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● *Original Contribution*

TRANSIENT ELASTOGRAPHY: A NEW NONINVASIVE METHOD FOR ASSESSMENT OF HEPATIC FIBROSIS

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Abstract—Chronic hepatitis is accompanied by progressive deposit of hepatic fibrosis, which may lead to cirrhosis. Evaluation of liver fibrosis is, thus, of great clinical interest and, up to now, has been assessed with liver biopsy. This work aims to evaluate a new noninvasive device to quantify liver fibrosis: the shear elasticity probe or fibroscan®. This device is based on one-dimensional (1-D) transient elastography, a technique that uses both ultrasound (US) (5 MHz) and low-frequency (50 Hz) elastic waves, whose propagation velocity is directly related to elasticity. The intra- and interoperator reproducibility of the technique, as well as its ability to quantify liver fibrosis, were evaluated in 106 patients with chronic hepatitis C. Liver elasticity measurements were reproducible (standardized coefficient of variation: 3%), operator-independent and well correlated (partial correlation coefficient = 0.71, $p < < 0.0001$) to fibrosis grade (METAVIR). The areas under the receiver operating characteristic (ROC) curves were 0.88 and 0.99 for the diagnosis of patients with significant fibrosis (≥ F2) and with cirrhosis (= F4), respectively. The Fibroscan® is a noninvasive, painless, rapid and objective method to quantify liver fibrosis. (E-mail: laurent.sandrin@echosens.com) © **2003 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **One-dimensional transient elastography, Hepatic fibrosis, Chronic hepatitis C, Liver stiffness, Quantitative palpation, Shear wave, Shear elasticity probe.**

INTRODUCTION

Chronic liver diseases often result in fibrosis that may eventually lead to cirrhosis, a state that carries a risk of lethal complications, including hepatocellular carcinoma. These facts point out the clinical interest in quantifying hepatic fibrosis and detecting patients with cirrhosis. At present, liver biopsy is the "gold standard" method to assess the grade of liver fibrosis. However, the use of liver biopsy has several limitations: physical and mental discomfort of the patients that may lead to a high percent of refusal, nonnegligible morbidity and occasional mortality (Cadranel et al. 2000; Poynard et al. 2000). Furthermore, due to the limited size of liver samples and the subjective assessment made by pathologists, accuracy and reproducibility of histologic grading has been questioned (Abdi et al. 1979; Maharaj et al. 1986; Soloway et al. 1971). Therefore, there is an increasing need for alternative noninvasive methods to estimate the grade of liver fibrosis (Friedman 2003). Among other potentially interesting approaches, elastography seems to be one of the most promising. Indeed, it is well known that liver stiffness is related to the degree of hepatic fibrosis, and palpation has been used from decades to establish a clinical diagnosis of hepatic fibrosis and cirrhosis. Recently, Yeh et al. (2002) found a correlation between liver elasticity measured on 19 liver samples obtained after hepatectomy and the fibrosis score determined by histologic analysis.

The development of elastographic techniques yielded four major approaches: static elastography, dynamic elastography, transient elastography and remote elastography. Static elastography (Ophir et al. 1991) has been applied to breast *in vivo* (Cespedes et al. 1993; Krouskop et al. 1998) and prostate *in vitro* (Krouskop et al. 1998). However, the use of this technique is limited by high sensitivity to boundary conditions that would

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certainly induce artefacts in the so-called elastogram, when applied to organs such as liver that can not be placed under controlled compression *in vivo*. Among dynamic elastographic techniques, magnetic resonance imaging (MRI) (Lorenzen et al. 2003; Muthupillai et al. 1995) has proven efficiency in measuring variations of the breast parenchyma elasticity during menstrual cycles. However, the technique requires long acquisition times that are incompatible with the study of organs moving during respiration, such as liver. Furthermore, the cost of MRI examination is prohibitive. Another dynamic technique termed sonoelasticity or sonoelastography (Lerner et al. 1987; Levinson et al. 1995; Parker et al. 1990; Yamakoshi et al. 1987, 1990) has been applied to *in vivo* liver elasticity measurements by Sanada et al. (2000) using a specifically adapted commercially available sonographic scanner. They showed that the average velocity of the low-frequency wave was higher among patients with chronic hepatitis or cirrhosis than among healthy volunteers. However, the results are likely to be biased by high boundary condition sensitivity, diffraction effects (Catheline et al. 1999b) and displacements of the liver during acquisition time that lasts about 90 s. More recently, remote elastography (Nightingale et al. 2001; Rudenko et al. 1996; Sarvazyan 1995) was proposed to image tissue elasticity by remotely inducing low-frequency vibrations in the tissues using acoustic radiation force. *In vivo* breast measurements were obtained with a combination of acoustic radiation force and ultrafast ultrasonic imaging (Bercoff et al. 2003).

In the present paper, we report *in vivo* liver elasticity measurements using the shear elasticity probe (Sandrin et al. 2002a), a device based on one-dimensional (1-D) transient elastography. The 1-D transient elastography technique is not intended to produce elasticity images because data are collected on the axis of a single-element transducer. However, elasticity images can be obtained with 2-D transient elastography, a technique that requires the use of ultrafast ultrasonic imaging (Sandrin et al. 1999; Sandrin et al. 2002a). Transient elastography differs from other US-based elastographic techniques by the kind of mechanical stimulation it relies on. The use of a transient vibration presents several advantages. First, the transmitted elastic wave can be temporally separated from reflected elastic waves. Thus, the technique is less sensitive to boundary conditions than other elastographic techniques. Second, the acquisition time is short (typically less than 100 ms), which enables measurements to be made on moving organs. Transient elastography is, thus, well adapted to the study of the liver.

