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● *Original Contribution*

LIVER ELASTICITY IN NASH PATIENTS EVALUATED WITH REAL-TIME ELASTOGRAPHY (RTE)

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Abstract—Liver elasticity as assessed by real-time elastography (RTE) has been shown to be correlated to liver fibrosis in various chronic liver diseases. The aim of our study was to assess the RTE performance in the evaluation of liver fibrosis in nonalcoholic steatohepatitis (NASH), as well as the histopathologic variables determining the eventual discordance between the RTE-predicted and the biopsy-proven fibrosis. Fifty-two consecutive biopsy proven NASH patients and 20 controls were studied. Liver tissue elasticity measurements were performed using the Hitachi EUB-8500 sonographer and the EUP-L52 Linear (3–7 MHz) probe. RTE liver tissue mean elasticity (TME) values were calculated and correlated to the histologic fibrosis, activity and steatosis scores. A decrease in TME was observed with increasing fibrosis ($r = -0.75$). Similarly, TME varied together consistently with steatosis ($r = -0.3$). In contrast, TME did not show any correlation with the severity of inflammation. Multiple regression analysis showed that fibrosis was the only variable able to significantly ($p < 0.0001$) modify TME values. The diagnostic accuracy of TME measurement for $F > 0$ evaluated by AUC-ROC analysis was 0.86. The diagnostic accuracy of TME measurement for $F \geq 2$ was 0.92. We suggest that RTE could be used as a complementary imaging method to evaluate liver fibrosis in NASH patients. Future studies of larger patient cohorts are necessary for the validation of the technique. (E-mail: aorlacchio@uniroma2.it) **© 2012 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Nonalcoholic steatohepatitis, Liver fibrosis, Real-time elastography, Liver steatosis.**

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognized cause of liver-related morbidity and mortality. It represents a spectrum of hepatic disorders that ranges from simple hepatic steatosis without fibrosis and concomitant inflammation to hepatic steatosis characterized by fibrosis associated to a necroinflammatory component (nonalcoholic steatohepatitis [NASH]) (Law and Brunt 2010). In NASH patients, liver fibrosis is an important factor associated with prognosis. Hence, a precise evaluation of the severity of fibrosis is necessary in NASH patients, to perform a correct staging and, eventually, to take a decision regarding the treatment (Brunt and Tiniakos 2010; Neuschwander-Tetri and Caldwell 2003; Kleiner et al. 2005). Currently, fibrosis is staged by liver biopsy (Brunt and Tiniakos 2010). However, liver biopsy is invasive and limited by hazard and discomfort to the patient (Bravo et al. 2001). In recent years, these limitations have led to the development of noninvasive procedures for the assessment of liver fibrosis. These include assays based on serum biomarkers (Manning and Afdhal 2008) and liver stiffness measurements such as magnetic resonance imaging (MRI) or ultrasound based elastography (Huwart et al. 2008; Ophir et al. 1991, 2000). Transient elastography (TE) (FibroScan, EchoSens, Paris, France) is easy to use and shows results immediately. TE evaluates the degree of liver fibrosis through the detection of the propagation speed of a shear wave generated by an ultrasonographic probe into the liver (Friedrich-Rust et al. 2009; Nguyen-Khac 2007). Real-time elastography (RTE) has recently been suggested to be a useful tool to estimate tissue elasticity. Indeed, RTE has mainly been used to study superficial organs such as breast and thyroid (Ophir et al.

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2000). RTE was developed in Japan for the visual assessment of tissue elasticity integrated in a sonography machine. RTE calculates tissue elasticity in real-time from the degree of tissue distortion when subtle compression is applied to the tissue (Dowell 2008; Shiina 1999). Only recently has RTE been employed to evaluate liver fibrosis by measuring liver elasticity (Friedrich-Rust et al. 2008; Fujimoto et al. 2007; Morikawa et al. 2011; Okada et al. 2005; Popescu et al. 2009; Săftoiu et al. 2007; Tatsumi et al. 2008). To our knowledge, there are no studies investigating the contribution of RTE in the assessment of fibrosis in NASH patients, since most of the authors who studied the accuracy of RTE in the diagnosis of liver fibrosis have focused on chronic viral hepatitis (Friedrich-Rust et al. 2008; Fujimoto et al. 2007; Morikawa et al. 2011; Okada et al. 2005; Popescu et al. 2009; Săftoiu et al. 2007; Tatsumi et al. 2008). With this in mind, we set out to assess the RTE performance in a cohort of NASH patients, as well as the histopathologic variables determining the eventual discordance between the RTE-predicted and the biopsy-proven fibrosis.

