Radiology-pathology correlation in staging of liver fibrosis using superb microvascular imaging

P PURPOSE
Progression of liver fibrosis to end-stage disease can potentially be prevented with antiviral treatment. Thus, diagnosis of fibrosis is important in determining treatment protocols. This study aims first, to determine the sensitivity of a novel Doppler method, superb microvascular imaging (SMI), in detecting small vascular structures of the liver compared with other Doppler methods; and second, to choose the best method among these Doppler applications to determine the morphologic changes that occur due to chronic fibrosis. By doing so, the study would be able to provide an ultrasound grading that might differentiate and predict mild and severe liver fibrosis, thus giving rise to a possible alternative to biopsy.

M METHODS
A total of 43 patients diagnosed with chronic hepatitis and scheduled for liver biopsy were included. Color Doppler, power Doppler, advanced dynamic flow (ADF) Doppler, color SMI (cSMI) and monochrome SMI (mSMI) Doppler were performed in subcapsular areas of right anterior lobe. Depth from the capsule of the most peripherally located detectable vessel was measured for each Doppler subgroup. Appearance of the vascular tree was categorized into four groups and correlated with pathology results. ROC curve analysis was used to determine if this Doppler classification was statistically significant in differentiating mild and severe forms of fibrosis. Finally, multiple regression analysis was used to determine which Doppler parameter can significantly predict severity.

R RESULTS
mSMI and cSMI were found to be superior to other Doppler techniques in detecting the most superficially located vessels of the liver, 4.4 mm and 3.3 mm deep from the capsule, respectively (P < 0.001). Among the changes identified in the vascular tree, small vessel blunting was the most prevalent finding in predicting the presence of severe fibrosis (multiple regression test, t=5.969, P < 0.0001). ROC analysis identified that the presence of at least two pathologic findings in the vascular tree was highly predictive of severe fibrosis (AUC=0.881, sensitivity 86.67%, specificity 89.29%, positive and negative predictive values 8.09 and 0.15, respectively).

C CONCLUSION
Our study proves that SMI is superior to other Doppler techniques in detecting the smallest vessels visible to ultrasound. Using this method, it is possible to determine the vascular changes in terms of blunting and tortuosity and thus predict the severity of fibrosis. This method might be a practical alternative to biopsy.

Progression of liver fibrosis to end-stage disease caused by hepatitis can potentially be prevented with antiviral treatment. Thus, assessing fibrosis is important in determining treatment protocols (1, 2). Chronic fibrotic disease causes distortion and compression of portal vein branches by connective tissue, and an increased number of tortuous arterioles surround cirrhotic nodules (3). Formerly, angiographic studies were performed to stage fibrosis and investigate intrahepatic vascular changes of chronic liver disease (4). Biopsy remains to be the gold standard for determining fibrosis stage (5). However, angiography and biopsy are both costly and demanding procedures with a certain mortality risk. Thus, several noninvasive imaging methods have been developed worldwide.

This study has two aims: first, to determine the sensitivity of a novel Doppler method, superb microvascular imaging (SMI), in comparison with other Doppler methods, in detecting small vascular structures of the liver; and second, to choose the best method among these Doppler applications to determine the morphologic changes, which occur due to chronic fibrosis, in peripheral vessels of the liver. By doing so, the study would be able to provide an ultrasound grading that might differentiate and predict mild and severe liver fibrosis; thus, giving rise to a possible alternative to biopsy.

Methods

Prospective study design and protocol were approved by the ethical committee of our institution (HNEAH-KAEK 2016/KK/92) and written informed consent was obtained. All patients were notified of the detailed research profile. A total of 43 patients diagnosed with chronic hepatitis and scheduled for liver biopsy were included in the study. Patients under 18 years of age, pregnant or lactating women, obese individuals with BMI >30 kg/m² and those with drug or alcohol addiction were excluded.