This work aimed to evaluate the interest of the shear elasticity probe or Fibroscan® in quantifying hepatic fibrosis and detecting cirrhosis in a cohort of patients with hepatitis C virus (HCV) chronic hepatitis. Hepatitis C is a worldwide health problem and hepatic fibrosis grade is a key parameter to assess prognosis and an indication of antiviral treatments in this disease. Furthermore, a semiquantitative score has been developed and validated for this condition (Bedossa and Poynard 1996). It allows comparison between elasticity measurement and histologic fibrosis staging. We evaluated, first, intra- and interoperator reproducibility of the liver elasticity measurement and, second, the ability of elasticity measurements to estimate the degree of hepatic fibrosis.

MATERIALS AND METHODS

Patients

Two cohorts of patients with HCV chronic hepatitis participated in the study after giving their informed consent. The intra- and interoperator reproducibility of the measurement technique were investigated in 15 patients. The ability of liver elasticity measurement to quantify hepatic fibrosis was investigated in 91 patients. Criteria for inclusion of patients were the following: 1. well documented HCV chronic hepatitis with the presence of HCV RNA in the serum and elevated serum transaminases levels, 2. absence of ascites, 3. absence of other detectable cause of liver disease (human immunodeficiency virus or HIV, hepatitis B virus or HBV, alcohol abuse, etc.), and 4. recently performed (less than 1 year) liver biopsy showing lesions compatible with HCV chronic hepatitis.

Histologic analysis

After biopsy, liver samples were fixed (4% formalin), paraffin-embedded, cut in 5-μm thick sections and stained with picrosirius red for fibrosis grading and with hematoxylin-eosin-safran (HES) for activity and steatosis grading. Both fibrosis and activity grading were established with the semiquantitative (METAVIR) score (Bedossa and Poynard 1996). This score ranges from F0 to F4 for fibrosis (F0: no fibrosis, F1: portal fibrosis without septa, F2: few septa, F3: numerous septa without cirrhosis and F4: cirrhosis) and from A0 to A3 for activity (A0: none, A1: mild, A2: moderate and A3: severe). Steatosis was estimated on a scale ranging from 0 to 100 according to the percentage of hepatocytes with fat droplets. Two pathologists experienced in liver diseases scored each histologic slide in a "blind" manner. In case of discordance, slides were reviewed and a consensus score was established. Only liver samples presenting at least 10 portal spaces or obvious cirrhosis were considered suitable for evaluation.

Elasticity measurement apparatus

The Fibroscan® was composed of a probe, a dedicated electronic system and a control unit (Fig. 1). The

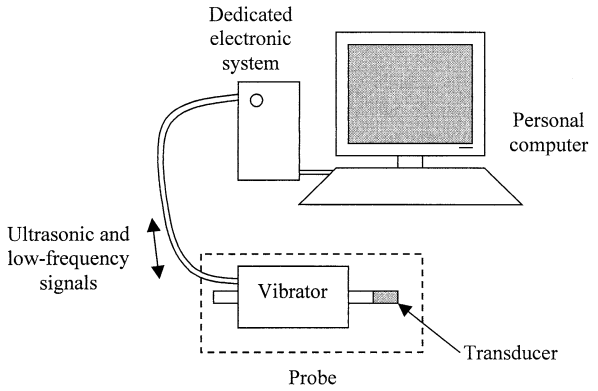

Fig. 1. The Fibroscan® is composed of a probe, a dedicated electronic system and a control unit.

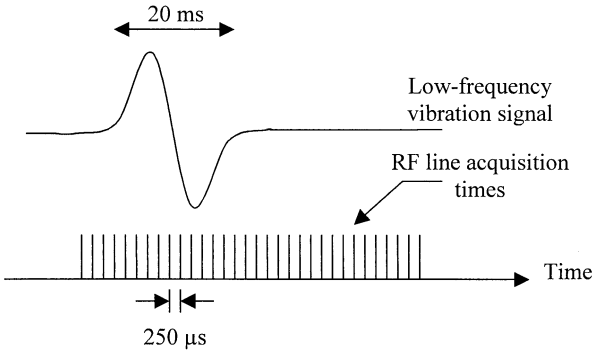

Fig. 2. Acquisition sequence: RF lines are acquired at a repetition frequency of 4000 Hz during the propagation of the low-frequency elastic wave.

probe contains a low-frequency vibrator (typically 50 Hz). An ultrasonic single-element transducer operating at 5 MHz was built on the axis of the vibrator. The 5-MHz center frequency is a compromise between the more specific 3.5-MHz transducer used for liver investigation and the need for high-resolution displacement estimation. This transducer was focused at 35 mm, which corresponds to the middle of the region of interest (ROI) (see below). The electronic system was specifically developed for this application. It sampled ultrasonic signals at 50 MHz with a 9-bit resolution and featured a 2-Mbytes random access memory (RAM). The whole system was operated with a personal computer (Pentium III, 800 MHz).

Details on the operation of the shear elasticity probe can be found in the literature (Sandrin et al. 2002b). Basically, a single ultrasonic transducer was both used as an ultrasonic emitter and receiver and as a low-frequency piston-like vibrator to generate a transient vibration. A total of 256 radiofrequency (RF) lines were acquired at a repetition frequency of 4000 Hz while a low-frequency elastic wave (one period of a 1-mm amplitude sinusoid) was sent through the medium under investigation by the vibrator (Fig. 2). Elasticity was derived from the velocity of the low-frequency elastic wave.

