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ABDOMINAL IMAGING

ORIGINAL ARTICLE

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

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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.

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.

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).

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.

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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.

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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/m^2 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 Aplio 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.

Then, using this best identifier method (which was mSMI), the vascular tree was qualitatively evaluated, searching for findings suggestive of parenchymal fibrosis reflected by tortuosity and blunting of various sizes of vessels. Findings were then graded and scored according to Table 1, where the portal vascular tree at the examination area was categorized into 4 main classes. According to this, normal vascular tree structure was defined as grade 0, thinning in the distal branches of the vascular tree as grade 1, marked tortuosity in distal branches as grade 2, blunting of distal small branches as grade 3 and in addition to the findings of grade 3, blunting of larger branches as grade 4. This grading was modified from Kuroda et al. (6) and Sato et al. (7) (Figs. 2–6).

Table 1. Vascular tree grading and scoring

	Grade 1	Grade 2	Grade 3	Grade 4	Score
Thinning of distal vessels	+				1
Tortuosity in distal vessels	+	+			2
Blunting of small vessels	+	+	+		3
Blunting of large vessels	+	+	+	+	4

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-

Main points

- Assessing liver fibrosis is important in determining treatment protocols.
- In chronic fibrotic disease, portal vein branches are distorted and compressed by connective tissue and an increased number of tortuous arterioles surrounding cirrhotic nodules. Vascularity is decreased and intrahepatic vessels typically show coiling and corkscrewing.
- SMI is superior to other noninvasive Doppler techniques in evaluating microvascular structures closest to the capsule, such that morphologic changes can be detected at an earlier stage.
- Blunting of small vessels was found to be the distinguishing feature of severe fibrosis as it was more prevalent in these patients. Changes detected only in peripheral areas of the liver closer to the capsule were also indicative of mild disease, while those that were present in more central parts tended to be severe.

