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Can early hepatic fibrosis stages be discriminated by combining ultrasonic parameters?

Razika Bouzitoune [a], Mahmoud Meziri [a,*], Christiano Bittencourt Machado [b], Frédéric Padilla [c], Wagner Coelho de Albuquerque Pereira [d]

[a] Laboratoire de Magnétisme et de Spectroscopie des Solides (LM2S), Université Badji Mokhtar, Annaba 23000, Algeria
[b] Biomedical Ultrasound Laboratory, Estácio de Sá University, Rio de Janeiro, Brazil
[c] LabTau, Unité Inserm1032, 141 Cours Albert Thomas, Lyon 69003, France
[d] Biomedical Engineering Program, COPPE/Federal University of Rio de Janeiro, RJ, Brazil

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ABSTRACT

In this study, we put forward a new approach to classify early stages of fibrosis based on a multiparametric characterization using backscatter ultrasonic signals. Ultrasonic parameters, such as backscatter coefficient (Bc), speed of sound (SoS), attenuation coefficient (Ac), mean scatterer spacing (MSS), and spectral slope (SS), have shown their potential to differentiate between healthy and pathologic samples in different organs (eye, breast, prostate, liver). Recently, our group looked into the characterization of stages of hepatic fibrosis using the parameters cited above. The results showed that none of them could individually distinguish between the different stages. Therefore, we explored a multiparametric approach by combining these parameters in two and three, to test their potential to discriminate between the stages of liver fibrosis: F0 (normal), F1, F3, and/without F4 (cirrhosis), according to METAVIR Score. Discriminant analysis showed that the most relevant individual parameter was Bc, followed by SoS, SS, MSS, and Ac. The combination of (Bc, SoS) along with the four stages was the best in differentiating between the stages of fibrosis and correctly classified 85% of the liver samples with a high level of significance ($p < 0.0001$). Nevertheless, when taking into account only stages F0, F1, and F3, the discriminant analysis showed that the parameters (Bc, SoS) and (Bc, Ac) had a better classification (93%) with a high level of significance ($p < 0.0001$). The combination of the three parameters (Bc, SoS, and Ac) led to a 100% correct classification. In conclusion, the current findings show that the multiparametric approach has great potential in differentiating between the stages of fibrosis, and thus could play an important role in the diagnosis and follow-up of hepatic fibrosis.

1. Introduction

It is known that the initiation of the hepatic fibrosis process alters the acoustical properties of the tissues. Therefore, techniques for biological tissue characterization with ultrasound (US) have been proposed in the last decades. Ultrasonic tissue characterization using radio frequency (RF) signals has shown the potential in supplying the data required for the assessment of tissue microstructures. In this context, different investigations have indicated that parameters such as Speed of Sound (SoS), Backscatter coefficient (Bc), Spectral Slope (SS), Attenuation coefficient (Ac) and Mean Scatterer Spacing (MSS) could differentiate normal from pathological tissues. For example, several studies have utilized these parameters for characterizing different organs, such as breast, kidney, skin, spleen, bone [1–6], and hepatic tissue [4,7–11]. So far, however, investigations of the differences between extreme liver structures (e.g., normal vs. cirrhosis or cancerous) [12–14] have neglected the detection of initial stages of fibrosis.

Chronic viral hepatic infections lead to progressive fibrosis. They may eventually lead to severe complications like cirrhosis, a state that may lead to serious problems like oesophageal varices and hepatocellular carcinoma. To obviate this, it would be interesting to quantify the stages of hepatic fibrosis. The methods for the diagnosis of fibrosis have made headway during the last decade (FibroTest, Elastography, and Ultrasound). However, liver biopsy remains the "gold standard" to assess the degree of liver fibrosis [15] despite certain limitations (i.e., high level of morbidity and

* Corresponding author at: BP 790 RP, Annaba 23000, Algeria. Tel.: +213 551 91 85 17; fax: +213 38 87 53 59.
E-mail addresses: mirazika75@yahoo.fr (R. Bouzitoune), mahmoud.meziri@gmail.com (M. Meziri), cbmfisio@gmail.com (C.B. Machado), frederic.pad@gmail.com (F. Padilla), wagner.coelho@ufrj.br (W.C.A. Pereira).

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mortality) [16]. In addition, liver biopsy has other drawbacks: possible errors of evaluation linked to the mixed development of fibrosis [17] without overlooking that this technique cannot be used routinely for medical supervision. The inconvenience of the liver biopsy leads many teams to develop alternate and non-invasive methods for diagnosing hepatic fibrosis [18]. Complementary methods of hepatic biopsy have been proposed by different authors for the quantification of fibrosis. Among these methods, we cite the FibroTest, which is a method based on serial scores combining various markers, giving an estimation of the stage of fibrosis according to the METAVIR scale [19,20]. Elastographic methods, such as transient elastography (Fibroscan), have taken on an important role in identifying the stages of liver fibrosis [21–23]. In these studies, the existence of an overlap between the stages of early fibrosis (F0, F1, and sometimes F2) has been discovered. Given the difficulties of elastographic methods to differentiate between F0 and F1, the authors have merged these two groups together as F0–1. However, elastographic methods are the most appropriate to identify the severe stages of fibrosis (F2, F3), and especially stage F4 (cirrhosis) [21–23].