Propagation of the low-frequency excitation

The displacements induced in the medium were measured using the standard cross-correlation technique (Jensen 1996; Ophir et al. 1991). RF lines were segmented vs. depth z into 1-mm slices with 50% overlap. The axial displacement was estimated in each segment by comparison between successive RF lines. Parabolic interpolation was performed to estimate the position of the maximum of the cross-correlation function. A precision of about 1 μm was achieved (Walker and Trahey 1994). Eventually, displacements were derived vs. depth to obtain strain estimates. Figure 3 shows the strains $\epsilon(z,t)$ induced in the liver of a healthy patient as a function of depth and time.

Elasticity estimation

Elasticity was derived from the velocity of the low-frequency elastic wave in an ROI located from 2.5 to 4.5 cm below the skin surface (Fig. 4). This ROI was chosen to avoid the subcutaneous tissue and the liver fibrous capsule in most of the patients, and to ensure that the signal to noise ratio (SNR) of the US allowed a good estimation of the tissue deformations.

The low-frequency elastic wave is mainly a shear wave, which presents a longitudinal component (Sandrin et al. 2002b). Its propagation velocity is of the order of 1 m/s whereas compressional ultrasonic waves are very fast (about 1500 m/s). We considered, in a first approx-

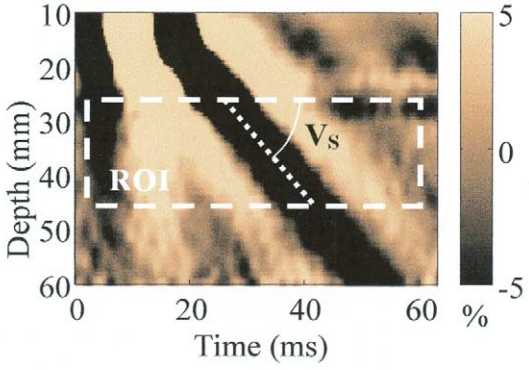

Fig. 3. Amplitude of the strains induced in a healthy patient liver as a function of depth and time. The elastic wave velocity, V_S, is the slope of the wave pattern.

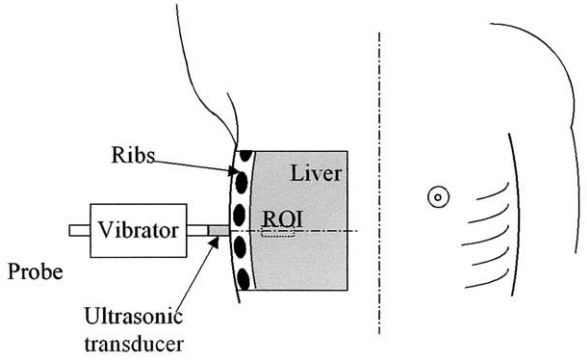

Fig. 4. Probe positioning for liver elasticity measurement.

imation, that liver is a nonviscous, isotropic, soft elastic medium. Under these approximations, the Young's modulus E is expressed as (Royer and Dieulesaint 2000):

$$E = 3\ \rho V_S^2, \tag{1}$$

where V_S is the shear velocity and ρ is the mass density. In soft tissues, mass density is approximately constant and close to the one of water. Therefore, a mass density of 1000 kg/m^3 was used throughout the present study.

In our experiments, shear velocity was estimated using a linear regression of the evolution of the phase delay of the strain estimates as a function of depth at the center frequency of the low frequency vibration. Indeed, as long as diffraction effects and dissipation can be ignored, the phase velocity does not differ from the shear velocity (Sandrin et al. 2002b) that can be expressed as:

$$V_S = 2\pi f_0 \left[\frac{\partial \phi(z, f_0)}{\partial z}\right]^{-1}, \tag{2}$$

where $\phi(z,f)$ is the phase delay of the Fourier transform of the strains induced in the medium $\epsilon(z,t)$ and f_0 is the center frequency of the low-frequency elastic wave (f_0 = 50 Hz).

Several measurements were performed on each patient. The algorithm automatically rejected shear velocity estimates obtained with a linear regression coefficient of determination (r^2) inferior to 0.85. The elasticity estimation was fully automated and took approximately 20 s on an 800-MHz Pentium personal computer. Finally, the median value of the validated ($r^2 \geq 0.85$) estimates was kept as the elasticity value of liver for a given patient.

Acquisition procedure

Because liver biopsies are performed on the right lobe of the liver, so were the elasticity measurements (Fig. 4). During the acquisition, patients were lying on their backs with their right arms behind their heads. The physician first proceeded to a sonographic examination to localize the best ultrasonic imaging window between the rib bones. Additionally, regions with large vessels were avoided and a minimal liver parenchyma thickness of 6 cm was sought. Afterward, the Fibroscan® was placed in intercostal position in front of the chosen ultrasonic window and measurements were performed.

To evaluate the intraoperator reproducibility of the measurement technique, 1 operator performed 3 consecutive series of 10 validated measurements, without moving the patient. The number of acquisition was a compromise between the examination duration and the statistical relevance of the elasticity measurement. In the same way, to study the interoperator reproducibility, 3 operators performed 10 validated measurements each on each patient on different days. Finally, to compare elasticity measurement to liver fibrosis histologic grading, 4 to 12 validated measurements were performed per patient, depending on the available examination time, which varied from 4 to 10 min.

