Estimation of reference values for liver elasticity in biopsy-proven normal liver using Supersonic Shear Wave imaging: measurement reliability and effect of steatosis

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Purpose

In the era of antiviral and antifibrotic therapy, accurate staging of hepatic fibrosis over time allows the determination of disease progression, response to therapy and optimization of treatment (1-4 on page ). Thus, noninvasive diagnosis of hepatic fibrosis is one of the fields which have been evolving rapidly in recent years. The non-invasive methods for assessing the severity of fibrosis include serum markers, magnetic resonance (MR) elastography and ultrasound (US) elastography (5 on page ). The serum markers are not sensitive enough to identify patients with mild degrees of fibrosis and cannot differentiate stages of fibrosis reliably (6 on page ). Even though MR elastography has clear advantages to evaluate the entire liver parenchyma, it is a time-consuming and an expensive test (7 on page ). In contrast, US are the most common imaging technique to produce elastogram of the tissue. US elastography is painless, rapid, and have no associated complication and is, therefore, very easily accepted by patient (8 on page ). Moreover, it is easy to perform, inexpensive, portable, and reliable. Thus, US is more likely to become a standard system used in clinical practice for population-based screening (9 on page ).

There are four types of techniques in US elastography including transient elastography (10 on page ,11 on page ) and acoustic radiation force impulse (ARFI) elastography (8 on page ,12 on page ), real-time tissue elastography, and real-time shear-wave elastography (SWE). Among these methods, SWE is the most recently introduced, and thus the least validate technique. SWE is based on the combination of a radiation force induced in tissues by focused ultrasonic beams and a very high frame rate ultrasound images capable of catching resulting shear waves in real time. It is integrated into a conventional US system with conventional US probes. Thus, SWE can be performed during standard US examination of the liver, which is routinely performed in patients with chronic liver disease (13 on page ,14 on page ).

Previous reports (13-15 on page ) demonstrated the possibility of SWE to be applied for staging of hepatic fibrosis and suggested the normal range of SWE in the subjects without hepatic fibrosis (F0). However, these previous studies have been mainly focused on making an accurate staging of hepatic fibrosis rather than finding the normal range of liver elasticity. In addition, these reports had several limitations including limited number of subjects without hepatic fibrosis and lack of histologically proved data on the reference range. These limitations are generally attributed to the ethical unacceptability of performing hepatic biopsy in individuals with healthy livers. However, the recent increase in living-donor liver transplantations represents a medical-and thus ethically acceptable-reason to perform hepatic biopsy in apparently healthy subjects during hepatic donor evaluation (16 on page ). This provided an opportunity to obtain a histologically proved reference range for liver elasticity. In addition, to use SWE in clinical practice, it should be accurate not only for the grading of fibrosis, but also for monitoring disease progression and treatment efficacy, i.e. reproducible. Treatment by antiviral or anti-fibrotic
agents would require multiple follow-up examinations. Therefore, reproducibility is an important prerequisite for the widespread application of SWE in follow-up tool. Several compounding factors such as age, hepatic steatosis, and obesity, have been suggested that it could possibly affect the results of US elastography using transient elastography and ARFI techniques (17-21). To our knowledge, no investigators have evaluated the compounding possible factors of SWE.

The purpose of our study was to determine the reference values of liver elasticity using SWE in the biopsy-proven normal livers. In addition, we aimed to evaluate the compounding factors such as steatosis and BMI in measuring the liver elasticity.
Methods and Materials

Our institutional review board approved this study and informed consent was waived due to the retrospective nature of the study.

Study Subjects

Between September 2011 and February 2012, we searched the electronic database of our institution and found 329 patients who underwent SWE and 559 patients who underwent US-guided liver biopsy. Among those patients, 239 patients underwent SWE and subsequent US-guided liver biopsy on the same day. After the exclusion of subjects with liver fibrosis (n=22), or other abnormal hepatic pathology (n=12) except for simple hepatic steatosis, and unreliable measurement (n=9), the 196 subjects (130 men, 66 women; mean age, 29.2 years) were finally included in this study. Of these 196 subjects, 123 subjects were normal nonsteatotic liver and 73 subjects were simple hepatic steatosis. BMI was calculated from the available data in the subject's medical records.