Ultrasound examination

Doppler ultrasound was performed on all patients before the biopsy on the same day. All ultrasound examinations were performed with an Apio 500 ultrasound system (Canon Medical Systems Corporation). Patients were fasting for at least 4 hours before the exam and the biopsy. They were placed in the left lateral decubitus position and the exam was performed through the intercostal area. To provide a matching image to pathology, all ultrasound measurements were made within the most peripheral part of the liver tissue, 2 cm underneath the liver capsule of the right anterior sector of the liver. Left liver lobe was not preferred since it was more difficult to obtain standard images and in some patients, it was not even possible to visualize the lobe itself.

Color Doppler, advanced dynamic flow (ADF) Doppler, power Doppler, color SMI (cSMI) and monochrome SMI (mSMI) Doppler samples of the portal vascular tree were obtained with a 3.5 MHz convex probe and recorded (Fig. 1). During the examination, care was taken to keep pulse repetition frequency values and sample area Doppler parameters at the same setting in all cases. All ultrasound exams were performed by the same radiologist and images were stored in the PACS system (Extreme PACS v.3.4, Turkey) to be evaluated and graded together by this radiologist and the interventional radiologist performing the biopsies (both with more than 15 years of experience in abdominal sonography) in consensus, blinded to pathology findings.

First, quantitative images were obtained with each Doppler technique. The most peripheral point, in other words the depth where a vessel was detected by each technique, was noted in terms of the depth of the vessel to the capsule in millimeters. Depth and standard deviation were calculated and compared, identifying the best method that is able to detect the most peripherally located, therefore the smallest vessel.

Table 1. Vascular tree grading and scoring

<table>
<thead>
<tr>
<th>Vascular tree grading</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thinning of distal vessels</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Tortuosity in distal vessels</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Blunting of small vessels</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Blunting of large vessels</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>4</td>
</tr>
</tbody>
</table>

Modified after Kuroda et al. (6) and Sato et al. (7).

Biopsy technique

Core biopsy was performed by a single interventional radiologist under ultrasound guidance using the intercostal approach from the right liver lobe with the patient in the left lateral decubitus position. The same area as Doppler examination was chosen for biopsy. Three samples of 2 cm length were obtained with an 18-gauge automatic core biopsy needle. Thus, the tissue sample corresponded to the same area in the right anterior sector of the liver that was screened by Doppler techniques.

Pathologic examination

Specimens were fixed in formalin and stained with hematoxylin-eosin and Masson trichrome. Histopathologic evaluation was performed by a single experienced pathologist who was blinded to ultrasound findings. The histologic grade and stage were evaluated semi-quantitatively according to the METAVIR scoring system (8). Severity of liver fibrosis was expressed on a 0–4 scale as follows: F0, no fibrosis; F1, portal fibrosis with no septa; F2, portal fibrosis with few septa; F3, bridging fibrosis with many septa; and F4, cirrhosis (9, 10). The overall results of liver histologic ex-
aminations were considered the reference standard for ascertaining the presence and degree of fibrosis. The number of patients in each histopathologic group were small, therefore they were grouped into two major headings; mild and severe groups. Thus, META-VIR F1 and F2 were considered in the mild group, while F3 and F4 were designated as the severe group (7, 11).

Statistical analysis
Statistical analyses were performed with MedCalc Statistical Software (version 11.2.1.0).

For comparing the sensitivity of various Doppler methods in identifying the depth
of small vessels, ANOVA test was applied. Levene’s test was used to test the homogeneity of variance prior to ANOVA. Student-Newman-Keuls test was applied as post hoc test. ROC curve was used to identify the cutoff point in terms of the Doppler score which was able to make such a differentiation. With standard error and 95% confidence interval (CI), the area under the ROC curve analysis determined whether the Doppler classification was statistically significant in differentiating mild and severe forms of fibrosis. Finally, multiple regression analysis determined which of the Doppler parameters was the most significant parameter in predicting presence of a severe stage of fibrosis. Statistical significance level was set at 0.05.

Results

Patient characteristics are summarized in Table 2. There were 43 patients, 25 males and 18 females. Mean age was 50±15 years. Etiology of chronic hepatitis was hepatitis C virus (HCV) in 18 patients (42%), hepatitis B virus (HBV) in 18 patients (42%), autoimmune hepatitis in 3 patients (7%), steatohepatitis, primary biliary cirrhosis (PBC), celiac disease and sarcoidosis in 1 patient each (9%).