Statistical analysis

Intra- and interoperator reproducibility. The intra- and interoperator reproducibility were evaluated over the studied population using the standardized coefficient of variation (CV), which is defined by (Glüeret al. 1995):

$$CV = \frac{\sqrt{\frac{1}{N}\sum_{n=1}^{N}(\sigma_n^2)}}{4 \cdot \sigma_{pop}} \cdot 100\%, \tag{3}$$

where σ_n is the SD of the three elasticity values obtained on patient n, N is the total number of patients and σ_{pop} is the SD of the first measurement made on each patient. If the same operator made the three elasticity measurements, the obtained CV evaluates the intraoperator reproducibility. If three different operators made the measurements, the CV evaluates a combination of both the intra- and interoperator reproducibility. The commonly used CV is the ratio of the SD over the mean. However, in our study, the mean value, which is an estimate of the distribution location, is strongly correlated to the proportion of patients with each fibrosis grade in the studied population. The chosen CV uses an estimate of the measurement dispersion, rather than the measurement location and is, thus, less sensitive to the population distribution among the fibrosis grade as long as it includes both F0 and F4 patients. Moreover, for a given measurement precision, numerator of eqn (3), the ability to differentiate patients according to their fibrosis grade

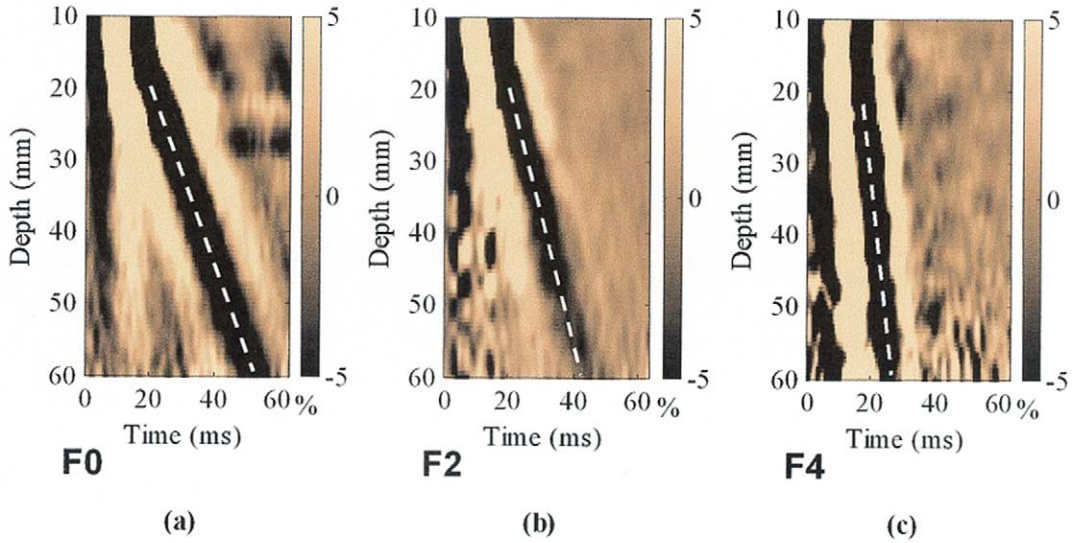

Fig. 5. Elastic waves propagation in livers with different fibrosis grades: (a) F0, (b) F2, and (c) F4. The slope of the white dotted lines represents the propagation velocity of the wave pattern that increases as a function of fibrosis grading.

will be better if the range of values from healthy to cirrhotic patients is large. The standardized CV defined in eqn (3) accounts for these points. Kruskal–Wallis tests were performed with the NCSS 2000 software (Statistical Systems, Kaysville, UT) to find possible significant ($p \leq 0.05$) differences among the acquisition series (same or different operator).

Multiple regression. The relationship between elasticity measurements and histologic parameters was investigated with a multiple regression analysis (NCSS 2000). Because this analysis requires that data follow a normal distribution, a logarithmic transform of the data had to be performed. The partial coefficient of correlation r_p and associated probability p were computed. Only correlations with a p value less than 0.05 were considered significant.

Box plots and ROC analysis. Box plots were used to study the elasticity value distribution according to the fibrosis grading. Finally, a receiver operating characteristic (ROC) analysis (Metz 1978) was performed (NCSS 2000). The area under each ROC curve was estimated using the trapezoidal rule.

RESULTS

Elasticity measurements

Examples of strain images (strain as a function of depth and time) are plotted in Fig. 5 for different liver fibrosis grades. As shown in Fig. 5. the slope of the wave pattern and, thus, the shear velocity seems to increase as the fibrosis grade increases. This indicates that liver gets harder as fibrosis spreads out. In both population cohorts, elasticity estimates vary from 3.35 to 69.1 kPa, which corresponds to shear wave velocity ranging from 1.06 to 4.80 m/s. Elasticity measurements were impossible in 5 patients with either narrow intercostal spaces or overweight.

Intra- and interoperator reproducibility

The intra- and interoperator reproducibility of the liver elasticity measurement technique were studied in 10 men: mean age 49.7 years (from 30 to 65 years) and 5 women: mean age 56.0 years (from 28 to 69 years). There was no significant difference between the three successive series of measurements made by the same operator (Kruskal–Wallis test). The intraoperator standardized CV was 3.2% over the studied population and varied from 2% to 18%. There was no significant difference between the three successive series of measurements made by the three different operators (Kruskal–Wallis test) and the corresponding CV was 3.3%.

