20-MHz ULTRASONIC INTEGRATED BACKSCATTER COEFFICIENT ESTIMATION OF IN VITRO HUMAN LIVER SAMPLES

M. Meziri[1], W.C.A. Pereira[2], A. Abdelwahab [3], P. Laugier[3] and J-A. Grimaud[3]

1 LSC Département de physique, Faculté des Sciences, Université M. Badji, Annaba Algérie.
[2]Biomedical Engineering Program, Federal University of Rio de Janeiro COPPE/UFRJ, Brazil.
[3]Laboratoire d'Imagerie Paramétrique (LIP) UMR CNRS 7623, University of Paris VI, Paris, France.
mmahmouddz@yahoo.fr

Abstract- The aim of this work is to test the discriminant power of the Integrated Backscattering Coefficient (IBC) for *in vitro* human liver fibrosis characterization. The backscattered RF signals from 21 human liver samples were collected with a 20MHz central frequency transducer (bandwidth 6-30MHz), within a 15mm² area of each specimen. To estimate the IBC the substitution method was used: the ratio of the echoes' power spectra with and without the specimen is obtained, after corrected for diffraction and attenuation, and then normalized by the sample thickness. The liver samples were grouped in five different disease stages (F0, F1, F2, F3, F4) by the Metavir Scoring System. The IBC mean and standard deviation were obtained for each group. The Kolmogorov-Smirnov statistical test was applied and pointed to significant differences ($p<0.05$) between the normal (F0) and pathological (F1, F3, F4) groups and also when comparing the groups F1, F3, F4 among themselves. On the other hand there was no significant difference ($p>0.05$) when comparing the normal (F0) and the cirrhosis (F4) groups. The originality of this study is based on the application of this bandwidth to liver characterization. Although in a limited number of specimens, the results point to two basic conclusions: within this bandwidth (a) it seems to be possible to differentiate degrees of liver fibrosis and (b) cirrhotic liver seems to have similar acoustic backscattering amplitudes as normal ones. The reason for this similarity is yet to be investigated so one is able to determine the limits of the IBC as a diagnostic tool. It is being considered a multiparametric approach using backscatter and attenuation aiming at better discrimination between groups.

Introduction

It is well known that the magnitude and frequency dependences of an acoustic echo signal spectrum are closely related to structural and elastic properties of the interacting medium [1]-[3]. Liver fibrosis causes an important modification in the tissue micro-architecture as well as in its elastic properties, thus changing its echographic signature. Some of these modifications can be identified in the image but they are usually not specific and too dependent on the judgment of the observer. Methods for detecting these modifications by ultrasound (US) have been a frequent topic of investigation in recent years. These modifications are accompanied by changes in US backscatter relative to that of healthy liver thus making the ultrasonic backscatter coefficient a potential parameter for characterization of the liver [4], [5]. Previous works on *in vivo* backscatter coefficient for healthy liver [6], [7] are in good agreement with *in vitro* work [4]. All these works have used frequency intervals below 10MHz. The objective of this work is to measure the Integrated Backscattering Coefficient (IBC) in 20MHz US signals from human liver *in vitro* samples with 4 different fibrosis degrees and test the capacity of this parameter to identify them.

Material and methods

Specimens

In this paper it was used 21 specimens of human liver. A slice of 0.5 cm thickness was carefully cut to insure uniform thickness and parallel cut surfaces. The sample degassed for 25 - 30 minutes at a low pressure while immersed in physiological saline solution. Before the acquisition, the sample and saline water were gently warmed to 25-35° C.

Imaging system for RF signal acquisition

Images and RF signals backscattered from the specimens were acquired with an *acoustic backscatter microscopy* system (ABM) with a 20-MHz center frequency transducer (Panametrics M316, 0.125" diameter, 0.75" focal length). A 6-to-30MHz bandwidth at -6dB was estimated from a specular echo from the plane steel reflector placed in a saline water bath at the focal position. The transducer was located above the steel plane reflector at its focal distance, adjusted perpendicularly to the the plane reflector (Fig. 1). It was mechanically displaced along a linear axis. A first scan was made in a 5x3-mm plane parallel to the reflector (matrix of 30 by 20 sites with 0.2-mm steps). At each site, the RF echo signal was received and amplified (Model 5052 PRX Sofranel, France), and sampled at 100 MHz with a digital oscilloscope (Lecroy 9350AL 500 MHz), then transferred to a computer for real-time acquisition and storage. The linearity of the acquisition gain was verified in the bandwidth.

