Usefulness of Liver and Spleen Acoustic Radiation Force Impulse (ARFI) for the Evaluation of Cirrhotic Patients

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Abstract

Objective: To evaluate the correlation between ARFI and Child-Pugh classification. Secondary Aims: 1) To compare ARFI values (hepatic, splenic and spleno-hepatic index) from cirrhotic to normal population; 2) To correlate biochemical parameters of liver function and ARFI. Materials and Methods: 58 cirrhotic patients (referred to US for surveillance or to clarify any hepatic decompensation) were included in this prospective study, as well as 38 healthy subjects who underwent ultrasonography for other reasons than hepatic evaluation. All had ARFI liver and spleen evaluation on ACUSON S2000 ARFI equipment. The best cut-off liver and spleen values for the diagnosis of cirrhosis in comparison to the normal subjects were determined using SPSS® v20. Results: Mean liver ARFI values in controls and cirrhotic patients were respectively 1.18 ± 0.22 m/s and 2.93 ± 0.50 m/s. The ROC curve demonstrated an AUC 0.998 and the best cut-off was 1.89. Mean spleen ARFI values in controls and cirrhotic patients were respectively 2.60 ± 0.42 m/s and 3.03 ± 0.71. The ROC curve demonstrated an AUC 0.766 and the best cut-off was 2.73 m/s. The splenohepatic index showed a worse AUC than ARFI liver. A weak correlation was found between the ARFI liver and Child-Pugh. We found no statistically significant differences for spleen ARFI values and Child-Pugh. We found a statistically significant correlation between liver ARFI and bilirubin, ALKP, GGT, AST and AST/ALT ratio; and with spleen ARFI and ALKP and AST/ALT ratio. Conclusion: We showed that there is a tendency of higher levels of liver ARFI values for higher Child-Pugh classification suggesting a definite trend for higher values with more severe disease.

Keywords

Liver Cirrhosis, Child-Pugh Classification, Liver Stiffness, Spleen Stiffness, ARFI

Subject Areas: Gastroenterology & Hepatology, Radiology & Medical Imaging

1. Introduction

Cirrhosis is a commonly encountered chronic liver disease characterized by the deposition of fibrous tissue within the liver. This causes the liver to become stiffer than normal, with distortion of normal liver architecture [1].

A precise estimation of the degree of liver fibrosis is important for the estimation of prognosis, surveillance, and treatment decision in patients with chronic liver disease [2] [3].

Biopsy provides an extremely valuable contribution to the assessment of liver status in the case of chronic disease, offering information both on fibrosis and necro-inflammatory activity. However, not only the risk of complications, which has been reported with a frequency of 5% - 20% for minor complications and 0.3% - 0.5% for major complications [4] including exceptional cases of mortality, but also contraindications, such as coagulopathy, large volume ascitis, poor patient cooperation and lack of patient consent, tend to limit its use, especially for repeated procedures over time [5].

Acoustic Radiation Force Impulse (ARFI) imaging Virtual Touch™ tissue quantification uses a software of the US Scanners Siemens Acuson S2000 (Siemens Healthcare, Erlangen, Germany) that offers the possibility of performing a quantitative measurement of the elasticity of the hepatic parenchyma during conventional US evaluations [6]. ARFI imaging technology involves the mechanical excitation of tissue using short-duration acoustic pulses in a region of interest chosen by the examiner, producing shear waves that spread away from the region of interest, generating localized, micron-scale displacements in the tissue [7] [8]. By recording the shear wave front at several locations and correlating these measurements with the elapsed time, the shear wave velocity (m/s) can be quantified; generally, the stiffer is a region in the tissue; the shear wave velocity will be greater as it travels through this region [9]-[12].

The Child-Pugh Score stratifies the severity of cirrhosis (from class A—better prognosis to class C—worst prognosis), and predicts the probability of decompensation and death, using clinical and analytical data (ascitis, encephalopathy, total bilirubin, serum albumin and international normalized ratio or prothrombin time). Originally, the Child-Pugh Score was used to predict mortality during surgery; however it is now used to assess prognosis and to evaluate the need of liver transplantation.

The primary aim of this study was to evaluate the correlation between ARFI results and Child-Pugh classification. Secondary aims were 1) to compare ARFI values (hepatic, splenic and spleno-hepatic index) from cirrhotic to normal population and 2) to correlate biochemical parameters of liver function and ARFI.

