut-off for detecting F= 4 was the same of F=3 (i.e.#2.75). Using this cut-off value for detecting F4 fibrosis stage, RTE showed a sensitivity of 87.5% (95% CI 47.3-99.7), a specificity of 84.62% (95% CI 71.9-93.1), a PPV of 46.7% (95% CI 21.3-73.4) and a NPV of 97.8% (95% CI 88.2-99.9).
**Fig. 3:** Box plots of liver elastic ratios for each fibrosis stage. The top and the bottom of the boxes are the first and third quartiles, respectively. The length of the box represents the interquartile range including 50% of the values. The line through the middle of each box represents the median. The error shows the minimum and maximum values (range). The increase of elastic ratios with increasing fibrosis is shown and there is an evident separation between F0/1-F2 and F3-F4 groups.
Fig. 4: (A) ROC curve analysis of RTE elastic ratios for patients with fibrosis F # 2 (F0/1 vs F2-F4). The area under the ROC curve is 0.86 (95% CI 0.74-0.94). (B) ROC curve analysis of RTE elastic ratios for patients with fibrosis F # 3 (F0/1-F2 vs F3-F4). The area under the ROC curve is 0.909 (95% CI 0.806-0.968).

Table 1
Patient characteristics. Values are expressed as percentages, means ± standard deviation and medians (min–max). Hepatic iron overload was categorized as mild (6.3–2.7 ms), moderate (2.6–1.4 ms) or severe (<1.4 ms).

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<tr>
<td><strong>Sex</strong></td>
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<tr>
<td>Males, n (%)</td>
<td>34 (56.7)</td>
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<tr>
<td>Females, n (%)</td>
<td>26 (43.3)</td>
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<tr>
<td>**Age (yr)</td>
<td>42 (21–76)</td>
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<tr>
<td>**BMI (kg/m²)</td>
<td>23.83 ± 3.67</td>
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<tr>
<td>**HCV+ n (%)</td>
<td>18 (32)</td>
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<tr>
<td>**Ferritin (ng/mL)</td>
<td>1690 (890–5242)</td>
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<tr>
<td>*<em>Liver MRI-T2</em> (ms)</td>
<td>3.7 (1.0–6.2)</td>
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<tr>
<td><strong>Iron overload grade n (%)</strong></td>
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<tr>
<td>Mild</td>
<td>39 (65)</td>
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<tr>
<td>Moderate</td>
<td>16 (26.7)</td>
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<tr>
<td>Severe</td>
<td>5 (8.3)</td>
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BMI, body mass index.

Table 2
Mean TE value ± standard deviation of liver stiffness is expressed in kPa. Patients were classified according to their METAVIR fibrosis stage using validated cutoff values (i.e. F0/F1 vs F2–F4 = 8.8 kPa; F0/F1–F2 vs F3–F4 = 9.6 kPa; F0/F1–F3 vs F4 = 14.6 kPa).

<table>
<thead>
<tr>
<th>TE stiffness (KPa)</th>
<th>9.6 ± 6.2</th>
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<tr>
<td><strong>METAVIR stage n (%)</strong></td>
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<tr>
<td>F0/1</td>
<td>28 (46.6)</td>
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<tr>
<td>F2</td>
<td>12 (20)</td>
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<td>F3</td>
<td>12 (20)</td>
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<tr>
<td>F4</td>
<td>8 (13.4)</td>
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TE, transient elastography.
Conclusion