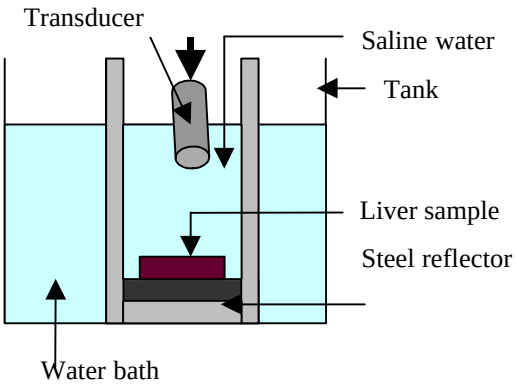

Figure 1. Experimental setup

Histology

Histological sections were made for each measured region of the liver. The sections for light microscopy were stained according to the Goldner trichrome technique. The liver samples were grouped in five different disease stages (F0, F1, F2, F3, F4) following the Metavir Scoring System. This classification was used as the gold-standard to compare with the IBC findings.

Backscatter coefficient estimation

The parameter used for measuring scattering for biological tissue is the backscatter coefficient (BSC). To correct for diffraction and system response each average backscattered power spectrum was normalized by the power spectrum of the specular echo from a perfect reflector placed in saline water at the same distance from the transducer. The next step was compensating the spectrum for attenuation. The estimation of BSC was evaluated using the following equation expressed in decibels [8]:

$$h(f,z) = 10\log_{10}\left\{\frac{\langle \cdot s(f,z)\cdot^2 \rangle}{\langle |s_{ref}(f,F)|^2 \rangle} C_A(f,z') \frac{1}{d} \frac{R_p^2 k^2 a^2}{8p\left[1+\left(\frac{ka^2}{4F}\right)^2\right]}\right\} \quad \textbf{(1)}$$

where:

f: the frequency.

z: the depth.

$h(f,z)$: backscatter coefficient in the liver.

$\langle |s(f,z)|^2 \rangle$: Average spectrum of the backscattered signal from the specimen placed at the focal region.

$|s_{ref}(f,F)|^2$: Average spectrum of the backscattered signal from plane reflector placed at the focal region.

$C_A(f,z')$: attenuation correction term.

R_p : Reflection coefficient from the plane reflector.

K :wave vector.

A : Transducer radius.

F : Transducer focal distance.

Five backscattered power spectra $h(f,z)$ were obtained at different depth z of each liver sample within the bandwidth. The integrated backscatter coefficients (IBC) were estimated using the equation below (reference for equation?):

$$IBC_{dB} = \frac{\int_{f_{min}}^{f_{max}} [h(f)]_{dB}\, df}{f_{max} - f_{min}} \qquad (2)$$

To calculate the IBC, the attenuation coefficient of the sample must be known. In this study we have used the attenuation coefficients measured on the same specimens in our previous work [9].

Statistical analysis

The statistical tests were made using the NCSS 97 software. The Kolmogorov-Smirnov test was used with a risk level $\alpha = 0.05$ to differentiate between healthy and fibrosis liver tissues. The same test was also used to classify the different fibrosis levels.

Results

In Fig. 2 one can see an example of the backscatter coefficient curves inside the frequency band pass, for different depth and corrected for the attenuation.

The 21 liver samples were scanned acquiring 416 backscattered US signals for each sample. Then the IBC was obtained for each sample by averaging the IBC for the different depths of each of them.

Figure 3 shows the IBC estimates from 5 specimens of normal human liver and 16 from fibrotic liver. The mean and the standard deviation of the measurements for each group are respectively –31.2 ± 2 dB and –24.6 ± 6 dB. One can see that the IBC for the normal samples are bigger than the ones for the fibrotic samples.

Figure 4 shows the measurements of the IBC from the four groups. The mean and standard deviation values for the IBC obtained were: -31.17 ± 1.98 dB, -24.73 ± 2.65 dB, -17.63 ± 1.97 dB and -30.27 ± 2.18 dB respectively for the groups F0, F1, F3 and F4.

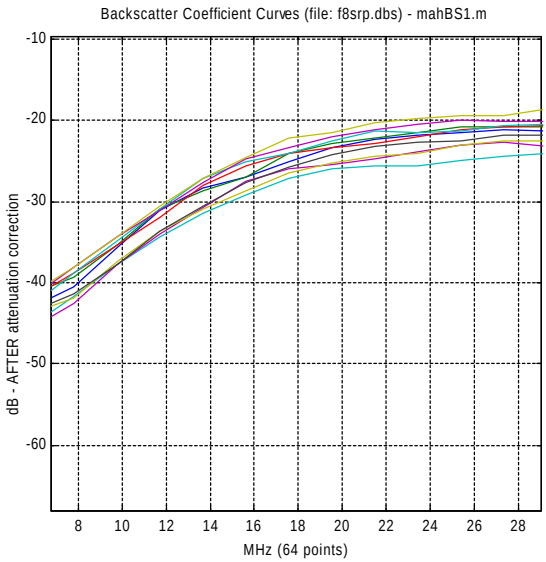

Figure 2. Plot of the Backscatter Coefficient curves for different depths, after attenuation correction, inside the frequency bandpass (6-30 MHz).

The Kolmogorov-Smirnov statistical test was applied to the two groups of Fig. 3 and the result pointed to significant differences ($p<0.05$) between the normal (F0) and fibrotic stages (F1, F3, F4). The same test was also applied to the results in Fig. 4 and showed significant differences when comparing the groups F1, F3, F4 among themselves. On the other hand there was no significant difference ($p>0.05$) when comparing the normal (F0) and the cirrhosis (F4) groups (Figure 5).