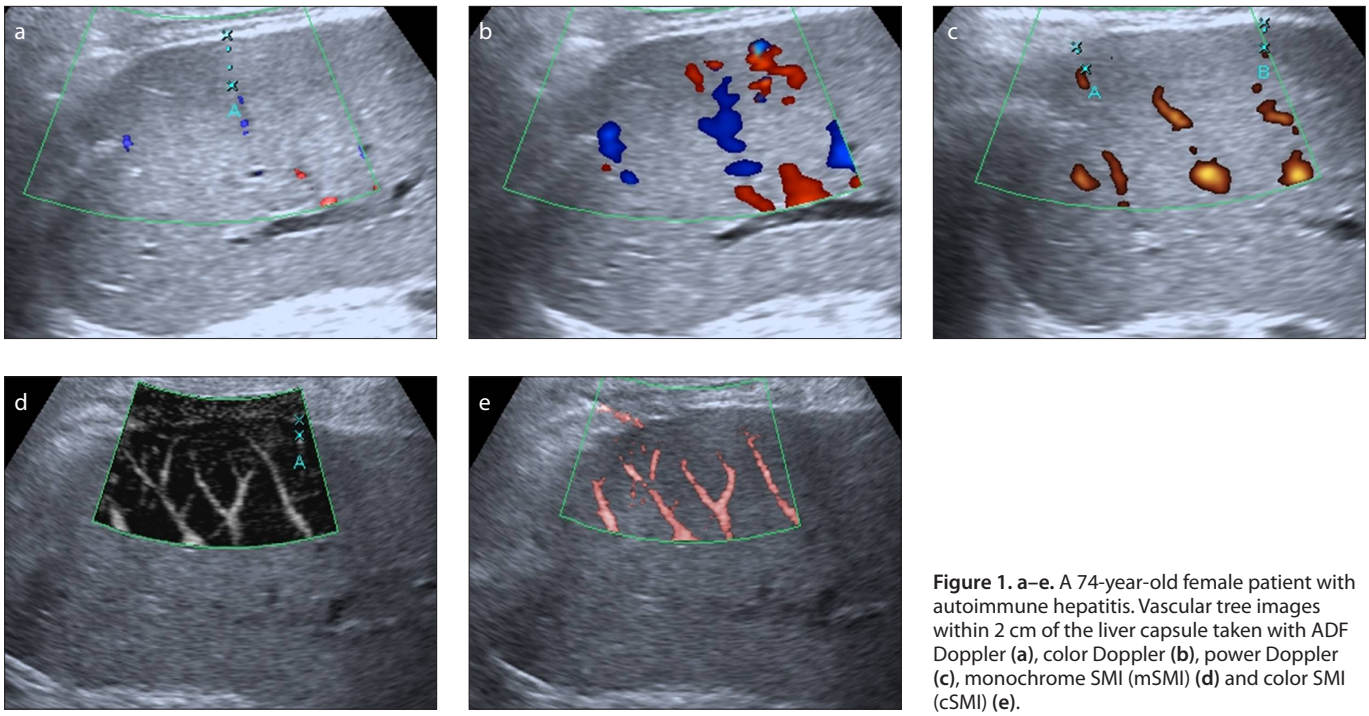

Figure 1. a–e. A 74-year-old female patient with autoimmune hepatitis. Vascular tree images within 2 cm of the liver capsule taken with ADF Doppler **(a)**, color Doppler **(b)**, power Doppler **(c)**, monochrome SMI (mSMI) **(d)** and color SMI (cSMI) **(e)**.

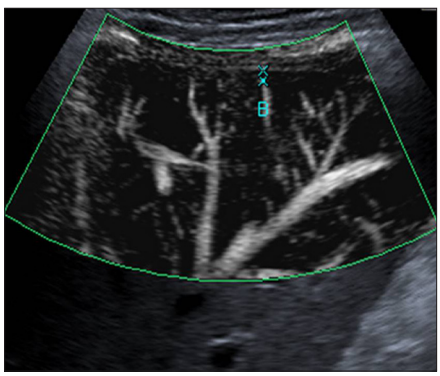

Figure 2. A 41-year-old male patient with chronic hepatitis C. Monochrome SMI of the vascular tree within 2 cm of the liver capsule. There is no evidence of thinning, tortuosity or blunting in branches. Doppler classification grade 0, METAVIR score 1.

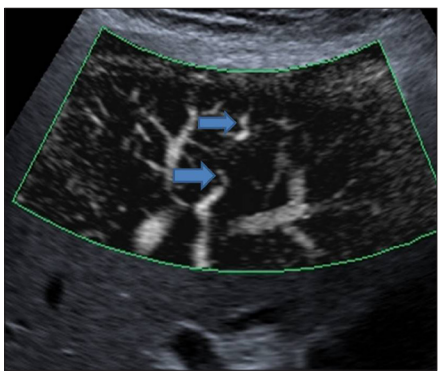

Figure 4. A 60-year-old female patient with chronic hepatitis B. Monochrome SMI demonstrating tortuosity *(arrows)* in distal branches but no evidence of blunting. Doppler classification grade 2, METAVIR score 2.

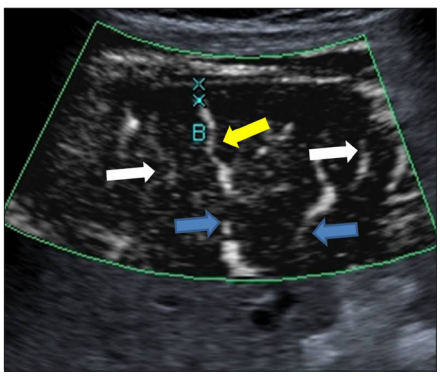

Figure 6. A 61-year-old male patient with chronic hepatitis B. Monochrome SMI image demonstrating tortuosity *(yellow arrow)*, blunting in small *(white arrows)* and large branches *(blue arrows)*. Doppler classification grade 4, METAVIR score 4.

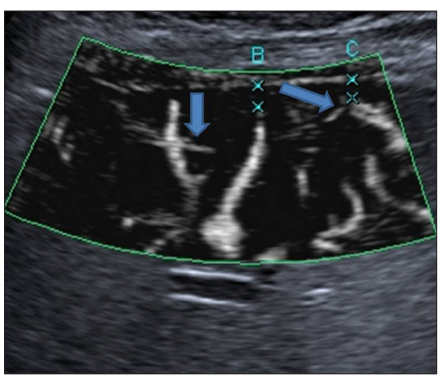

Figure 3. A 37-year-old female patient with chronic hepatitis B. Monochrome SMI demonstrating thinning *(arrows)* in distal branches. Doppler classification grade 1, METAVIR score 2.