2. Methods and Materials

2.1. Subjects

We obtained written informed consent from all the subjects in the study and the approval of the Hospital Ethics Committee.

In this prospective study 96 examinees were categorized into two groups: 1) 58 patients with previously diagnosed liver cirrhosis (biopsy-proven cirrhosis or clinically presumed cirrhosis—combination of clinical, analytical, endoscopic and imaging data); 2) 38 healthy individuals, with no hepatic disease, who undergone ultrasonography study for various reasons—abdominal pain, vomiting, renal colic and some volunteer members of hospital staff (the inclusion criteria were no history of liver disease, no diseases such as diabetes, renal insufficiency, and congestive heart failure, and laboratory tests done within 30 days. The exclusion criteria were age under 18 years old, use of chronic medication and refusal to participate in the study); Diagnosis of cirrhosis by non-invasive imaging, including ultrasound, magnetic resonance or a combination of the two, was established based on typical imaging findings, including liver surface nodularity, liver segment I hypertrophy, splenomegaly, hepatofugal portal venous flow and portosystemic vascular shunts. The exclusion criteria were: no laboratory tests done within 30 days and portal vein thrombosis.

The 58 cirrhotic patients were categorized using the Child-Pugh classification: Child A (34, 58.6%), Child B (13, 22.4%), Child C (10, 19%).
Etiology of liver cirrhosis included alcohol (n = 47, 81%), hepatitis B virus (HBV) infection (n = 4, 6.9%), hepatitis C virus (HCV) infection (n = 4, 6.9%) and primary biliary cirrhosis (PBC) (n = 3, 5.2%).

Demographic data, including age and gender were recorded for each individual patient at the time of ARFI imaging.

2.2. Acoustic Radiation Force Impulse Imaging Examination

All participants (n = 96) were examined using the Siemens ACUSON S2000 Ultrasound ARFI Virtual Touch™ Tissue Quantification System (Erlangen, Germany). During real-time B-mode imaging, a region-of-interest was placed in right hepatic lobe or the spleen on vessel- and tumor-free parenchyma, at a depth of 2 - 8 cm, during suspended respiration (Figure 1(a) and Figure 1(b)). In 6 hours fasting examinees, six measurements were performed from both referenced organs. The final ARFI value for each participant and for each organ was calculated as a mean of these 6 measurements. We used a 4 MHz convex probe that applies short duration acoustic pulses with a fixed transmitter frequency to generate localized displacements in tissue. The results are expressed in meters per second (m/s).

ARFI values in m/s obtained in the liver were multiplied by those of the spleen and divided by 100, producing the spleno-hepatic index.

2.3. Biochemical Parameters of Liver Function

The following biological parameters were recorded in each individual patient at the same month of ARFI examination: total bilirubin (Bilirubin) (µmol/L), alkaline phosphatase (ALKP) (U/L), γ-glutamyl transpeptidase (GGT) (U/L), aspartate aminotransferase (AST) (U/L) and alanine aminotransferase (ALT) (U/L). We calculated also the AST/ALT ratio.

2.4. Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). The verification of the assumptions of normality for the application of parametric tests on the variables of interest was done resorting to the Kolmogorov-Smirnov test with Lilliefors correction. Non-parametric tests were performed whenever normality could not be assumed. All tests were two-tailed and a p-value of less than 0.05 was deemed to be statistically significant.

For ARFI imaging, the mean of 6 successful measurements obtained in each patient for liver and for spleen was calculated and used for further analyses. Overall comparison of ARFI liver and spleen values between different Child-Pugh classification groups performed using the Mann-Whitney U-test. These results were expressed as mean ± standard deviation for the sake of comparability with published data. Laboratory tests were compared using the Mann-Whitney U-test and the results were expressed as median (range).

For the purpose of evaluating the performance of hepatic fibrosis tests in predicting cirrhosis, using clinical assessment as the gold standard, ROC analyses were performed. This included the calculation of the area under the ROC curves (AUC), as well as related sensitivity (Se) and specificity (Sp) values.
The Spearman correlation between Child-Pugh classification and ARFI measurements for liver and spleen was calculated only for cirrhotic patients. The chi-square test was used to compare categorical variables, expressed as percentages. Logistic regression analysis was used to confirm predictors of cirrhosis while removing the nuisance influence of sex and age.

