Evaluation of Sound Speed for Detection of Liver Fibrosis

Prospective Comparison With Transient Dynamic Elastography and Histology

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Objective. The degree of liver fibrosis determines the prognosis and treatment of patients with chronic viral hepatitis. Transient elastography (TE) has been accepted as a noninvasive method for assessment of liver fibrosis. Sound velocity (SV) changes are also dependent on elastic properties of tissue. The aim of this pilot study was to evaluate whether SV estimation of liver tissue allows the determination of fibrosis stages in patients with chronic viral hepatitis. ***Methods.*** Prospectively, 50 healthy volunteers and 149 patients received stiffness (TE, 50-Hz vibrator, 5-MHz array) and SV (conventional ultrasound, C5–2-MHz transducer) measurements. Eighty-four patients received representative liver biopsies. The estimated SV and stiffness were compared using liver biopsy as a reference (METAVIR fibrosis stage [F] scoring system [Hepatology 1996; 24:289–293]). Descriptive statistics, analysis of variance, receiver operating characteristic curve analysis, and box plot analysis as well as intraoperator and interoperator reproducibility analyses were performed. ***Results.*** The SV ranged from 1540 to 1650 m/s. The mean SV ± SD was significantly different between healthy volunteers (1559 ± 11 m/s) and patients with F0–F3 (1575 ± 21 mm/s) and F4 (1594 ± 18 m/s) disease ($P < .001$). For detection of liver cirrhosis, the area under the receiver operating characteristic curve for SV was 0.80 (95% confidence interval, 0.69–0.89). With a cutoff value of 1589 m/s, the sensitivity, specificity, and positive and negative predictive values of SV for detection of liver cirrhosis were 82%, 76%, 70%, and 86%, respectively. Sound velocity measurements were reproducible (15%) and had acceptable operator independence (19%). ***Conclusions.*** The SV of liver tissue depends on the fibrosis stage. An SV of 1589 m/s or higher detects cirrhosis with high sensitivity. Therefore, SV measurement appears to be a promising new method for noninvasive quantification of liver fibrosis.
Key words: FibroScan; fibrosis; liver stiffness; ultrasound velocity.

Abbreviations
AUROC, area under the receiver operating characteristic curve; BMI, body mass index; CI, confidence interval; CV, coefficient of variation; F, fibrosis stage; HBV, hepatitis B virus; HCV, hepatitis C virus; ROC, receiver operating characteristic; ROI, region of interest; SV, sound velocity; TE, transient elastography; ZSI, zone speed index

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Progressive chronic hepatitis is characterized by the development of liver fibrosis and eventually cirrhosis with associated complications such as hepatocellular carcinoma.[1,2] The stage of fibrosis is the main determinant of the prognosis and influences treatment decisions.[3] With regard to the global burden of disease, timely detection of liver fibrosis appears of immense importance given that just for hepatitis C virus

(HCV), the estimated number of carriers is about 130 million worldwide.[4] Furthermore, most patients with chronic hepatitis will not be satisfactorily monitored by a single staging examination but will need lifelong follow-up.[5,6] Apart from the quality of results, the aim therefore should be that a routine examination setup ensures the lowest side effects for the patients at affordable costs for the health system.

Today, liver biopsy using a standardized semiquantitative scoring system is still the reference standard for staging fibrosis in patients with chronic hepatitis.[7–9] Biopsy is invasive, painful, and afflicted with sampling errors and serious complications.[10–12] A milestone was the introduction of transient elastography (TE) using the FibroScan device (Echosens, Paris, France).[13,14] Transient elastography calculates liver stiffness, which highly correlates with the fibrosis stage of the liver.[15] The pooled sensitivity and specificity of TE for detection of liver fibrosis depend on the fibrosis stage (F) and are reported to be 87% (95% confidence interval [CI], 0.84–0.9) and 91% (95% CI, 0.89–0.92) for METAVIR[16] stage F4 and 70% (95% CI, 0.67–0.73) and 84% (95% CI, 0.8–0.88) for METAVIR stages F2–F4, respectively.[17] Meanwhile, the excellent diagnostic accuracy of TE for cirrhosis detection has been confirmed in a meta-analysis.[18] However, in differentiation of lower fibrosis stages ($F \geq 2$) TE is not sufficient in clinical practice.[18]