Liver biopsy remains the reference method in order to determine the grade of fibrosis in chronic liver diseases, but it is an invasive and painful procedure, which can study only 1/50,000 of the total volume of the liver [5,10,19]. Liver biopsy has also several complications related to its invasiveness (e.g. patient’s dis-comfort and bleeding), and an overall procedure-related mortality of 1/1000-10,000 [6]. A heterogeneous distribution of fibrosis in the liver may become a limitation for biopsy, since only a small portion of parenchyma is sampled during the procedure. In more than half of cases, the specimens obtained with a 17-gauge needle have an average length and number of portal tracts well below the minimum sample size requirements (i.e. 10 or more portal tracts) [7]. In the field of non-invasive assessment of liver fibrosis, ultrasound-based methods play a pivotal role. TE has been validated in different chronic liver diseases, including chronic viral hepatitis (HCV and HBV), alcoholic steatohepatitis, non-alcoholicsteatohepatitis (NASH), autoimmune hepatitis, primary biliary cirrhosis and hepatopathy related to iron overload [10-13,19,21]. Di Marco et al. [18] performed TE examinations in a cohort of 56 homozygous-#-thalassemic patients, finding that liver stiffness increased proportionally to liver fibrosis stages detected by liver biopsy (r = 0.70; P > 0.001), without lack of interference by iron deposits in the parenchyma (i.e. LIC). Adhoute et al. [11] studied the utility of TE and other non-invasive methods in patients with genetic hemochromatosis (C282Y homozygosity). They included 57 cases with 46 controls, obtaining a strong correlation between TE and many biochemical markers, although ferritin levels did not correlate with TE values. The prevalence of patients with TE values higher than 7.1 kPa (cut-off level for significant fibrosis), was22.8% in patients with hemochromatosis and 0% in the controls (P < 0.0001). Fraquelli et al. [12] evaluated 115 adult thalassemicpatients (59 with #-thalassemia major and 56 with thalassemiaintermedia) with TE, confirming the significant positive correlation between liver stiffness and fibrosis stage (r = 0.73, P = 0.003). In a cohort of 42 transfusion-independent adult patients with #-thalassemia intermedia, it has been demonstrated that longitudinal changes in serum ferritin levels correlate with TE values of hepatic stiffness during a 4-year follow-up (r= 0.836,P< 0.001) [25]. To our knowledge, no study investigated the role of RTE in the non-invasive assessment of liver fibrosis in patients with primary and secondary hemochromatosis. While TE requires a dedicated equipment, RTE has been implemented on high-end ultrasound systems by different manufacturers. In our study we used the RTE module of the ultrasound system MyLab Twice (Esaote, Genoa, Italy). This RTE module differs from that produced by Hitachi due to a shortest range of pixel’s values (i.e. from 0 to 100 vs the Hitachi 256 step-wise grading) to represent strain distribution and tissue elasticity. It uses a conventional ultrasound probe to compare and analyze echosignals from the tissue under examination before and under slight compression [26,27], generating a color-coded map of strain distribution, called elastogram. Since elastic ratio is the ratio of strain distribution in two selected ROIs, some Authors prefer to use small parenchymal blood vessels (<3 mm) as internal control [30]. In our study, we selected as internal control a homogeneous red (soft) area in the diaphragm, considering this
perihepatic muscular structure less prone to inter-individual variability. Using this method of elastogram analysis, mean elasticity ratios significantly differed from patients with severe fibrosis (METAVIR stages F3, F4) to those with mild fibrosis (METAVIR stages F0/1, F2) (P < 0.05). Elastic ratios above the cut-off value of 2.75 identified patients at risk for severe fibrosis (F3 and F4) with a sensitivity of 70% and a specificity of 97.5%. From the results of the AUC-ROC curve analysis, it is evident that the best cut-off value for detecting F= 4 is the same of that for identifying patients with F#3 (i.e., #2.75). This result may be due to different factors, including the small sample size of F4 patients and the lack of a significant difference between the mean elastic ratios of F3 and F4 fibrosis stages. This latter result may be reasonably related to the exclusion of patients with decompensated liver cirrhosis from the study. Different investigators criticized the lack of inter-observer agreement in hepatic RTE [28,31], because the operator's freehand compression of the probe is a parameter difficult to be standardized. In order to overcome this problem, newer RTE modules are able to produce elastograms in response to the pressure generated by heartbeats [22,23]. However, some Authors found good inter-observer agreement (intra-class correlation coefficient = 0.906, P < 0.001) performing RTE by