Comparison between elasticity measurements and histologic parameters

Patient selection. Among the 91 patients included in this part of the study, elasticity measurements failed in 5 and 19 biopsies were considered not suitable for fibrosis grading. The statistical analysis to compare the elasticity measurement to the histologic parameters was, thus, conducted in 67 patients: 41 men, mean ages 47.7 years (30

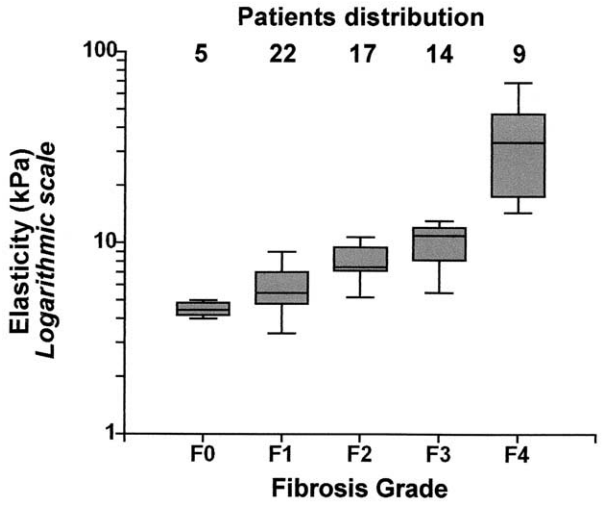

Fig. 6. Elasticity measurements for each fibrosis grade (log scale for the vertical axis). The top and bottom of the boxes are the 25th and 75th percentiles. The length of the box is, thus, the interquartile range (IQR). The line through the middle of the box represents the median (the 50th percentile). The upper adjacent value is the largest observation that is less than or equal to the 75th percentile plus 1.5 times IQR. The lower adjacent value is the smallest observation that is greater than or equal to the 25th percentile minus 1.5 times IQR.

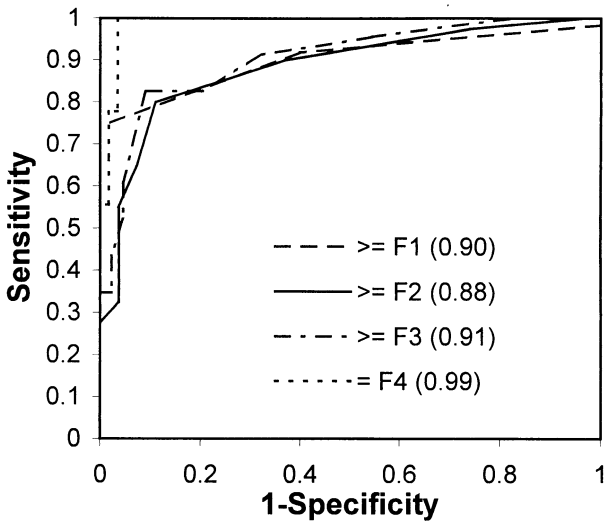

Fig. 7. ROC curves of liver elasticity for the prognostic of METAVIR grade superior or equal to F1, F2, F3 and F4. The area under each ROC curve is indicated in parentheses next to the graph legend.

to 78 years) and 26 women, ages 54.5 years (28 to 78 years).

Histologic parameters. The fibrosis grade distribution among the studied patients was as follows: 5 patients were F0, 22 patients F1, 17 patients F2, 14 patients F3 and 9 patients F4. Concerning activity, the distribution was 4 patients A0, 31 patients A1, 27 patients A2 and 5 patients A3. The steatosis grade varied from 0% to 60% and 40 patients had a 5% or less steatosis, 23 patients had a 10% to 40% steatosis and 4 patients had a 50% to 60% steatosis.

Multiple regression. The multiple regression analysis of the relationship between the logarithmic transform of elasticity measurements and the fibrosis, activity and steatosis grade gave the following results. The partial correlation coefficients (p value) were 0.71 ($< < 0.001$), -0.08 (0.55) and 0.05 (0.70) for the fibrosis, the activity and the steatosis, respectively.

Box plots. To represent the elasticity measurements according to the histologic fibrosis grading, box plots were made (Fig. 6). Because the distribution of the elasticity measurements in the cirrhotic grade (F4) is very large (14.4 to 69.1 kPa), the ordinate axis uses a logarithmic scale for a better visualization of the elasticity values distribution in the F0 to F3 fibrosis grading.

ROC analysis. The ROC curves of liver elasticity measurement were plotted for the hepatic fibrosis grade superior and equal to F1, F2 (which is the minimum fibrosis grade leading to patient treatment), F3 and for hepatic fibrosis grade at F4, which correspond to cirrhosis (Fig. 7). In other words, the first ROC curve (legend $\geq$ F1) depicts the ability of the technique to discriminate between patients with grade F0 and those with grades $\geq$ F1. The second ROC curve (legend $\geq$ F2) depicts the ability of the technique to discriminate between patients with grade F0 or F1 and those with grades $\geq$ F2 (etc.). The areas under the ROC curves, which estimate the performance diagnostic of elasticity measurement, were 0.90, 0.88, 0.91 and 0.99 for the hepatic fibrosis grade superior and equal to F1, F2, F3 and F4, respectively. Based on the elasticity measurement distribution according to fibrosis grade and the ROC curves, thresholds can be chosen to split the patients into those without significant fibrosis (F0 and F1) and those with advanced fibrosis for which antiviral treatment should be performed ($\geq$ F2). Of the studied patients with elasticity inferior or equal to 5.1 kPa, 93% were F0 or F1. Of the studied patients with elasticity superior or equal to 7.6 kPa, 94% were F2 or more.