Among other interesting approaches, the US characterization method seems to be one of the most promising because it offers many advantages: lack of radiation, lower price, and easy transportability, compared with liver biopsy. Fibrosis identification in the early stages is very important for estimating adequate treatment, prognosis, and for surveillance in patients with chronic hepatitis. For these reasons, efforts have been made by Meziri et al. [8–10], to evaluate the ultrasonic parameters and to test their capacity to discriminate between different stages of hepatic fibrosis, according to the METAVIR Score [24]. They have shown that none of these parameters were able to discriminate the different stages of fibrosis [8,9]. The same authors also tested a multiparametric approach. However, an overlap was observed, especially between stages F0 and F4 and 85% of samples were correctly classified, whatever the combination of ultrasonic parameters. The characterization of extreme stages such as the F4 has never been a challenge for the different methods of characterization (elastography, ultrasound technique). Differentiating the early stages of fibrosis seems very challenging, since the change in liver tissue from one stage to another is very subtle. Nevertheless, the classification of such changes is essential for the clinician to choose the appropriate medication, and the medical follow up. To our knowledge, no work has been devoted to the beginning of the installation of liver fibrosis. The novelty of this study lies in its potential to differentiate the early stages, F0–F3, by combining the acoustic parameters. The rest of the paper is organized as follows: Section 2 presents the methodology of the experimental setup, recalls briefly the different methods of ultrasonic parametric evaluations, and presents a statistical analysis. Section 3 presents the results. The discussion of the results and final conclusions are given in Sections 4 and 5, respectively.

2. Materials and methods

2.1. Liver specimens

Twenty human liver specimens were obtained during hepatectomy. They were immediately frozen and kept at −20 °C. It has already been found that freezing the liver sample may not affect ultrasound measurements. The effect of the freeze/thaw cycle has no significant difference between the acoustic parameters such as speed of sound and attenuation ($p > 0.05$) [25,26]. A slice of 5 mm thickness was carefully cut to assure uniform thickness and parallel surfaces. The sample was big enough to allow a region-of-interest (ROI) of 4×4 mm^2 of homogeneous tissue. The samples were then unfrozen at room temperature. Each sample was degassed for 30 min at a low pressure while being immersed in physiological serum. Lastly, the sample and the liquid were warmed to an average temperature of 35 ± 2 °C under the monitoring of the digital thermometer. It is known that liver vessels may generate strong echoes that are usually easily identified. Therefore, we used homemade software, which is capable of producing B-mode images from the acquired RF signals. Hence, it is possible to find the B-mode plans when there are strong echoes coming from structures like vessels, and avoid them, by displacing the ROI away from the vessel. As the ROI is small, it was always possible to find one without strong echoes, and hence all the signals with strong reflections were discarded from our analysis. After ultrasonic measurements, each sample underwent histological examination. The details of the histological preparation can be found in [8]. The stages of fibrosis were classified by an experienced pathologist according to the METAVIR criteria: F0 for the absence of fibrosis; F1 for portal fibrosis without septa; F2 for portal fibrosis with few septa; F3 for septal fibrosis without cirrhosis and F4 for cirrhosis. Fig. 1 represents the hematoxylin and eosin (H&E) stained section images (6× magnification) of the different stages of fibrosis (F0, F1, F3, and F4).

2.2. Ultrasonic acquisition

Detailed information about data acquisition can be found in [8]. The samples were positioned on a polished steel plate (6 cm × 10 cm) beneath a thin plastic membrane. Then, it was placed in a saline–water-filled reservoir kept at 35 ± 2 °C by immerging it in a temperature-controlled water bath. A 20 MHz transducer was placed above the plate at its focal distance F (Panametrics M316, 0.125″ diameter, 0.75″ focal length, 6-dB bandwidth 6–30 MHz, 460 μm spatial resolution of −6 dB). The transducer was moved with steps of 200 μm, and a total of 421 signals were then acquired for each sample. Radiofrequency (RF) signals were received and amplified (Model 5052 PRX Panametrics amplifier Waltham, MA, USA), and then sampled at 100 MHz using a digital oscilloscope (Lecroy 9350AL 500 MHz, Geneva,

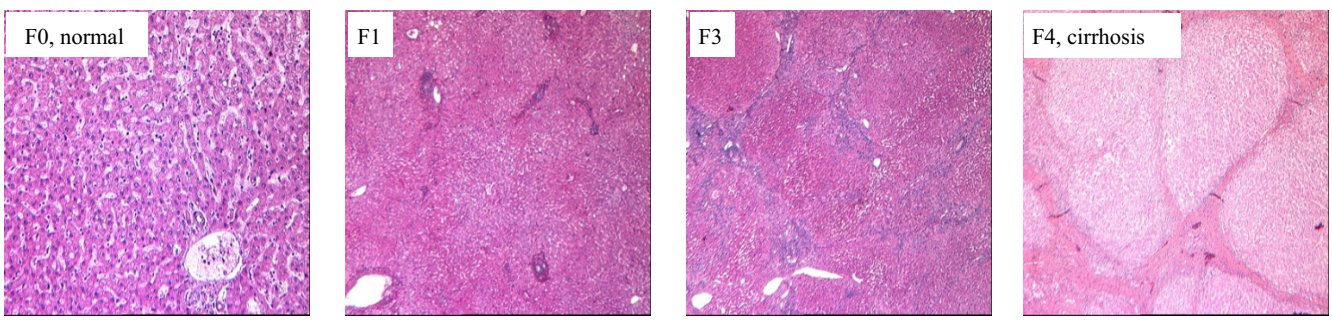

Fig. 1. H&E stained section images (6× magnifications) of the different fibrosis stages F0, F1, F3 and F4).