Real-Time Shear Wave Elastography: Image Acquisition and Analysis

Real time SWE was performed by using ShearWave™ Elastography on Aixplorer® (Supersonic Imagine, Aix Provence, France) and the SC6-1 probe. All measurements were performed by one of five radiologists with 5 ~ 10 years of experience of abdominal US. In all patients, the right lobe of the liver was examined through the intercostal view with the patient lying in a supine or semi-decubitus position with the right arm in maximal abduction. We set the SWE™ default box size to 2.5 cm X 2.0 cm and the Q-box™ size to 1 cm. The Q-box™ was located in an area of relatively uniform elasticity (as judged by the SWE™ image and standard deviation value less than 30% of the mean elasticity) and uniform liver parenchyma (as judged by the B-mode image in the dual-display format) where the liver tissue was at least 6cm thick and 2cm of depth below the liver capsule and vessel-free liver parenchyma (8 on page ). Three consecutive liver elasticity measurements were obtained in each patient at the similar scanning view, and perform one Q-box™ measurement per image. Each measurement was performed on single breath-hold duration. A median value of three measurements expressed in kPa was used as a representative measurement of the liver elasticity in a subject.
Fig. 1: Real-time shear wave elastography was performed by using ShearWave™ Elastography

References: RADIOLOGY, ASAN MEDICAL CENTER, ASAN MEDICAL CENTER - Seoul/KR
Fig. 2: SWE™ default box and Q-box™ ROI was chosen in an area where the liver tissue was at least 6cm thick and 2cm deep below the liver capsule and vessel-free liver parenchyma.

**References:** RADIOLOGY, ASAN MEDICAL CENTER, ASAN MEDICAL CENTER - Seoul/KR

**Reference Standard: US-guided Hepatic Needle Biopsy and Histologic Analysis**

After undergoing SWE, each subject on the same day underwent US-guided liver biopsy, which was performed by one of five radiologists with 5 ~ 10 years of experience of abdominal US. With 18-gauge needles (Stericut 18G Coaxial; TSK Laboratory, Tochigi,
Japan), biopsy specimens were obtained twice (at two sites) in the right hepatic lobe of each patient. Each biopsy specimen was approximately 1.5 cm in length.

The histologic results were reviewed by an experienced hepatic pathologist more than 20 years who was blinded to the US findings. The degree of steatosis was assessed on the basis of the percentage of hepatocytes containing macrovesicular and/or microvesicular fat droplets, as follows: Grade 0 indicated no steatosis; grade 1, fewer than 33% of hepatocytes containing macrovesicular and/or microvesicular fat droplets; grade 2, 33% ~ 66% of hepatocytes containing macrovesicular and/or microvesicular fat droplets; and grade 3, more than 66% of hepatocytes containing macrovesicular and/or microvesicular fat droplets (22 on page ).
**Fig. 3:** Biopsy specimens were obtained twice in the right hepatic lobe of each patient with 18-gauge needles.

**References:** RADIOLOGY, ASAN MEDICAL CENTER, ASAN MEDICAL CENTER - Seoul/KR

**Statistical Analyses**

Repeated measures ANOVA were performed to evaluate the difference between three measurements of the liver elasticity in donor group and fibrosis group, respectively. To determine the reference values for liver elasticity, we adopted the method specified in Clinical and Laboratory Standards Institute (CLSI) guideline C28-A3 for determining reference intervals for quantitative clinical laboratory tests(23 on page, 24 on page). The reference population, representing the group of all patients included to establish the reference range, was defined as the group of the subjects with normal hepatic histology except for simple hepatic steatosis at histologic analysis. A significant difference in mean liver elasticity between the reference population and the subjects with biopsy-proven liver fibrosis was established by using the t test. Because use of a parametric method for establishing a reference range when the values in the reference population follow a normal distribution is recommended in the CLSI guideline, the normality of the mean liver elasticity in the reference population was checked by using the Shapiro-Wilks test and the Q-Q plot. The lower and upper limits of the reference range were estimated to be the 2.5 and 97.5 percentiles, respectively, of the distribution of mean liver elasticity values in the reference population, covering the middle 95% of subjects in the reference population. Therefore, if the reference population values are conformed to a normal distribution, the lower and upper limits of the reference range could be calculated as follows: mean liver elasticity - 1.96 X SD and mean liver elasticity + 1.96 X SD, respectively, where SD is the standard deviation of mean liver elasticity values.