DISCUSSION

Liver elasticity in patients with HCV chronic hepatitis is highly correlated with the degree of liver fibrosis assessed by biopsy. The diagnostic performances of the

Fibroscan® are good. Even though the studied population is too limited to define absolute thresholds, the result presented here show that elasticity measurements are promising to split the patients into different subgroups. Patients with cirrhosis or advanced fibrosis, who need to be treated to prevent the evolution to cirrhosis, are clearly identified, as well as patients without significant fibrosis. The elasticity values in patients with cirrhosis (F4) are well separated from the others and present a very large range (from 14 to 69 kPa). Further studies in patients with cirrhosis will investigate potential relationships between these values and the degree of portal hypertension or the overall outcome. The multiple regression analysis showed that elasticity measurements were related only to the fibrosis grade and not to the activity and steatosis grades. In a first approximation, activity, which integrates the different basic necroinflammatory lesions of the hepatic tissue, was not expected to be related to liver elasticity. Fatty tissues are softer than healthy liver parenchyma; thus, steatosis would be expected to induce a decrease of liver elasticity, but it was not the case in the studied population where, it must be pointed out, no patient had massive steatosis ($> 60\%$).

The intra- and interoperator reproducibility of the elasticity measurement technique were evaluated in a small population. However, these patients presented elasticity values ranging from 4 to 61.3 kPa, which corresponds to the value range encountered in the larger population cohort. The intraoperator standardized CV of the Fibroscan®, obtained without moving the patient, is low and indicates that the technique is reproducible. For one patient, the standardized CV was 18%. This may be attributed to a heterogeneous distribution of fibrosis in the liver. The standardized CV obtained by changing the operator and the measurement day, thus, the patient position, is similar to the intraoperator standardized CV. This suggests that changing operator or the chosen acoustic window, which may vary from one operator to another, does not add variability to the measured elasticity value. The operator influence is, thus, negligible. However, operators had a several month experience with the Fibroscan®, and we believe that a short training is appropriate before operating the device.

No elasticity measurements were obtained on 5 patients out of the total 91 patients. On these 5 patients, the acquisition system automatically rejected all elasticity estimates because they did not satisfy the criterion on r^2. This criterion rejects the acquisitions if the low-frequency elastic wave propagation is not measured properly. Elasticity measurement can be difficult or even impossible in patients who are obese, have narrow intercostal spaces or have ascites. In the case of obese patients, the low-frequency vibration induced by the probe and/or the US wave can be strongly attenuated by the fatty tissue. It results in a poor SNR that affects the elasticity measurement algorithm. Moreover, in those patients, the chosen ROI needs to be shifted deeper below the skin surface to avoid fatty tissue. In the case of patients with a narrow intercostal space, the 9-mm probe diameter may be too large to fit between costal bones. Elasticity measurements are impossible in patients with ascites because low frequency elastic waves do not propagate through liquids. This last limitation is not practically important because ascites is a circumstance where the diagnosis of cirrhosis is usually clinically obvious. Finally, in this study, the number of patients for whom the elasticity measurement failed is low compared to the 19 biopsies that were not suitable for METAVIR grading.

Assuming that the fibrosis semiquantitative score obtained by liver biopsy is a "gold standard," 1-D transient elastography is 99% efficient for the detection of patients with cirrhosis and 88% efficient for the detection of patients with fibrosis grade superior or equal to F2. However, discrepancies were observed. This is probably not due to morphologic conditions. Indeed, the measurement of the elasticity is averaged between 25 and 45 mm from the skin, a distance that precludes taking into account subcutaneous tissues except for frankly overweight patients, where the measurement is technically impossible. It seems more likely that these discrepancies are mainly due to limitations of hepatic biopsy and to the design of the METAVIR grading system. The latter takes into account the degree of distortion of hepatic architecture as well as the extent of fibrosis, which are not necessarily well correlated because it happens to find patients with cirrhosis that present only thin bundles of fibrotic tissue surrounding the liver nodules. Presumably, liver elasticity would be more closely related to the amount of fibrosis than to the degree of architecture distortion. This point needs to be further evaluated by morphometric studies. However, the limited size of liver biopsy provides an even more simple explanation. Reproducibility of liver biopsy is poor due to the sample size and to the heterogeneity of liver fibrosis. Actually, the optimal length of a liver biopsy has been estimated to be 3 cm or more, a size that is reached in only a minimal number of cases. The size limitation of hepatic biopsies, particularly those performed by the transjugular route, results in a limitation of the method and emphasizes the need for more reliable tools.

The measurements presented in this paper were obtained using a 1-D transient elastography approach. The most importnat limit of the 1-D approach is that it provides an average elasticity estimated over the measurement depth. The technique is, thus, well adapted to diffuse processes such as chronic liver diseases. Using transient elastography to provide a 2-D image of liver

elasticity would require the use of ultrafast ultrasonic imaging (Sandrin et al. 1999, 2002a), which will be addressed in further studies. In the present study, the elasticity estimate was averaged over a volume that can be approximated by a cylinder of length 20 mm (between 25 mm and 45 mm below skin surface) and diameter 20 mm (the shear wavelength at 50 Hz with $V_s = 1$ m/s in normal liver). This volume represents 1% of the liver total volume, which is much more relevant than the biopsy sample size, which is only of 1/50,000 (Bravo et al. 2001).