Conventional ultrasound systems assume a constant speed of sound of 1540 m/s in human tissue. It is well known that the ultrasound velocity varies depending on the type of tissue and its elastic properties (Young modulus). The Young modulus is roughly 3 times the shear modulus assuming soft tissue is incompressible. It is in the kilopascal range for the liver. The ultrasound speed is dominated by the bulk modulus of tissue (compressibility), which is in the gigapascal range. Because tissue can be regarded as isotropic, we assumed a correlation between the shear wave modulus and Young modulus. Therefore, the ultrasound velocity should reflect elastic properties closely. Sound velocity (SV) aberrations affect the quality of the dynamic focus of ultrasonic lenses and blur the images. To improve the image quality, the SV aberrations within the tissue can be corrected. For this correction, a measurement procedure is used, which is well known from the focusing process of digital single-lens reflection cameras.[19] The difference between the calibrated SV (1540 m/s) and the estimated true SV within a region of interest (ROI) is displayed as the zone speed index (ZSI).[19,20]

The hypothesis of this study was that ultrasound velocity estimation as a byproduct of image optimization in a commercial ultrasound device (Zonare Medical Systems, Inc, Mountain View, CA) reflects elastic properties of liver tissue and is in principle suitable for elastography.

The aim of this prospective pilot study was to evaluate whether SV measurements of the liver reflect the liver stiffness and corresponding fibrosis stage in patients with chronic viral hepatitis.

Materials and Methods

The study design was prospective. The study protocol was approved by the local Ethics Committee according to the Declaration of Helsinki. Written informed consent was obtained from each patient.

Patients with chronic hepatitis who were referred to our ultrasound unit were consecutively included from July 2008 to March 2009. The inclusion criterion was chronic viral hepatitis B or C. Exclusion criteria were ascites, liver tumors, and concomitant additional liver disease. Ascites was an exclusion criterion because TE measurements are not possible with ascites.[14] A tumor was defined as an exclusion criterion because it may cause variation in tissue density and compressibility. The patients included in the study were referred from our outpatient liver clinic, liver transplant unit, external practitioners, and the internal wards of Hannover Medical School. From 353 patients with chronic viral hepatitis, 134 had ascites and 69 had liver tumors. One patient was excluded because of coincident autoimmune hepatitis. The study group consisted of 149 patients. Each of them received TE and SV measurements using a conventional ultrasound system. Measurements were performed immediately one after another.

Fibrosis stage F4 was defined as cirrhosis on liver biopsy according to the METAVIR scoring system.[16] For 65 of 149 patients with fibrosis

stages lower than F4, liver biopsies were not of representative size or had been performed more than 1 year before the initiation of the study. Because of ethical concerns, biopsies were not repeated in these patients. Of the remaining 84 patients, 57 received liver biopsies within a maximum time span of 8 months from other examinations. Twenty-seven patients had older biopsies but had proven F4 fibrosis, who were on the waiting list for liver transplantation and had proven portal hypertension.

For each patient, the following clinical data were recorded at the time of TE and ultrasound examinations: age, sex, body mass index (BMI), and serum aspartate aminotransferase, serum alanin aminotransferase, bilirubin, and platelet count values. We used histology as an invasive reference standard and TE as a noninvasive established method for staging of liver fibrosis. On the basis of published data, a cutoff value of 17.6 kPa was defined as cirrhosis on TE.[15]

Fifty healthy volunteers without liver disease constituted the control group. All control participants received a medical survey, had blood tests excluding hepatitis A, B, and C, had normal livers on ultrasound imaging, and received measurements with the Zonare system (Table 1).

The intraoperator and interoperator reproducibility of the SV measurements was studied in 10 patients (7 men and 3 women; 6 with HCV and 4 with hepatitis B virus [HBV]; mean age ± SD, 45 ± 9 years [range, 29–59 years]; and BMI, 24.4 ± 2.7 kg/m^2 [range, 19.5–28.3 kg/m^2]).