To analyze the effects of compounding factors including subject age, steatosis, and BMI on the measurement of median liver elasticity, univariate and multifactorial linear regression analysis was performed. The association between parametric and continuous variables was analyzed using the Pearson correlation test. Continuous variables were compared using the t-test. Measurement reliability of the liver elasticity was evaluated by using intra-class correlation coefficient (ICC). An ICC value greater than 0.75 indicates good reproducibility (25 on page, 26 on page). To evaluate the effects of the candidate compounding factors on the reliability, we modeled the data as two levels (measurement level and subject level). Multi-level regression models with each candidate factor or combination of factors were applied and ICCs with candidate factors were estimated and compared. SAS 9.2 was used to perform all statistical analyses. P < .05 was considered to indicate statistical significance. P < .05 was considered to indicate statistical significance.
Results

Study Population

Of these 196 subjects, we found that 123 (62.8%) subjects did not have hepatic steatosis and 65 (33.2%) subjects had grade 1 steatosis. The remaining 8 subjects had grade 2 (6 subjects) and grade 3 (2 subjects) steatosis. The mean body weight, height, and body index of these 196 subjects were 167.8 kg, 64.98 cm, and 22.78 kg/m², respectively.

Reference Range for Mean Liver Elasticity

The mean liver elasticity values in the subjects with the biopsy-proven normal (nonsteatotic and steatotic) livers were 4.37 kPa. There was a significant difference in mean liver elasticity values between subjects with biopsy-proven normal livers (4.37 kPa±0.91) and biopsy-proven liver fibrosis (9.60kPa±3.17) (P< .0001). According to the Clinical and Laboratory Standards Institute guideline C28-A3, the estimated reference range of the liver elasticity was 2.58 kPa ~ 6.16 kPa. There was no significant difference between three measurement values in biopsy-proven normal livers (P = .29) (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Mean Values for Each Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measure 1</td>
</tr>
<tr>
<td>Donor</td>
</tr>
<tr>
<td>Mean±SD (kPa)</td>
</tr>
<tr>
<td>(n=196)</td>
</tr>
<tr>
<td>95% CI</td>
</tr>
<tr>
<td>Fibrosis</td>
</tr>
<tr>
<td>Mean±SD (kPa)</td>
</tr>
<tr>
<td>(n=22)</td>
</tr>
<tr>
<td>95% CI</td>
</tr>
</tbody>
</table>

Effects of Subject Age, Steatosis, and BMI on liver elasticity

Table 2 showed univariate and multivariate analyses for correlation between clinical factors and median liver liver elasticity of three measures. Multifactorial linear regression analysis revealed no significant correlation between the mean elasticity values and subject age, steatosis. In contrast, BMI was independently correlated with liver elasticity.
### TABLE 2: Univariate and Multivariate Analyses for correlation between Clinical Factors and Liver elasticity

<table>
<thead>
<tr>
<th>Clinical factors</th>
<th>Tissue elasticity</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation coefficient</td>
<td>P-values</td>
<td>Estimated B</td>
</tr>
<tr>
<td>Age</td>
<td>-0.094a</td>
<td>0.192</td>
<td>-0.011±0.007</td>
</tr>
<tr>
<td>Steatosis</td>
<td>-0.028</td>
<td>0.694</td>
<td>-0.221±0.156</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>0.149a</td>
<td>0.037</td>
<td>0.072±0.025</td>
</tr>
</tbody>
</table>

A = not available (values are not presented for factors that were not included in the multivariate analysis)

a Correlation coefficients were obtained by the Pearson correlation test

b Correlation coefficients were obtained by the Spearman non-parametric correlation test

c Multivariate analysis was performed using the multifactorial linear regression analysis

d Multivariate analysis was performed using the multifactorial ordinal logistic regression analysis

### Effects of Subject Age, Steatosis, and BMI on Measurement reliability

Total population showed a high ICC value (0.92) which indicated high measurement reliability (Table 3). Univariate and multivariate analyses using multi-level linear regression model for correlation between clinical factors and ICC were demonstrated in Table 4. On multi-level linear regression model, ICC based on the age as a covariance was 0.9235, ICC based on the steatosis was 0.9242, and ICC based on the BMI was 0.9233. ICC based on all three covariances was 0.9219. ICC values ranged from 0.9219 to 0.9242 and the percentage differences between ICC values was very low (0.0007577). Therefore, ICC values did not significantly differ according to the compounding factors either on comparison of each ICC value on multi-level linear regression model.

Table 3. Measurement Reliability & Effects of Steatosis
<table>
<thead>
<tr>
<th></th>
<th>Total population</th>
<th>Nonsteatotic group</th>
<th>Steatotic group</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>196</td>
<td>123</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.37±0.91</td>
<td>4.39±0.90</td>
<td>4.33±0.94</td>
<td>0.69</td>
</tr>
<tr>
<td>95% CI</td>
<td>(4.23, 4.50)</td>
<td>(4.23, 4.55)</td>
<td>(4.12, 4.55)</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4: Univariate and Multivariate Analyses for correlation between Clinical Factors and ICC**