3. Results

3.1. Patient Population Characteristics

In total 96 consecutive patients were evaluated by ARFI. ARFI was able to provide valid results in all of these patients. The etiology of liver disease (alcohol, HBV, HCV and PBC) and other patient characteristics are listed in Table 1.

58 cirrhotic patients (A) and 38 healthy individuals (B), were included in the study. Mean age for group A was 65.5 ± 11.8 and for group B was 56.7 ± 16.3. As expected, the patients with liver cirrhosis were older compared to the other group. In the group A 73.9% were male and 19.4% were female, while in group B 34.2% were male and 65.8% were female.

There was a significant difference for age and gender between the two groups; however, applying a logistic regression, we found that these differences do not interfere in the predictive capability of ARFI liver and spleen values.

We found statistically differences between the serum markers results of the two groups (healthy and cirrhotic), with exception for ALT (Table 2).

3.2. ARFI Liver Values

Six ARFI values were successfully obtained from the right lobe of the liver in all 96 patients.

There was a significant difference in the ARFI hepatic values between the two groups. The mean ARFI hepatic values of cirrhotic and healthy groups were 2.93 ± 0.50 m/s and 1.18 ± 0.22 m/s, respectively (p < 0.001) (Figure 2 and Table 3). The ROC curve analysis demonstrated an AUC of 0.998 (95% Confidence Interval 0.994 - 1.000). The best cut-off for establishing the presence of cirrhosis proved to be 1.89 m/s for liver values (98.3% Se and 100% Sp).

3.3. ARFI Spleen Values

Six ARFI values were successfully obtained from the spleen of all 96 patients, regardless of splenic size (whether normal or enlarged). The mean ARFI spleen values were 2.60 ± 0.42 m/s in normal subjects and 3.03 ± 0.71 m/s in cirrhotic patients.

Table 1. Patients characteristics, values are expressed as mean ± SD or n (%).

<table>
<thead>
<tr>
<th></th>
<th>Cirrhosis (A)</th>
<th>Healthy (B)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>58</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>65.5 ± 11.8</td>
<td>56.7 ± 16.3</td>
<td>p = 0.003*</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>M: 73.9%; F: 19.4%</td>
<td>M: 34.2%; F: 65.8%</td>
<td>p &lt; 0.001**</td>
</tr>
<tr>
<td>Etiology of liver disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>47 (81%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>4 (6.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>4 (6.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBC</td>
<td>3 (5.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child-Pugh classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>34 (58.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>13 (22.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>10 (19%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*t-Test; **Test chi-square (F—female; HBV—hepatitis B virus; HCV—hepatitis C virus; M—male; PBC—primary biliary cirrhosis).
Table 2. Laboratory tests results of healthy and cirrhotic patients. Results are express as median and min-max.

<table>
<thead>
<tr>
<th></th>
<th>Cirrhosis (A)</th>
<th>Healthy (B)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>20.5 (7.0 - 4539)</td>
<td>9.5 (3.8 - 34.4)</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td>ALKP (U/L)</td>
<td>109 (39 - 387)</td>
<td>65.5 (41 - 146)</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>92.5 (13 - 647)</td>
<td>22 (10 - 163)</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>41 (18 - 273)</td>
<td>27 (13 - 56)</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>35 (10 - 220)</td>
<td>30 (12 - 63)</td>
<td>p = 0.259*</td>
</tr>
<tr>
<td>AST/ALT</td>
<td>1.1 (0.32 - 4.88)</td>
<td>0.9 (0.39 - 1.44)</td>
<td>p = 0.001*</td>
</tr>
</tbody>
</table>

*Mann Whitney test (ALKP—alkaline phosphatase; ALT—alanine aminotransferase; AST—aspartate aminotransferase; Bilirubin—total bilirubin; GGT—γ-glutamyl transpeptidase).

Table 3. Liver and splenic stiffness values in healthy and cirrhotic patients. Values are expressed as mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Cirrhosis (A)</th>
<th>Healthy (B)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARFI Liver (m/s)</td>
<td>2.93 ± 0.50</td>
<td>1.18 ± 0.22</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td>ARFI Spleen (m/s)</td>
<td>3.03 ± 0.71</td>
<td>2.60 ± 0.42</td>
<td>p &lt; 0.001*</td>
</tr>
</tbody>
</table>

*Mann Whitney test.

in cirrhotic patients.