Pathology results included normal hepatic parenchyma in 4 patients (F0, 9%), F1 in 14 patients (33%) and F2 in 10 patients (23%), comprising the mild fibrosis group together with the normal parenchyma group, 28 patients in total (65%). Severe forms of fibrosis were observed in a total of 15 patients (35%) among whom 3 patients (7%) had F3 and 12 patients (28%) had F4 fibrosis levels (Table 2).

Among the Doppler parameters evaluated (color Doppler, ADF Doppler, power Doppler, cSMI, and mSMI), mSMI technique was significantly better in identifying small vessels within the most peripheral 20 mm under the liver capsule. Area under the ROC curve (AUC) determined that ultrasound classification of vascular changes were able to distinguish between mild and severe fibrosis. ROC curve analysis also indicated that at least two pathologic parameters are necessary to determine a severe stage of disease (AUC=0.881, 95% CI 0.75–0.96, specificity 89.3%, sensitivity 86.7%, positive and negative predictive values 8.09 and 0.15, respectively) (Fig. 8).

Multiple regression equation demonstrated that among the studied Doppler parameters (such as thinning, tortuosity, small and large vessel blunting), small vessel blunting was the most significant parameter that contributes to the differentiation of severe fibrosis (t=5.969, P<0.0001) (Table 4).

Discussion

Staging liver fibrosis is crucial to determine whether antiviral treatment would be beneficial and to evaluate the response to therapy. The gold standard for staging liver fibrosis is histopathology, but the invasive nature, the need for an experienced interventional radiologist and risk of complications like bleeding may render biopsy not preferable in certain patient groups.

In chronic fibrotic disease, portal vein branches are distorted and compressed by connective tissue and an increased number of tortuous arterioles surround cirrhotic vessels.
Liver fibrosis staging using superb microvascular imaging

Table 3. Doppler methods and their sensitivity in vessel detection, followed by the statistical data from Levene’s test and ANOVA test

<table>
<thead>
<tr>
<th>Doppler method</th>
<th>Distance to liver capsule (mm) (mean±SD)</th>
<th>Post hoc comparison</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADF</td>
<td>8.10±2.12</td>
<td>Color Doppler</td>
<td>0.282</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Power Doppler</td>
<td>0.856</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cSMI</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mSMI</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Color Doppler</td>
<td>8.82±2.85</td>
<td>ADF</td>
<td>0.282</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Power Doppler</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cSMI</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mSMI</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Power Doppler</td>
<td>8.35±2.79</td>
<td>ADF</td>
<td>0.856</td>
</tr>
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<td></td>
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<td>Color Doppler</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>cSMI</td>
<td>0.361</td>
</tr>
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<td></td>
<td></td>
<td>mSMI</td>
<td>0.010</td>
</tr>
<tr>
<td>cSMI</td>
<td>4.36±1.55</td>
<td>ADF</td>
<td>0.041</td>
</tr>
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<td>Power Doppler</td>
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<td>cSMI</td>
<td>0.361</td>
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<tr>
<td></td>
<td></td>
<td>mSMI</td>
<td>0.589</td>
</tr>
<tr>
<td>mSMI</td>
<td>3.30±1.07</td>
<td>ADF</td>
<td>&lt;0.001</td>
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<td></td>
<td>Power Doppler</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>cSMI</td>
<td>0.010</td>
</tr>
</tbody>
</table>

| F ratio | 58.693 |
| Significance | P < 0.001 |

ANOVA, analysis of variance; SD, standard deviation; ADF, advanced dynamic flow; cSMI, color superb microvascular imaging; mSMI, monochrome superb microvascular imaging.

nodules (3). Vascularity is decreased and the intrahepatic vessels typically show coil- ing and corkscrewing (4). Invasive and noninvasive diagnostic methods can be used to evaluate these morphologic changes.