Velocity Sound Measurements

All patients received an SV measurement with the Zonare system using a convex array C5–2-MHz transducer. The examination was performed by 2 gastroenterologists highly experienced in ultrasound who were both blinded to the results of TE and biopsy. Measurements were performed on the right liver lobe through the intercostal spaces with the patient lying in a supine position with the right arm in maximal abduction. An ROI was positioned in the right lobe of the liver located 1 cm below the liver capsule.

Table 1. Clinical Data of the Patients Included in This Study

Parameter	n (%)	Mean ± SD	Median	Range
Total patients	149 (100)			
Female	58 (38.9)			
Male	91 (61.1)			
Age, y		47.4 ± 12.2	48	22–72
BMI, kg/m^2		24.9 ± 3.7	24.7	15.8–36.5
Platelet count, /μL		170.6 ± 75.4	170	21–427
AST, U/L		67.9 ± 59.7	46	18–372
ALT, U/L		83.9 ± 102.3	52	11–917
Bilirubin, U/L		13.3 ± 11.2	10	3–92
Hepatitis C	102 (68.5)			
HCV RNA, ×10^6 U/L		1.9 ± 2.7	8.7	0–10
Hepatitis B	42 (28.2)			
HBV DNA, ×10^6 U/L		20 ± 90	0.34	0–600
Hepatitis C and B	5 (3.4)			
FibroScan data				
Interquartile range		3.5 ± 4.5	1.8	0–27
Interquartile range/ mean stiffness, kPa		0.2 ± 0.2	0.2	0–1.2
Success rate, %		90.2 ± 14.1	100	31–100
Valid measurements		10 ± 1.4	10	4–19
Control group				
Total patients	50 (100)			
Female	28 (56)			
Male	22 (44)			
Age, y		33.2 ± 7.7	30.5	22–56
BMI, kg/m^2		22.7 ± 2.3	22.8	17.7–28.4
SV, m/s	1559.9 ± 10.7	1560	1530–1580	

ALT indicates alanine aminotransferase; and AST, aspartite aminotransferase.

The ROI had a standard size of 35 × 35 mm for each patient. When the target area had been located, the operator pressed a button to commence the measurements. The ZSI of the ROI was determined by the ultrasound machine. Trail speeds ranged from 1400 to 1650 m/s with a step value of 10 m/s. For example, a ZSI of 10 means an estimated SV of 1550 m/s. Six successful measurements were performed on each patient in apnea. The median value was considered representative of the SV of the liver. Only procedures with 6 successful measurements were considered reliable.

Liver Stiffness Measurements

All patients received TE (sample size, 4 × 1 cm) with the FibroScan using a low-frequency vibrator (50 Hz) with an ultrasonic single-element transducer operating at 5 MHz on the axis of the vibrator. The examination was performed by 1 experienced physician (>100 examinations) who was blinded to the results of ZSI measurements and liver biopsy. Measurements were performed on the right lobe of the liver through intercostal spaces with the patient lying in a supine position with the right arm in maximal abduction. The tip of the transducer probe was placed in an intercostal space at the level of the right liver lobe. The measurement depth was between 25 and 65 mm below the skin surface. At least 10 successful measurements were performed on each patient. The results were expressed in kilopascals. The success rate was calculated as the number of successful measurements divided by the total number of measurements. The median value was considered representative of the elastic modulus of the liver.

Biopsy

Liver biopsy was performed with a Menghini needle (17 gauge, 9 cm) by 2 experienced gastroenterologists who were blinded to the ultrasound and TE results. The liver tissue was fixed in formalin and embedded in paraffin. Only samples with a minimal length of 20 mm were considered eligible. The biopsy specimens were examined by experienced liver pathologists who were blinded to the ultrasound and FibroScan results. The biopsies were read by 2 independent pathologists. Liver fibrosis and steatosis were semiquantitatively evaluated by the METAVIR scoring system.[16] The fibrosis stage was staged on a 5-point scale according to the METAVIR system (F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis). In cases of uncertainty between two stages, only the more severe was considered. Steatosis in liver specimens was graded from 0 to 3 (0, none; 1, 1%–10%; 2, 10%–30%; and 3, 30%–100% of hepatocytes).

