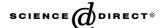


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In vitro chronic hepatic disease characterization with a multiparametric ultrasonic approach

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Abstract

Although, high resolution, real-time ultrasonic (US) imaging is routinely available, image interpretation is based on grey-level and texture and quantitative evaluation is limited. Other potentially useful diagnostic information from US echoes may include modifications in tissue acoustic parameters (speed, attenuation and backscattering) resulting from disease development. Changes in acoustical parameters can be detected using time-of-flight and spectral analysis techniques.

The objective of this study is to explore the potential of three parameters together (attenuation coefficient, US speed and integrated backscatter coefficient—IBC) to discriminate healthy and fibrosis subgroups in liver tissue. Echoes from 21 fresh in vitro samples of human liver and from a plane reflector were obtained using a 20-MHz central frequency transducer (6–30 MHz bandpass). The scan plane was parallel to the reflector placed beneath the liver. A 30×20 matrix of A-scans was obtained, with a 200- μ m step. The samples were classified according to the Metavir scale in five different degrees of fibrosis.

US speed, attenuation and IBC were estimated from standard methods described in the literature. Statistical tests were applied to the results of each parameter individually and indicated that it was not possible to identify all the fibrosis groups. Then a discriminant analysis was performed for the three parameters together resulting in a reasonable separation of fibrotic groups. Although the number of tissue samples is limited, this study opens the possibility of enhancing the discriminant capability of ultrasonic parameters of liver tissue disease when they are combined together.

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1. Introduction

Nowadays ultrasonic (US) imaging at high frequency and real-time is clinically available, nevertheless image interpretation is mainly based on grey-level and texture visual inspection. Literature points to potentially useful quantitative diagnostic information from US echoes

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that may include modifications in tissue acoustic parameters from disease development [1].

The basic underlying hypothesis is that pathologic changes in biological tissues produce alteration in their fundamental physical or micro-architectural properties (e.g., density, elasticity, viscosity, and inhomogeneity) and thus affect the propagation of ultrasonic waves that can be quantified by measuring wave speed, attenuation and backscattering or other parameter such as elasticity over a range of frequencies. In the liver, which is the focus of the present work, ultrasound tissue

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characterization has the potential to become an objective means for diagnosis. It is difficult to differentiate diffuse liver diseases (fibrotic, cirrhotic and fatty liver) by visual inspection from the US images because the change in image is frequently very subtle [2]. The visual criteria for differentiating diffuse diseases are not objectively defined and thus are highly dependent upon the professional's skills. This may cause a biased diagnostics and a serious limitation in its reproducibility [3]. To date, liver biopsy remains the gold standard to grade liver fibrosis, and there is a well identified need for the development of alternative non invasive means of liver characterization.

In the past three decades, researchers have worked on different methods to characterize in vitro and in vivo liver tissue with ultrasound. The three most common US parameters are the ultrasonic wavespeed, attenuation coefficient and integrated backscatter coefficient [4–6]. Multiparametric studies for liver characterization have been conducted by several authors. One of the most complete studies is from [7] that investigated the performance of first and second order statistics and texture parameters from in vivo RF and B-mode scans (3 MHz center frequency). From the 18 parameters tested it was found that nine of them were reasonably uncorrelated. In a following paper of the same group [8], these parameters have been applied to discrimination between normal and various classes of diffuse liver disease. The area under the Receiver Operating Characteristic (ROC) curve ranged from 88% to 97%, depending on the disease class, obtained with only two or three parameters (selected by discriminant analysis). On the same study, it was tested the performance of the three parameters proposed by Lizzi et al. [9], resulting in essentially the same range ROC curve areas. Finally, the parameters proposed by Insana et al. [10] and Garra et al. [11]—attenuation coefficient and texture from a diffuse/structural scattering model were also applied to the same data bearing a performance 5–15% lower than the others.

Another kind of multiparametric approach was presented by Sarvazyan et al. [12] in which a new concept of medical imaging is proposed. The idea is to iteratively compare the received radiation field with the one calculated by a simulation model constructed from a knowledge database (anatomy, histology, pathology, physical tissue properties, etc.). A pictorial 2D or 3D model of the object would come up as a result of the iterative minimization of the difference signal, which would be more exact for inherently including the relevant biomedical data.

More recently, elastography was proposed to image tissue elasticity and has been applied to in vitro and in vivo liver fibrosis grading [13,14]. In these studies, liver elasticity measurements were correlated to fibrosis grade.

The early diagnosis and grading of fibrosis is important because it offers the opportunity of a complete

recovery with proper medication and also opens the possibility to following up its evolution thus improving the chances for the patient.

On the other hand the availability of diagnostic ultrasonic devices at very high frequencies (10–20 MHz) for clinical applications in dermatology, ophthalmology, endoluminal imaging and laparoscopic imaging encouraged the investigation of high frequency quantitative ultrasonography for liver characterization [15]. Although the penetration of ultrasound at 20 MHz is not sufficient to exam the liver non invasively, applications such as laparoscopic or intraoperative imaging (IOUS) in human may be accessible. In addition, such high ultrasonic frequencies may be used in small animal studies to test new antifibrotic therapeutic strategies.

The main purpose of this paper is to investigate the possibility to discriminate between differents stages of liver fibrosis with the simultaneous use of three ultrasonic parameters namely: wavespeed, attenuation coefficient and integrated backscattered coefficient with a 20-MHz pulse-echo system applied to in vitro human liver samples at different pathological degrees.

This paper is organized as follows. Section 2 describes materials and methods to evaluate the ultrasonic parameters for the human liver samples. We briefly present the histological aspects as well as statistical tests performed. Then, in Section 3 we present the results. Discussion and conclusion are in Section 4.

2. Materials and methods

2.1. Specimens

In this paper 21 specimens of human liver obtained during hepatectomy were used. The samples were immediately frozen to $-20\,^{\circ}\mathrm{C}$ and stored at this temperature until experiments. For ultrasonic measurements, the samples were left at room temperature and let be unfrozen naturally. A slice of 0.5-cm thickness was then carefully cut to assure uniform thickness and parallel surfaces. Each sample was degassed for 25–30 min at a low pressure while immersed in physiological saline solution. Before the signal acquisition, the sample and saline water were gently warmed to an average temperature of 35 \pm 2.0 °C.

2.2. Imaging system for RF signal acquisition

Images and radiofrequency (RF) signals backscattered from the specimens were acquired with an *acoustic backscatter microscopy* system with a 20-MHz center frequency transducer (Panametrics M316, 0.125 in. diameter, 0.75 in. focal length) (Panametrics, Waltham, MA, USA). The transducer, placed in a saline water bath, was located above a steel plane reflector at its focal

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