<table>
<thead>
<tr>
<th>Clinical factors</th>
<th>Tissue elasticity</th>
<th></th>
<th></th>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Univariate analysis</td>
<td></td>
<td>Multivariate analysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estimated B</td>
<td>P-values</td>
<td>ICC</td>
<td>Estimated B</td>
</tr>
<tr>
<td>Age</td>
<td>-0.0104±0.0070</td>
<td>0.070388</td>
<td>0.9235</td>
<td>-0.0115±0.0071</td>
</tr>
<tr>
<td>Steatosis</td>
<td>0.0827±0.1301</td>
<td>0.5121</td>
<td>0.9242</td>
<td>0.2143±0.1511</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>0.0351±0.0212</td>
<td>0.9295</td>
<td>0.9233</td>
<td>0.0600±0.0245</td>
</tr>
</tbody>
</table>
Conclusion

In our study, the reference range of the liver elasticity values for biopsy-proven normal livers in 196 patients using SWE was 2.58kPa ~ 6.16 kPa, and the mean liver elasticity value was 4.37kPa. BMI had a significant effect on the value of liver elasticity, while subject age and hepatic steatosis had no effects on the liver elasticity. Liver elasticity measured by SWE in normal liver showed high measurement reliability (ICC= 0.92) regardless of the presence of possible compounding factors such as age, BMI or steatosis.

To the best of our knowledge, this has been no study at determining the reference range of biopsy-proven normal liver elasticity value using SWE. Our study depicted the reference range of normal liver elasticity. Our histologically proved criterion was fortuitously almost similar to previously proposed values (13-15 on page ), which, however, lacked histologic correlation. A previous report(14 on page ) demonstrated that liver elasticity values on 15 healthy volunteers using SWE ranging from 4 to 7.5 kPa. Also, several reports (13 on page ,15 on page ) assessed that liver fibrosis staging using SWE in chronic hepatic C patient, mean liver elasticity was 4.78 kPa and 6.2 kPa for the patient with F0-1, respectively. Hepatic elasticity measured with SWE in our study can be used as the reference values for diagnosing hepatic fibrosis. A previous study(15 on page ) showed that SWE is more accurate than transient elastography assessing early liver fibrosis (F#2). With the reference values established in our study, SWE is expected to have a role in diagnosis of early hepatic fibrosis.

Measurement reliability is also an important issue when biomarkers are used as a follow-up tool. Since treatment by antiviral or anti-fibrotic agents would require multiple follow-up examination of US elastography, high measurement reliability is essential for US elastography. In this study, liver elasticity measured by SWE in normal liver showed high measurement reliability (ICC: 0.92), which was correlated well with a previous study (27 on page ) (ICC: 0.93 ~ 0.93). Possible reasons for this high reliability of SWE include the facts that the Q-box™ judged by the SWE™ image and standard deviation value less than 30% of the mean elasticity and uniform liver parenchyma judged by the B-mode image in the dual-display format.

Known compounding factors to measure liver stiffness were suggested using TE and ARFI. Age, BMI, steatosis and ascites have been suggested as compounding factors (17-21 on page ). Considering the differences of underlying mechanism to measure liver stiffness according to the methods of US elastagrophy, there can be some differences in the compounding factors according the methods. Therefore, in our study, we investigated the effect of the compounding factors using TE and ARFI, since there was no study which analyzed the effect of compounding factors using SWE. We found that BMI to be independently correlated with liver elasticity, while that the liver elasticity value could be generalized across subject age and steatosis. Although the differences in
the interobserver agreement due to increased BMI, our data suggest that SWE should be used cautiously as a surrogate of liver biopsy for assessing liver fibrosis in obese patients. A previous study suggested that the interaction of fat with low-frequency vibrations of TE may affect the signal to noise ratio—that is, the relevant parameter for assessing liver fibrosis in patients with fat problems (18 on page ).

Our study had limitations. First, although we evaluated the patients who had normal livers or simple steatosis, the reliability of the liver elasticity could differ according to the stage of fibrosis. Second, our study did not include the intraobserver variability study. Third, our study included a cohort of Korean patients, mainly young and relatively low-weight adult subjects. However, we found that age was not a confounding factor, and ethnic difference probably does not influence liver elasticity values. Last, liver biopsy may involve some degree of sampling error, as it enables one to sample only a tiny portion of the liver. However, hepatic needle biopsy is probably the most practical gold-standard procedure currently available (16 on page ).

In conclusion, hepatic elasticity values measured with real-time SWE in histologically proven normal liver ranged from 2.58 to 6.16 kPa with high measurement reliability. There was a no significant correlation between the mean elasticity values and subject age, steatosis, however, BMI was independently correlated with liver elasticity.
References