Statistical Analysis

The statistical evaluation was performed using SPSS version 11.5 for Windows software (SPSS Inc, Chicago, IL). Descriptive statistics such as frequencies, mean values, and SDs were calculated. Box plots were used to study the SV and stiffness value distribution according to the fibrosis grading. Coefficients of variation (CVs) of the mean values were calculated. Univariate one-way analysis of variance was combined with the closing test procedure to control the experiment-wise error ($\alpha = .05$) for multiple comparisons (post hoc, Tukey, least significant difference, and Bonferroni tests). The study was designed with a total sample size of 150 because an unpaired t test with a $P < .05$ two-sided significance level and difference in mean values of –14.739 will have 90% power to detect the difference between group 1 (mean value, 1576.461) and a group 2 (mean value, 1591.200), assuming that the common SD is 22.045, when the sample sizes in the two groups are 30 and 120, respectively. Finally, a receiver operating characteristic (ROC) curve analysis was performed. The area under each receiver operating characteristic curve (AUROC) was estimated using the trapezoidal rule. The cutoff values were calculated by ROC analysis. The intraoperator and interoperator reproducibility was evaluated over the studied population using the standardized CV. A CV of less than 30% reflects an acceptable rate for an operator-independent method. The significance level of the CV was measured by the Kruskal-Wallis test.

Results

Table 1 shows the clinical data of the patients and the control group. Sound velocity measurements were successfully performed in all participants. No one was excluded because of technical difficulties.

Table 2 shows the mean values of the measured parameters for each method for stiffness in a dichotomized separation of patients with liver biopsy: F0–F3 (n = 50) and F4 (n = 34) and for the SV in 3 groups: control (n = 50), F0–F3 (total n = 50; F0 = 10; F1 = 26; F2 = 11; and F3 = 3), and F4 (n = 34).

The estimated SV values ranged from 1540 to 1650 m/s. The mean SV values in the different groups were as follows: control, 1559 ± 11 m/s; F0–F3, 1575 ± 21 m/s; and F4, 1594 ± 18 m/s ($P < .001$).

Univariate Analysis of Variance

To detect significant pair differences between the investigated SV in different groups, we applied post hoc tests with the SV as a dependent parameter and the fibrosis stage as a factor (n = 124). There was a significant difference between the SV in the control group, patients with F0–F3 disease, and patients with F4 disease ($P < .001$). There was a negative, nonsignificant correlation between steatosis of the liver on biopsy and the SV (n = 149; $r = –0.20$; $P = .09$).

Box Plot Analysis

Figure 1 shows the distribution of the SV values in the control group, patients with lower fibrosis stages (F0–F3), and patients with liver cirrhosis (F4). The outlier in the group of patients with chronic hepatitis (patient 82) was a patient with obesity and fatty liver on biopsy. In the group of patients with liver cirrhosis, 5 had obesity and fatty livers on biopsy. One (patient 18) had high liver enzymes and was on the waiting list for liver transplantation.

We did not measure the stiffness in the control group because it has already been done in many previous publications. Compared to TE, the SV

Table 2. Clinical Data of Patients With Liver Biopsy

Parameter	n (%)	Mean ± SD	Median	Range	*P*
Total patients	84 (100)				
Female	29 (34.5)				NS
Male	55 (65.5)				NS
BMI, kg/m^2					
F4		25.9 ± 4.5	25.1	17.7–36.5	
F0–F3		24.4 ± 3.4	24.3	17.6–36.5	NS
Age, y					
F4		52.4 ± 8	51	36–68	
F0–F3		44.1 ± 13.1	45	22–67	.001
METAVIR fibrosis stage					
F0	10 (11.9)				
F1	26 (30.9)				
F2	11 (13.1)				
F3	3 (3.6)				
F4	34 (40.5)				
Steatosis at biopsy	41 (48.8)				
FibroScan data					
Stiffness, kPa					
F4	34 (40.5)	35.9 ± 17.5	30.4	8.8–75	
F0–F3	50 (59.5)	8.0 ± 3.7	6.9	3.1–16.8	<.0001
Interquartile range					
F4		7.7 ± 6.2	5.9	0–27	
F0–F3		1.9 ± 1.8	1.3	0.3–8.5	<.0001
Interquartile range/mean stiffness					
F4		0.2 ± 0.2	0.2	0–0.7	
F0–F3		0.2 ± 0.2	0.2	0.03–1.2	NS
Success rate, %					
F4		87.1 ± 13.9	91	62–100	
F0–F3		91.7 ± 12.3	100	55–100	NS
Valid measurements					
F4		10.1 ± 1.8	10	6–16	
F0–F3		10 ± 0.7	10	8–14	NS

NS indicates not significant.

was the more stable measurement for detection of cirrhosis, with a significantly lower rate of variation around the mean value (CV = 0.97 for TE and 0.014 for SV).