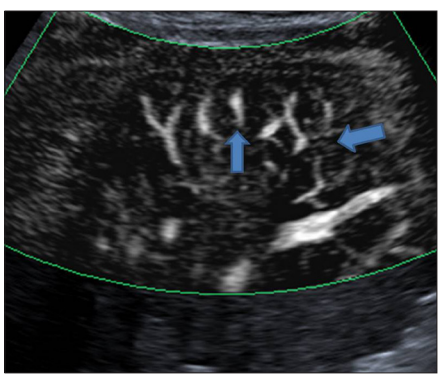

Figure 5. A 36-year-old male patient with chronic hepatitis C. Color SMI image showing blunting in small branches *(arrows)* but not in large branches. Doppler classification grade 3, METAVIR score 3.

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, METAVIR 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

Table 2. Patient characteristics	
n=43	
Age (yrs), mean±SD	50±15
Gender	
Male	25
Female	18
BMI (kg/m^2), mean±SD	26.8±3.3
Etiology, n (%)	
HCV	18 (42)
HBV	18 (42)
Autoimmune	3 (7)
Other	4 (9)
METAVIR fibrosis score, n (%)	
F0	4 (9)
F1	14 (33)
F2	10 (23)
F3	3 (7)
F4	12 (28)
ALT (IU/L), mean±SD	61±51
Total bilirubin (mg/dL), mean±SD	2.85±2.88
Albumin (g/dL), mean±SD	4.6±4.2
INR, mean±SD	1.4±1.6

SD, standard deviation; BMI, body mass index; HCV, hepatitis C virus; HBV, hepatitis B virus; ALT, alanine aminotransferase; INR, international normalized ratio.

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.

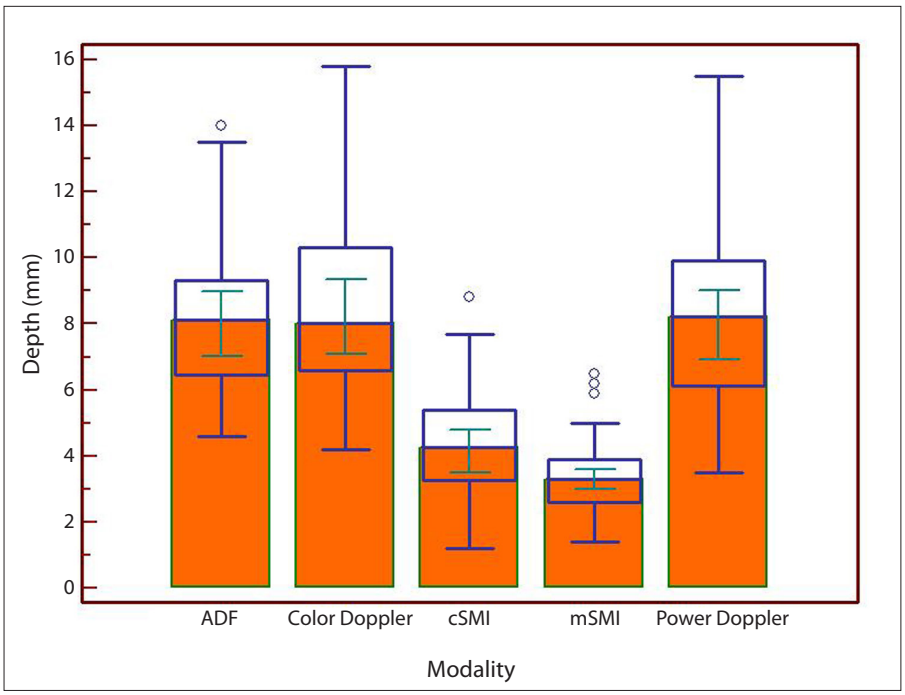

Figure 7. Graph demonstrating the ANOVA test results, showing the difference between Doppler groups in terms of depth measurements of the smallest visible vessels.