We observed a statistically significant difference between ARFI spleen values in subjects without hepatic pathology and those with liver cirrhosis (p < 0.001) (Figure 3 and Table 3). The ROC curve analysis demonstrated an AUC of 0.766 (95% Confidence Interval 0.671 - 0.862). The best cut-off for establishing the presence of cirrhosis proved to be 2.73 m/s for spleen values (78.9% Se and 65.8% Sp).

3.4. Spleno-Hepatic Index

We calculated the spleno-hepatic index (liver ARFI values multiplied by splenic values divided by 100) and concluded that the AUC for this index is subtle worse than AUC ARFI liver (data not shown).

3.5. Correlation between ARFI and Child-Pugh Classification

We studied 34 patients in Child-Pugh A group, 13 in Child-Pugh B and 10 in Child-Pugh C (Table 1). The mean ARFI liver values were 2.83 ± 0.54 m/s, 2.95 ± 0.40 and 3.24 ± 0.46, respectively. A weak Spearman correlation was found between the ARFI liver values and the Child-Pugh classification (ρ = 0.280; p = 0.039) (Figure 4).
On a closer inspection, there were no statistically significant correlation between spleen ARFI values and the Child-Pugh classification.

3.6. Correlation between ARFI and Biochemical Parameters of Liver Function

We examined the correlation between the ARFI liver and spleen values and results from laboratory tests (Table 4). We found that there is a statistically significant direct correlation between liver ARFI values and the laboratory results of bilirubin, ALKP, GGT, AST and AST/ALT ratio. In what concerns to spleen, we found a statistically significant direct correlation between the ARFI values and ALKP and AST/ALT ratio.

4. Discussion

Our results were consistent with those found on recent literature (Table 5). Yu et al. [1] and Piscaglia et al. [5] compared ARFI liver values in normal and cirrhotic patients. Yu deter-
Table 4. Correlation between ARFI results and laboratory tests results.

<table>
<thead>
<tr>
<th></th>
<th>ARFI Liver</th>
<th>ARFI Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bilirubin</strong></td>
<td>$\rho = 0.528$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td><strong>ALKP</strong></td>
<td>$\rho = 0.529$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td><strong>GGT</strong></td>
<td>$\rho = 0.479$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>$\rho = 0.395$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>$\rho = 0.084$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td><strong>AST/ALT</strong></td>
<td>$\rho = 0.367$</td>
<td>$p &lt; 0.001$</td>
</tr>
</tbody>
</table>

$\rho$—Spearman correlation coefficient; ALKP—alkaline phosphatase; ALT—alanine aminotransferase; AST—aspartate aminotransferase; Bilirubin—total bilirubin; GGT—$\gamma$-glutamyl transpeptidase.

Table 5. Comparison between our results and previous studies results.

<table>
<thead>
<tr>
<th></th>
<th>ARFI Liver (m/s)</th>
<th>ARFI Spleen (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Cirrhotic</td>
</tr>
<tr>
<td>A. Gallotti [13]</td>
<td>2010</td>
<td>1.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simona Bota [14]</td>
<td>2010</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. Piscaglia [5]</td>
<td>2011</td>
<td>1.13 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Grgurevic [16]</td>
<td>2011</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. Rifai [17]</td>
<td>2011</td>
<td>1.09 ± 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hojun Yu [1]</td>
<td>2012</td>
<td>1.16 ± 0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vermehren J. [20]</td>
<td>2012</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yoshitaka Takuma [15]</td>
<td>2013</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Our study</td>
<td>2013</td>
<td>1.18 ± 0.22</td>
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</tr>
</tbody>
</table>

AUC—a rea under curve; Se—sensitivity; Sp—specificity.

mine 1.13 m/s for healthy and 2.55 m/s for cirrhosis and Piscaglia determined 1.16 m/s for healthy and 2.35 m/s for cirrhotic patients. Our study found values slightly higher than those.

Gallotti et al. [13] performed the first measurements of the abdominal organs stiffness evaluating 35 healthy volunteers, the mean values being 1.59 m/s for liver and 2.44 m/s for spleen. In our study we obtained lower values for liver in patients without liver pathology (1.18 ± 0.22) and slightly higher spleen values (2.60 ± 0.42) for the same health group.