Receiver Operating Characteristic Curve Analysis

Using TE as the reference (n = 149) and a cutoff value of 17.4 kPa for liver cirrhosis, the AUROC for the SV was 0.72 (95% CI, 0.63–0.80). With a cutoff value of 1589 m/s, the sensitivity and specificity of the SV for detection of liver cirrhosis were 75% and 70%, respectively.

For a more objective analysis of SV measurements, the ROC analysis was repeated with histology as the reference (n = 84). The AUROC values for SV and TE were 0.80 (95% CI, 0.69–0.89) and 0.9 (95% CI, 0.97–1), respectively. The sensitivity and specificity of the SV for detection of liver cirrhosis rose to 82% and 76% with a cutoff value of 1589 m/s or higher for the SV versus 94% and 100% for TE with a cutoff value of 17.6 kPa for the stiffness. The AUROCs were significantly different. The positive and negative predictive values for the SV in this population were 70% and 86% (Figure 2).

Intraoperator and Interoperator Reproducibility

The intraoperator and interoperator reproducibility of the SV measurements was studied in 10 patients (see "Materials and Methods"). There was no significant difference between the 3 successive series of measurements performed by the same operator. The intraoperator standardized CV was 0.15 (15%). There was no significant difference between the 3 successive series of measurements performed by the 3 different operators, and the corresponding CV was 0.19 (19%).

Discussion

In this study, we were able to prove the hypothesis that the SV allows the determination of fibrosis stages in patients with chronic viral hepatitis, which to our knowledge has not been reported previously. Conventional ultrasound devices with the ability of SV measurement have the potential to be used as noninvasive approaches for detection of liver fibrosis. Using a cutoff value of 1589 m/s, the sensitivity and specificity of this method for detection of liver cirrhosis were 82% and 76%, respectively. The cutoff value of 1589 m/s missed only 7% (n = 6) of the patients with cirrhosis. Further analysis showed that 5 of these patients were overweight and had an elevated BMI of greater than 26 kg/m^2. We observed a negative correlation between steatosis of the liver parenchyma and the SV. Although this correlation was not significant in this study population, it is well known that obese patients present diagnostic difficulties for ultrasound and TE.[21,22] In the study by Foucher et al,[21] a BMI of greater than

Figure 1. Distribution of the SV values in the control group and patients with fibrosis (F0–F3) and cirrhosis (F4) on liver biopsy according to the METAVIR scoring system.

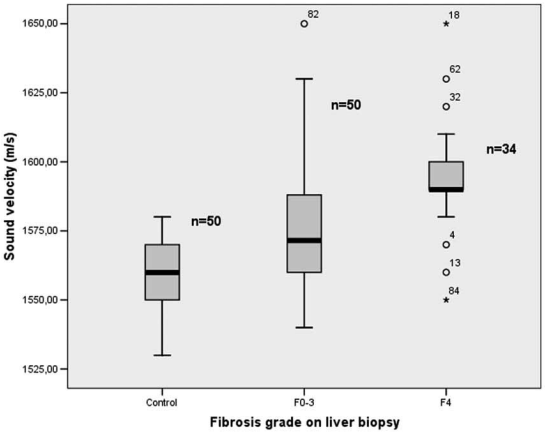
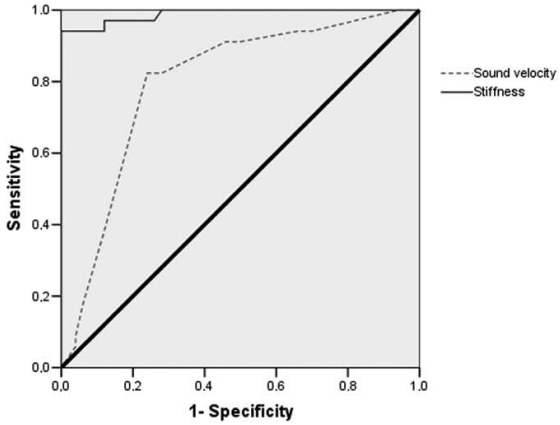


Figure 2. Areas under the ROC curves for SV and stiffness for the detection of liver cirrhosis in patients with chronic viral hepatitis.