Angiographic analysis of liver vasculature has historically laid the basis for describing the secondary effects of parenchymal distortion on liver vessels. However, angiographic examination requires patient compliance, appropriate technical conditions and an experienced team, and is only able to demonstrate large vessels that would be affected in later stages of the disease.

Noninvasive methods of fibrosis staging include various ultrasound techniques like contrast Doppler ultrasound, fibroscan, ultrasound elastography and others like magnetic resonance elastography (MRE). Contrast Doppler ultrasound may be performed to demonstrate small vascular structures but requires the use of contrast media which may not be readily available throughout the world. Another method for evaluating liver vascularity is the microflow imaging (MFI) obtained by contrast-enhanced ultrasonography (CEUS) technique, which is costly and is also not used worldwide.

Fibroscan is a noninvasive method of examining the degree of hepatic fibrosis; however, the diagnostic accuracy is affected by its nonradiologic nature, morbid obesity, ascites and small intercostal spaces (12, 13).

Both ultrasound elastography and MRE techniques report very good to excellent diagnostic performance for diagnosis of severe fibrosis (14). However, technical and biologic confounding factors may affect the feasibility or fibrosis classification accuracy of these techniques. They are influenced by cardiac dysfunction, hepatic congestion, acute inflammation, and cholestasis (15). Future standardization of elastography techniques is necessary to improve reproducibility of elastography measurements, facilitate comparison of diagnostic thresholds, and improve patient care (14).

SMI is an innovative ultrasound Doppler technique developed by Canon Medical Systems, which analyzes the clutter motion from stationary tissues, employs a new adaptive algorithm to identify and remove this motion and thus reveals true blood flow. It also features high frame rates (>50 FPS) and high resolution. All of these features enable SMI to demonstrate very small vessels, which were previously invisible to imaging (7, 16–18).

In this study, when testing for the sensitivity of several Doppler methods like color Doppler, ADF Doppler, power Doppler, and SMI in detecting small vessels of the liver, a statistically significant difference was found in favor of SMI. The smallest vessels observed on color SMI and monochrome SMI were only 4.4 and 3.3 mm deep from the capsule, being superior to the other Doppler techniques in review for evaluating the most peripheral microvascular structures.

In a study with chronic liver disease patients, it is reported that SMI displayed a detailed map of the vascular architecture and there were significant differences in the distribution of the SMI patterns regarding fibrosis stages (7). This study also shows that increasing stages of fibrosis demonstrate various signs. SMI is able to distinguish the changes that occur in the cirrhotic liver by their impressions on surrounding vascular structures. Changes detected in cirrhotic livers include thinning and increased tortuosity of peripheral vessels as well as blunting at various levels of the vascular tree. Since it can demonstrate the vascular tree structure better than other Doppler techniques, SMI, especially mSMI can distinguish mild and severe forms of fibrosis by differentiating blunting of small vessels. As demonstrated...
in this study, blunting of small branches was more prevalent in severe fibrosis. Changes detected closer to the capsule were also indicative of mild disease, while those that were present in more central parts tended to be severe. Moreover, the presence of at least two pathologic findings on SMI were found to be highly suggestive of severe fibrosis stages.

There are some limitations to our study. First, the study involves a small number of patients. Due to its ultrasonographic nature, obese patients were not good candidates for the examination. Since our study excluded those with a BMI >30 kg/m² and did not include any patients with ascites, SMI findings in these individuals were not assessed.

In conclusion, we believe that with the addition of SMI into the armamentarium, ultrasonography will regain its well-deserved place in the study of chronic liver disease, compared with the other highly sophisticated and expensive imaging methods. Follow-up of patients with liver fibrosis who receive antiviral treatment will be more convenient and unnecessary liver biopsies and use of contrast media will decrease.

Conflict of interest disclosure
The authors declared no conflicts of interest.

References
8. Garcia-Tsao G, Friedman S, Iredale J, Pinzani M. Now there are many (stages) where before there was one: In search of a pathophysiological classification of cirrhosis. Hepatology 2010; 51:1445–1449. [CrossRef]

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