a free-hand compression approach and calculating the elastic ratio using a method similar to ours [29]. In our work, the mean value of the 10 elastic ratios obtained from each patient by a single experienced operator (who performed more than 100 RTE evaluations according to [30]) was significantly correlated with TE values (r = 0.645, 95% CI 0.468-0.772, P < 0.0001). With TE, tissue stiffness is estimated along an ultrasonic A-line, in a fixed region, which is neither user adjustable nor image guided [31]. The most important limit of this one-dimensional approach is that provides an average elasticity estimated over the measurement depth; for this reason we decided to exclude patients with an inhomogeneous pattern of liver iron overload, which may lead to a patchy deposition of fibrous tissue within the liver parenchyma. On the other hand, RTE allows the study of a wider portion of liver parenchyma, allowing to precisely detect large vessels and nodular lesions inside the sampling area, which can influence the strain response [26]. The common limitations of both TE and RTE include obesity, narrow intercostal spaces and ascites [10,20]. The major drawback of our study is that liver biopsy was not systematically used as reference standard; however, TE has been proven to be 99% efficient for the detection of patients with cirrhosis and 88% efficient for the detection of patients with fibrosis grade superior or equal to F2 [21]. When performing TE, the elasticity estimate is averaged over a volume that can be approximated by a cylinder of length 20 mm (between 25 mm and 45 mm below skin surface) and diameter 20 mm. This volume represents 1% of the liver total volume, which is much more relevant than the biopsy sample size, which is only of 1/50,000 [19]. TE cut-off values used in our study were formerly obtained by Ziol et al. [21] from a large cohort of 327 patients with chronic viral hepatitis C and validated using liver biopsy as reference standard. In a more recent work comparing TE, RTE and aspartate-to-platelet ratio index in the assessment of liver fibrosis [20], Ferraioli et al. found that TE is able to accurately assess significant fibrosis (F#2) with a high specificity 91.4%, offering an excellent diagnostic performance in the discrimination of severe fibrosis and cirrhosis with a NPV of 99%. Focusing on differentiating non-significant from significant fibrosis (F#2), TE and aspartate-to-platelet
ratio index, with an overlapping AUC (0.88 and 0.86), performed better than RTE. Even thought is still considered the reference standard, liver biopsy may fail in the assessment of the degree of liver fibrosis because it is subject to intra- and interobserver variability and to sampling errors, even when the biopsy length is adequate [32]. In addition, the METAVIR scoring system takes into account not only parenchymal fibrosis, but also architectural changes in the liver, without reference to quantitative changes in liver collagen [32]. So, like MRI is rapidly replacing liver biopsy for LIC quantification, TE is increasingly being used to study liver fibrosis in hematologic disorders leading to iron overload. The use of TE in this clinical concern has raised the question about the possible interference of iron deposition with liver stiffness measurement. Fraquelli et al. [12] examined the cross-sectional association between MRI T2* values of liver iron overload and TE measurements in 73 adult thalassemia patients (47 with thalassemia major and 26 with thalassemia intermedia). Median LIC values were 4.58 mg/gdw(range 1.02-19.7) in patients with thalassemia major and 5.98 mg/gdw(range 1.11-19.02) in those with thalassemia intermedia. In both groups, no correlation was found between LIC and TE results (r=#1.4257 andr= 0.09). In their cohort of 56 thalassemia major patients, Di Marco et al. [18] have found that TE values of liver stiffness are independently correlated only with the stage of fibrosis (P= 0.002) and aspartate aminotransferase(P= 0.006) values, concluding that TE measurements are not influenced by iron deposition. Examining a smaller cohort of patients, other Authors [33] have found a significant correlation of TE values with ferritin (r= 0.34,P= 0.01), T2* (r=#0.46,P= 0.003) and LIC values retrieved from MRI (r= 0.54,P< 0.0001), suggesting that it is not possible to completely exclude the interference of iron deposition with liver stiffness measurements. However, these Authors did not perform a multivariate analysis in their study.

We suggest that RTE could be used as a complementary imaging method to TE for a preliminary assessment of liver fibrosis. In particular, RTE may be performed immediately after a standard B-mode ultrasound examination of the liver, allowing to discriminate between F0/1-F2 vs F3-F4 with a reasonable diagnostic accuracy. However, in its present form, RTE cannot replace TE for assessing liver fibrosis in patients with iron overload.

**Personal information**

**References**