All acquisitions were performed on the right lobe of the liver in intercostal position. Sanada et al. (2000) performed elasticity measurements on the left lobe of the liver. Actually, the diameter of their stick-like vibration head (20 mm) was too large to fit between the costal bones, and the forced vibration could not be properly sent through. In our experiments, the diameter of the transiently vibrating transducer is only 9 mm. It fits between the costal bones of most patients to transmit elastic waves properly. The important contribution of the chest wall is that it prevents the liver from being directly compressed by the probe itself. Indeed, pressing on the liver during the measurement would modify its elasticity because soft tissues react in a highly nonlinear way. The second contribution of the chest wall is that it gives a static, almost plane surface for the probe positioning. In fact, the surface on which the low frerquency vibration is sent should be as plane as possible. Diffraction effects occur at the surface of the transducer due to the large shear wavelength. The study of these effects is far beyond the scope of this paper and has been addressed by others (Catheline et al. 1999a; and unpublished observations).

The stiffness of the liver was expressed using the Young's modulus. Theoretically, two parameters are necessary to characterize a linear homogeneous and isotropic solid. Assuming that liver is a purely elastic solid, the Young's modulus and the Poisson's ratio may be used as elastic parameters to describe its mechanical properties. In the particular case of nearly incompressible media, such as soft tissues, the Poisson's ratio is almost constant ($\sigma \approx 0.5$) (Fung 1981; Parker et al. 1990). Thus, only one parameter is necessary. The shear modulus and the shear velocity could be used instead of the Young's modulus. Due to the exponential variation of the latter vs. the fibrosis grade and to the normal distribution of elasticity measurements under logarithmic transform, a logarithmic index is under investigation (Fig. 6).

The liver elasticity estimates obtained using 1-D transient elastography are different from those found in the literature. They are larger than those obtained with direct mechanical measurements (Chen et al. 1996; Yeh et al. 2002). Yeh and colleagues found values ranging from 640 Pa to 20 kPa in fresh liver samples obtained in patients with no fibrosis and in patients with cirrhosis, respectively. On the contrary, using dynamic elastography, Sanada et al. (2000) measured *in vivo* low-frequency elastic waves velocities and found much larger velocities (between 5 m/s and 12 m/s) than those obtained with 1-D transient elastography. These discrepancies point out the difficulty of measuring soft tissue elasticity and the important role of the kind of mechanical excitation used because elasticity varies as a function of frequency, strain amplitude, etc.

Results presented in this paper show a very good correlation between the fibrosis grade assessed by histologic examination of liver biopsy sample and the elasticity measurements performed using the 1-D transient elastography approach. This is in good agreement with the well-known relationship between the liver fibrosis and the stiffness of the liver by palpation. However, soft tissues are very complex media in terms of biomechanical features. The constitution of hepatic fibrosis can be considered to be homogeneous from a macroscopic point of view, but is highly heterogeneous and anisotropic on the microscopic level. Viscous loss, anisotropy, heterogeneity and nonlinearity are particularly interesting properties that cannot be measured using the present form of the Fibroscan®.

Conclusion and future work

The aim of this preliminary study was to evaluate the potential application of 1-D transient elastography as a means to measure liver elasticity and to quantify liver fibrosis. Although the number of studied patients is limited, the results are very promising and provide the basis for a larger ongoing multicenter clinical trial.

The Fibroscan® is a simple and low-cost device that could be used to assess instantaneously and directly the elasticity of the liver. The measurements are fully noninvasive, reproducible and may be performed by physicians or even nonphysicians after a short training period. Correlation with fibrosis grade is good despite the high false-negative rate of the biopsy itself (Nord 1982). Elasticity measurements are difficult or impossible in obese patients and patients with narrow intercostal spaces, but this last limitation is to be overcome by technical improvements such as the development of new probes. Presumably, in the future, the Fibroscan® could be used instead of the liver biopsy for assessment and monitoring of liver fibrosis.

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REFERENCES

Abdi W, Millan JC, Mezey E. Sampling variability on percutaneous liver biopsy. Arch Intern Med 1979;139:667–669.

Bedossa P, Poynard T. An algorithm for grading of activity in chronic hepatitis C. Hepatology 1996;24:289–293.

Bercoff J, Tanter M, Fink M. Supersonic imaging: a new technique for mapping of the visco-elastic properties of tissues. 28th International Symposium on Ultrasonic Imaging and Tissue Characterization, Arlington, VA, May 28–30, 2003. Ultrason Imaging 2003.

Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med 2001; 344:495–500.

Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: Results of a prospective nationwide survey. Hepatology 2000;32: 477–481.

Catheline S, Thomas JL, Wu F, Fink M. Diffraction field of a low-frequency vibrator in soft tissues using transient elastography. IEEE Trans Ultrason Ferroelectr Freq Control 1999a;46:1013–1020.

Catheline S, Wu F, Fink M. A solution to diffraction biases in sono-elasticity: The acoustic impulse technique. J Acoust Soc Am 1999b; 105:2941–2950.

Cespedes I, Ophir J, Ponnekanti H, Maklad N. Elastography: Elasticity imaging using ultrasound with application to muscle and breast *in vivo*. Ultrason Imaging 1993;15:73–88.