PATIENTS AND METHODS

Patients

This is a retrospective single-centre study, conducted at a diagnostic imaging department in collaboration with a liver unit from July 2008 until December 2010 investigating the impact of liver fibrosis, steatosis and inflammation on liver elasticity as measured by real-time elastography (RTE) in NASH patients. Fifty-two consecutive NASH patients (18 men and 34 women, mean age 53 $\pm$ 13 years) were selected from a pretreatment cohort of biopsy proven NASH patients (Kleiner et al. 2005). All patients had a histologic NAFLD activity score (NAS) $\geq$5, alcohol intake $<$30 g/day, no history of blood transfusion, no positive tests for anti-HCV, no anti-hepatitis B virus (HBV) or anti-human immunodeficiency virus (HIV) antibodies. Patients with a history of alcohol consumption ($\geq$30 g/day in men and $\geq$20 g/day in women) were also excluded from the study. The study was approved by our institutional review board (IRB). The patients provided written informed consent before the beginning of the study in accordance with the Declaration of Helsinki. Twenty control subjects were included in the study. All controls had no history of liver disease, alcoholism, blood transfusion, positive tests for anti-HCV, anti-hepatitis B virus (HBV) or anti-human immunodeficiency virus (HIV) antibodies and type 2 diabetes mellitus. All controls had a body mass index (Kg/m^2) less than 27, cholesterol level (mg/dL) less than 200, triglyceride level (mg/dL) less than 170 and no evidence of fatty liver at ultrasound examination.

Liver histology

Core liver biopsy specimens minimum 15 mm in length deep to Glisson's capsule in the right liver lobe were obtained 3 weeks before the RTE examination from each NASH patient. Liver biopsies were performed in the right liver lobe under ultrasonographic guidance. The specimens were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin (H&E) and Masson trichrome stains for histology evaluation. The slides were evaluated by two expert pathologists in consensus. Histologic fibrosis, activity and steatosis were scored according to NASH Clinical Research Network Scoring System Definitions and Scores (Kleiner et al. 2005). The patients were stratified according to their steatosis grade, lobular inflammation grade and fibrosis stage. Fibrosis stage 1, 1A, 1B and 1C were grouped together. Only patients with a NAS score $\geq$5 were included in the study (Kleiner et al. 2005).

Real-time elastography (RTE)

The Hitachi EUB-8500 ultrasound scanner and the EUP-L52 Linear (3-7 MHz) (Esaote/Hitachi Medical Corporation, Tokyo, Japan) probe were used. Patients were examined in supine position with the right arm elevated above the head. Liver regions of interests (ROIs) free of large vessels (20 mm length $\times$ 20 mm breadth placed 10 mm below the surface of the liver) were evaluated both in the left and in the right lobe of the liver. The equipment displays real-time tissue elasticity images showing the ROI as a semitransparent, colored area, juxtaposed with B-mode images (color coded images). The colors in the ROI range from blue to red to show the relative hardness and softness of tissues inside the ROI.

Color coded images were acquired under the heartbeat or abdominal aorta. Since a single frame corresponds to a cardiac cycle, and given the fact that a cardiac cycle lasts approximately 0.85 s and that our examination lasts 5 s, a single elastosonographic image was the result of the mean of six different elastosonographic frames.

The probe was kept in position manually while the subject briefly held his or her breath. We were sure that the liver moved in an axial direction with B-mode image and the software automatically analyzed the frames corresponding to heartbeat. The acquisitions were considered reliable only if a pressure of 3–4 on a scale of 0–6 arbitrary units was recorded. The three measurements were performed 10 mm below the liver surface in right and left liver lobe, respectively, at the level of the V, VI and VIII segments and of the II-III segments on the medial and lateral site. The position varied slightly from patient to patient since major vessels had to be excluded from the ROI analysis. Thus, a fixed position for each patient could not be determined. These