28 kg/m^2 was the only factor associated with measurement failure. Some authors see the interference caused by thoracic subcutaneous fat as the limiting factor for success in these patients.[21] Other groups have suggested that the optimal cutoff values for the diagnosis of extensive fibrosis and cirrhosis differ depending on the underlying liver disease and are higher in patients with alcoholic liver disease (fatty livers).[23] In experimental settings, it is has been shown that the SV depends on the fat content of the tissue and is slower in fat compared to water and human tissue.[24–26] In this study, all patients with cirrhosis who were missed by the SV measurements were not only obese but also had a lower SV in the liver compared to the cutoff of value of 1589 m/s and the mean SV value for the population with cirrhosis.

In the implementation of this algorithm, there is potentially a bias in that the sound speed estimate is taken from the surface of the transducer to the ROI to produce the estimate. The tissue between the transducer and the ROI (eg, thoracic subcutaneous fat) will potentially produce a bias; hence, this might be another reason for the larger variations in sound speed. An algorithmic improvement in this regard is possible and may remove the potential bias generated from the non-ROI tissue. Optimizations of processing algorithms for SV measurements in fatty tissue are further goals that should be achieved in the future.

The smallest possible sample size of 35 × 35 mm was 3 times larger than the sample size of the FibroScan. This may be why the SV values were more closely correlated with histology than with the FibroScan. Because the SV in the Zonare system allows measurements in 10-m/s steps only, it seems that this may be another obstacle to achieving the same or even better results than the FibroScan.

The intraoperator and interoperator reproducibility of the SV measurements was evaluated in a small population. However, these patients had corrected SV values ranging from 1540 to 1600 m/s, which corresponded to the value range encountered in the larger population cohort. The intraoperator standardized CV of an SV measurement, obtained without moving the patient, was good and indicates that the technique is reproducible. The higher deviations may have been attributed to a heterogeneous nodular liver texture as well as the patient's breathing status. The standardized CV obtained by changing the operator was similar to the intraoperator standardized CV. This suggests that changing the operator does not add variability to the measured elasticity value. However, 2 operators had several months of experience with SV measurements. The greatest deviations were seen in the operator with less experience. We believe that a short training period is appropriate before operating the device.

For patients with chronic hepatitis, this diagnostic setup provided quantitative ultrasound imaging and less subjective information in characterization of liver tissue. In this pilot study, TE was the more accurate modality; nevertheless, the principal advantages of SV measurement compared to TE are as follows: (1) SV measurements can be performed in all patients without any technical difficulties; (2) the measurements can be obtained in different parts of the liver, even if the soft tissue path to the liver exceeds 2.5 cm; (3) SV measurements can be obtained with a routine ultrasound device and need no additional investment, and additional information about the nodular texture of the liver and extrahepatic complications can be obtained; and (4) this method may obviate major financial investments for longitudinal follow-up examinations of patients with chronic viral hepatitis, especially for general practitioners and physicians not working in specialized tertiary centers.

A limitation of our study was the small number of F3 fibrosis cases. This was due to the character of our center as a leading transplant center in Germany. We have a high proportion of transplant candidates; therefore, our cohort does not reflect a real-life cohort.

In conclusion, the first steps for establishing SV measurement as a noninvasive novel method in the staging of liver fibrosis are promising. We have shown that the SV indeed reflects the elastic properties of liver tissue and is suitable for elastography. With further development of the processing algorithms, a 10-fold increase in SV resolution can be expected. Our measured SV values reflect liver stiffness and are able to indicate higher-grade fibrosis under clinical conditions, findings that to our knowledge have not been reported previously.

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