Chen EJ, Novakofski J, Jenkins WK, O'Brien W. Young's modulus measurements of soft tissues with application to elasticity imaging. IEEE Trans Ultrason Ferroelectr Freq Control 1996;43:191–194.

Friedman SL. Liver fibrosis—from bench to bedside. J Hepatol 2003; 38:S38–S53.

Fung YC. Biomechanics—Mechanical properties of living tissues. New York: Springer Verlag, 1981.

Glüer CC, Blake G, Lu Y, et al. Accurate assessment of precision errors: How to measure the reproducibility of bone densitometry techniques? Osteopor Int 1995;5:262–270.

Jensen JA. Estimation of blood velocities using ultrasound. Cambridge: Cambridge University Press, 1996.

Krouskop TA, Wheeler TM, Kallel F, Garra BS, Hall T. Elastic moduli of breast and prostate tissue under compression. Ultrason Imaging 1998;20:260–274.

Lerner RM, Parker KJ, Holen J, Gramiak R, Waag RC. Sono-elasticity: medical elasticity images derived from ultrasound signals in mechanically vibrated targets. Acoust Imaging 1987;16:317–327.

Levinson SF, Shinagawa M, Sato T. Sonoelastic determination of human skeletal muscle elasticity. J Biomech 1995;28:1145–1154.

Lorenzen J, Sinkus R, Biesterfeldt M, Adam G. Menstrual-cycle dependence of breast parenchyma elasticity: Estimation with magnetic resonance elastography of breast tissue during the menstrual cycle. Invest Radiol 2003;38:236–240.

Maharaj B, Maharaj RJ, Leary WP, et al. Sampling variability and the influence on the diagnostic yield of percutaneous needle biopsy of the liver. Lancet 1986;1:523–525.

Metz CE. Basic principles of ROC analysis. Semin Nucl Med 1978;8: 283–298.

Muthupillai R, Lomas DJ, Rossman PJ, et al. Magnetic resonance elastography by direct visualization of propagating acoustic strain waves. Science 1995;269:1854–1857.

Nightingale KR, Palmeri NL, Nightingale RW, Trahey GE. On the feasibility of remote palpation using acoustic radiation force. J Acoust Soc Am 2001;110:625–634.

Nord JH. Biopsy diagnosis of cirrhosis: Blind percutaneous versus guided direct vision techniques. A review. Gastrointest Endosc 1982;28:102–104.

Ophir J, Cespedes EI, Ponnekanti H, Yazdi Y, Li X. Elastography: A method for imaging the elasticity in biological tissues. Ultrason Imaging 1991;13:111–134.

Parker KJ, Dongshan F, Graceswki M, Fai Y, Levinson SF. Vibration sonoelastography and the detectability of lesions. Ultrasound Med Biol 1998;24:1437–1447.

Parker KJ, Huang SR, Musulin RA, Lerner RM. Tissue response to mechanical vibrations for sonoelasticity imaging. Ultrasound Med Biol 1990;16:241–246.

Poynard T, Ratziu V, Bedossa P. Appropriateness of liver biopsy. Can J Gastroenterol 2000;14:543–548.

Royer D, Dieulesaint E. Elastic waves in solids. New York: Springer Verlag, 2000.

Rudenko OV, Sarvazyan AP, Emilianov SY. Acoustic radiation force and streaming induced by focused nonlinear ultrasound in a dissipative medium. J Acoust Soc Am 1996;99:2791–2798.

Sanada M, Ebara M, Fukuda H, et al. Clinical evaluation of sonoelasticity measurement in liver using ultrasonic imaging of internal forced low-frequency vibration. Ultrasound Med Biol 2000;26: 1455–1460.

Sandrin L, Catheline S, Tanter M, Hennequin X, Fink M. Time-resolved pulsed elastography. Ultrason Imaging 1999;21:259–272.

Sandrin L, Tanter M, Catheline S, Fink M. Shear modulus imaging with 2D transient elastography. IEEE Trans Ultrason Ferroelec Freq Control 2002a;49:426–435.

Sandrin L, Tanter M, Gennisson JL, Catheline S, Fink M. Shear elasticity probe for soft tissues with 1D transient elastography. IEEE Trans Ultrason Ferroelec Freq Control 2002b;49:436–446.

Sarvazyan AP. A new approach to remote ultrasonic evaluation of viscoelastic properties of tissues for diagnostics and healing monitoring. Abstract of ARPA/ONR Medical Ultrasonic Imaging Technology Workshop, Landsdowne, Virginia, January, 1995. 1995:24-26.

Soloway RD, Baggenstoss AH, Schoenfield LJ, Summerskill WH. Observer error and sampling variability tested in evaluation of hepatitis and cirrhosis by liver biopsy. Am J Dig Dis 1971;16: 1082–1086.

Walker WF, Trahey GE. A fundamental limit on the performance of correlation based on phase correction and flow estimation techniques. IEEE Trans Ultrason Ferroelec Freq Control 1994;41:644–654.

Yamakoshi Y, Suzuki M, Sato T. Imaging the elastic properties using low frequency vibration and probing ultrasonic wave. Japanese Meeting of Applied Physics, Tokyo, 1987.

Yamakoshi Y, Sato J, Sato T. Ultrasonic imaging of internal vibration of soft tissue under forced vibration. IEEE Trans Ultrason Ferroelec Freq Control 1990;37:45–53.

Yeh WC, Li PC, Jeng YM, et al. Elastic modulus measurements of human liver and correlation with pathology. Ultrasound Med Biol 2002;28:467–474.