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 (PBS), 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. Color Doppler, ADF Doppler and power Doppler techniques could demonstrate vessels approximately 8 mm deep from the liver capsule, while cSMI and mSMI techniques were able to demonstrate vessels only 4.4 and 3.3 mm in depth, respectively ($P < 0.001$) (Table 3 and Fig. 7).

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

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

Doppler method	Distance to liver capsule (mm) (mean±SD)	Post hoc comparison	*P*
ADF	8.10±2.12	Color Doppler	0.282
		Power Doppler	0.856
		cSMI	0.041
		mSMI	<0.001
Color Doppler	8.82±2.85	ADF	0.282
		Power Doppler	0.027
		cSMI	<0.001
		mSMI	<0.001
Power Doppler	8.35±2.79	ADF	0.856
		Color Doppler	0.027
		cSMI	0.361
		mSMI	0.010
cSMI	4.36±1.55	ADF	0.041
		Color Doppler	<0.001
		Power Doppler	0.361
		mSMI	0.589
mSMI	3.30±1.07	ADF	<0.001
		Color Doppler	<0.001
		Power Doppler	0.010
		cSMI	0.589
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 coiling 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

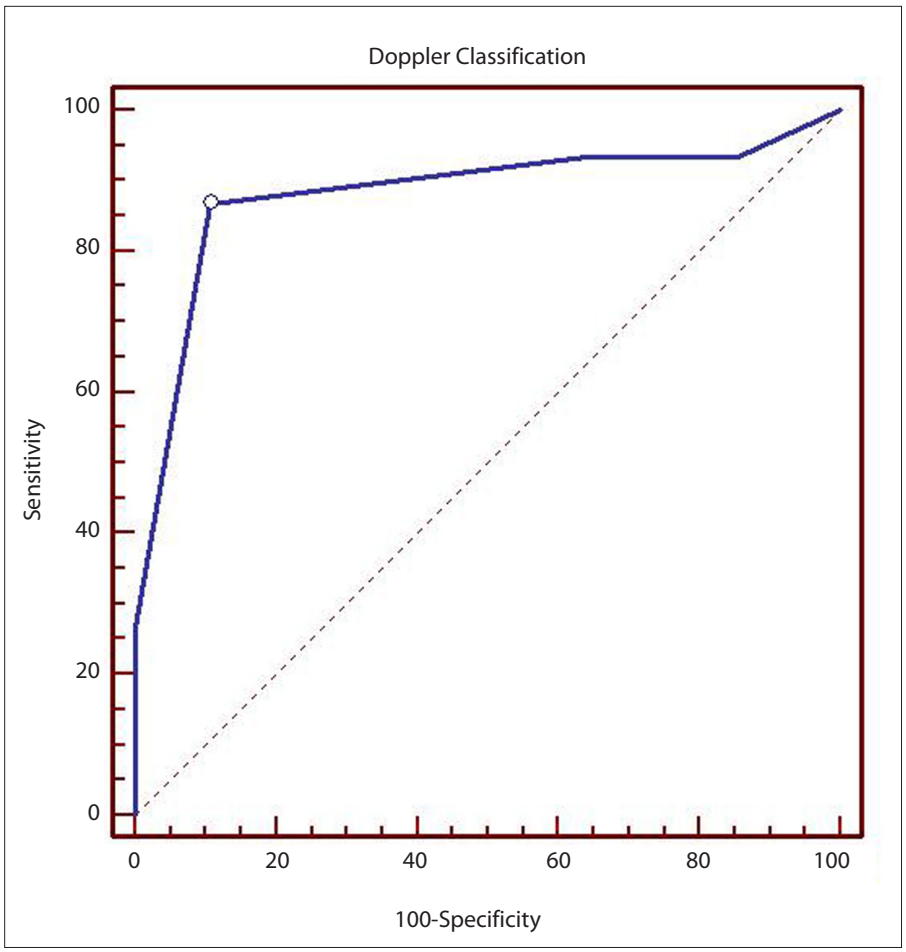

Figure 8. ROC curve to distinguish mild from severe fibrosis. The presence of at least two findings of vascular distortion (cutoff of ≥2 positive findings) yielded 86.7% sensitivity, 89.3% specificity, and 0.881 AUC (95% CI, 0.746–0.960; $P < 0.0001$).