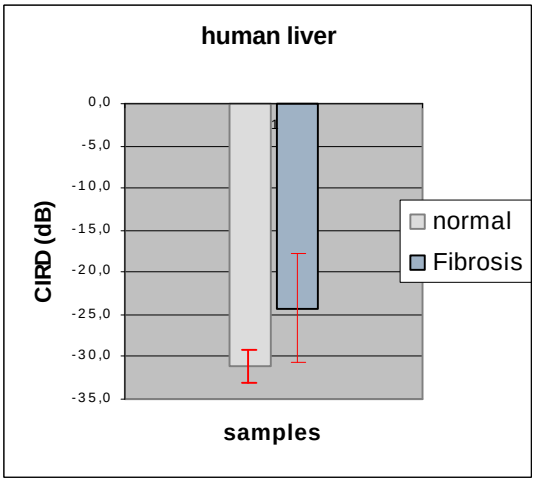

Figure 3. Comparison of the integrated backscatter coefficients for normal human liver n=5) and fibrotic liver (n=16) samples. Error bars represent ± 1 SD of results among sample of each group.

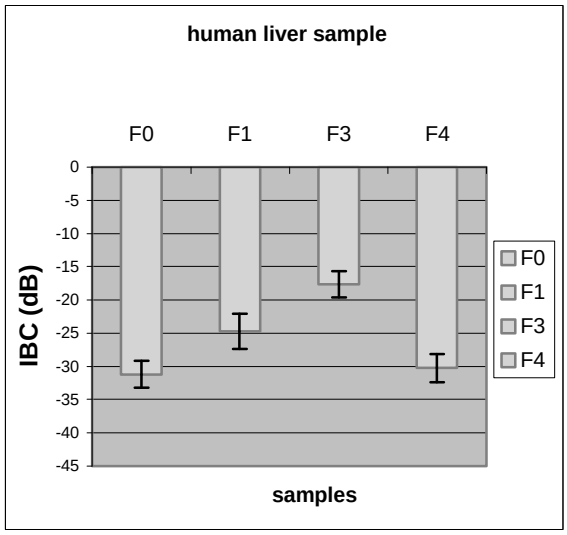

Figure. 4: The integrated backscatter coefficient with the 20-MHz transducer for healthy and fibrotic human liver. Error bars represent ± 1 SD of results among sample of each group.

Discussion and Conclusion

Diffuse liver diseases are a common clinical condition. The severity and extent of the disease ranges from mild fatty infiltration to cirrhosis.

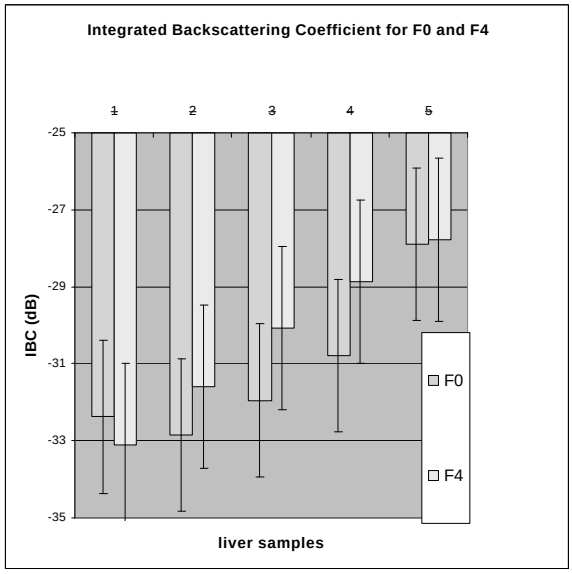

Figure 5: IBC in healthy human liver (Fo) and in cirrhotic liver (F4) samples. A large overlap exists between the two groups.

Integrated Backscatter Coefficient has been measured for 21 *in vitro* human liver samples with a 20-MHz central frequency transducer (bandwidth 6-30MHz) for the first time.

We remarked that the IBC exhibited a discriminating power to differentiate between healthy and pathological samples. Perhaps it can be used

during their treatment follow up [12]. Nevertheless care must be taken when dealing with the extreme cases (Normal X Cirrhosis) when the IBC alone is inconclusive. This has also been mentioned by Zheng [10].

The results presented here cannot be directly compared to previously reported results obtained for lower frequency bandwidth, for example [2] and [11].

Although obtained in a limited number of specimens, the results point to two basic conclusions: within this bandwidth (a) it seems to be possible to differentiate several degrees of liver fibrosis and (b) the cirrhotic liver seems to have similar acoustic backscatter amplitudes as the normal one. The reason for this similarity is yet to be investigated so one is able to determine the limits of the IBC as a diagnostic tool. Further developments will be based on a multiparametric approach using backscatter and attenuation aiming at better discrimination between groups.

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