Bota et al. [14] used ARFI to determine ARFI spleen values in cirrhotic patients and its use in predicting the presence of portal hypertension signs (esophageal varices). The study included 57 cirrhotic patients and for a cut-off value of 2.51 m/s (AUROC 0.91, $p < 0.0001$, with 85.2% Se, 91.7% Sp, 95.8% PPV, 73.3% NPV and 87.1% accuracy). Thus confirming the hypothesis that an increase in splenic stiffness occurs with the develop-
ment of portal hypertension. In our study, we obtained slightly higher values for spleen values in cirrhotic pa-

tients (3.03 ± 0.71).

Takuma et al. [15], Grgurevic et al. [16] and Rifai et al. [17] evaluated ARFI of the liver and the spleen of
groups of healthy and cirrhotic patients. As our study, all obtained significant higher ARFI values for both or-
gans in cirrhotic patients (for ARFI liver values the results were respectively 1.21 m/s, 1.13 ± 0.13 m/s, 1.09 ±
0.18 m/s for healthy and 2.16 m/s, 2.27 ± 0.35 m/s, 2.86 ± 0.58 m/s for cirrhotic and for ARFI spleen values the
results were respectively 2.58 m/s, 2.77 ± 0.68 m/s, 2.55 ± 1.02 m/s for healthy and 3.36 m/s, 3.29 ± 0.65 m/s,
2.35 ± 0.56 for cirrhotic).

Spleno-hepatic index has been studied in same literature [18] [19] and had found same contradictory results.
Our results showed that, when we combine the 2 values of ARFI (hepatic and splenic) in that index, we do not
find a better AUC for the diagnosis of cirrhosis.

Vermehren et al. [20] performed a study with 166 cirrhotic patients and correlate, among other hypothesis,
the correlation between hepatic and splenic ARFI and the prediction of Child-Pugh B/C vs. A. They concluded that
there were significant overall differences between those clinical groups. In our study, we found that there is a
tendency of higher levels of liver ARFI values for higher the Child-Pugh classification (A to B and B to C),
suggesting a definite trend for higher values with more severe disease.

ARFI also produced results correlated with those obtained laboratory tests: there was a statistically significant
direct correlation between liver ARFI values and the laboratory results of bilirubin, ALKP, GGT, AST and
AST/ALT ratio and a statistically significant direct correlation between the spleen ARFI and ALKP and
AST/ALT ratio.

Furthermore, an advantage of using both techniques (B mode and ARFI) is that they are fast and convenient
to perform and use a conventional US machine. Moreover, ultrasound permits the evaluation of other features
such as portal vein diameter, splenomegaly and liver surface, which has been shown to be highly accurate in the
diagnosis of early cirrhosis and seems to provide complementary information to that of liver stiffness.

The limitations of our study include the following: unavoidable selection bias caused by clinical diagnosis of
cirrhosis without a liver biopsy; a single center study without external validation.

5. Conclusions

In the present study, we have found that ARFI showed excellent accuracy to determine the presence of cirrhosis.
We also found that liver ARFI was better than both spleen ARFI and spleno-hepatic index for the diagnosis of
cirrhosis.

We showed that there is a tendency of higher levels of liver ARFI values for higher Child-Pugh classification
suggesting a definite trend for higher values with more severe disease.

We found that there is a statistically significant direct correlation between liver ARFI values and the laboratory results of bilirubin, ALKP, GGT, AST and AST/ALT ratio. In what concerns spleen ARFI values, we found
a statistically significant direct correlation between the ARFI and ALKP and AST/ALT ratio.

According to the limitations mentioned, further studies are needed to overcome them, with a larger number of
cirrhotic patients that will provide us with a cut-off between the groups of this classification, as a good diagno-
sic tool for the prediction of worse prognosis that will help in clinical practice.

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http://dx.doi.org/10.1002/hep.21513


Abbreviations

ALKP—Alkaline Phosphatase
ALT—Alanine Aminotransferase
ARFI—Acoustic Radiation Force Impulse
AST—Aspartate Aminotransferase
AUC—Area under Curve
Bilirubin—Total Bilirubin
GGT—γ-Glutamyl Transpeptidase
HBV—Hepatitis B Virus
HCV—Hepatitis C Virus
PBC—Primary Biliary Cirrhosis
Se—Sensitivity
Sp—Specificity