Table 4. Multiple regression analysis

Variable	Coefficient	Standard error	*P*
(Constant)	0.1250	0.4795	0.013
Thinning	0.3750	0.5872	0.5269
Tortuosity	-0.4250	0.4198	0.3178
Blunting, small vessel	2.2167	0.3714	<0.0001
Blunting, large vessel	0.08333	0.5636	0.8811
R^2- adjusted	0.5198		
F- ratio			12.3643
Significance level			<0.001

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/m^2 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

1. Vallet-Pichard A, Pol S. Hepatitis B virus treatment beyond the guidelines: special populations and consideration of treatment withdrawal. Therap Adv Gastroenterol 2014; 7:148–155. [CrossRef]
2. Jieanu CF, Ungureanu BS, Sandulescu DL, et al. Quantification of liver fibrosis in chronic hepatitis B virus infection. J Med Life 2015; 8:285–290.
3. Yamamoto T, Kobayashi T, Phillips MJ. Perinodular arterior plexus in liver cirrhosis. Scanning electron microscopy of microvascular casts. Liver 1984; 4:50–54. [CrossRef]
4. Bosniak MA, Phanthumachinda P. Value of arteriography in the study of hepatic disease. Am J Surg 1966; 112:348–355. [CrossRef]
5. Poynard T, Munteanu M, Imbert-Bismut F, et al. Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. Clin Chem 2004; 50:1344–1355. [CrossRef]
6. Sato K. Angiographic study of the liver cirrhosis. Nihon Igaku Hoshasen Gakkai Zasshi 1969; 28:1612–1628.
7. Kuroda H, Abe T, Kakisaka K, Fujiwara Y, et al. Visualizing the hepatic vascular architecture using superb microvascular imaging in patients with hepatitis C virus: A novel technique. World J Gastroenterol 2016; 22:6057–6064. [CrossRef]
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]
9. Goodman ZD. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol 2007; 47:598–607. [CrossRef]
10. Alvarado-Mora MV, Locarnini S, Rizzetto M, Pinho JR. An update on HDV: virology, pathogenesis and treatment. Antivir Ther 2013; 18:541–548. [CrossRef]
11. Sugimoto K, Shiraishi J, Moriyasu F, Ichimura S, Metoki R, Doi K. Analysis of intrahepatic vascular morphological changes of chronic liver disease for assessment of liver fibrosis stages by micro-flow imaging with contrast-enhanced ultrasound: preliminary experience. Eur Radiol 2010; 20:2749–2757. [CrossRef]

12. Ridolfi F, Abbattista T, Busilacchi P, Brunelli E. Contrast-enhanced ultrasound evaluation of hepatic microvascular changes in liver diseases. World J Gastroenterol 2012; 18:5225-5230.
13. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet 2008; 371:838–851. [CrossRef]
14. Tang A, Cloutier G, Szeverenyi NM, Sirlin CB. Ultrasound elastography and MR elastography for assessing liver fibrosis: part 2, diagnostic performance, confounders, and future directions. AJR Am J Roentgenol 2015; 205:33–40. [CrossRef]
15. Arena U, Vizzutti F, Corti G, et al. Acute viral hepatitis increases liver stiffness values measured by transient elastography. Hepatology 2008; 47:380–384. [CrossRef]
16. Dong Ho L JYL, Joon Koo H. Superb microvascular imaging technology for ultrasound examinations: Initial experiences for hepatic tumors. Eur J Radiol 2016; 85:2090–2095. [CrossRef]
17. Meng-Na H, Ke L, Yu-Xin J, Tian-An J. Application of superb microvascular imaging in focal liver lesions. World J Gastroenterol 2017; 23:7765–7775. [CrossRef]
18. Zhan J, Diao XH, Jin JM, Chen L, Chen Y. Superb microvascular imaging -a new vascular detecting ultrasonographic technique for avascular breast masses: A preliminary study. Eur J Radiol 2016; 85:915–921. [CrossRef]