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● *Original Contribution*

SUPERSONIC SHEAR WAVE IMAGING OF THE SPLEEN FOR STAGING OF LIVER FIBROSIS IN RATS

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Abstract—The goal of the work described here was to explore the cause of spleen stiffness (SS) in hepatic fibrogenesis and evaluate the value of SS in liver fibrosis (LF) staging. LF was induced with carbon tetrachloride (CCl_4) in rats (n = 40). Supersonic shear wave imaging and contrast-enhanced ultrasound were performed to determine liver stiffness (LS), SS and splenic hemodynamics. SS, LS and free portal pressure exhibited moderate correlations with fibrosis stage ($r = 0.744–0.835$, $p < 0.001$). Time–intensity curves of contrast-enhanced ultrasound for the spleen were presented as decreasing peak intensity and slope of decrease, and increasing time to peak. Splenic sinus dilation and congestion were observed on histopathologic analysis. The area under the receiver operating characteristic curve of SS was higher than that of LS for differentiating LF stages 0–2 from stages 3–4 ($Z = 2.293$, $p = 0.02$). SS is a reliable diagnostic marker for the assessment of LF in the CCl_4 model, especially for severe fibrosis. Elevated portal pressure is the cause of increasing SS. (E-mail: qianlinxue2002@163.com) © **2017 Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Liver fibrosis, Spleen stiffness, Liver stiffness, Portal pressure, Contrast-enhanced ultrasound.**

INTRODUCTION

Liver fibrosis is a pathologic process caused by various etiologies and characterized by intra-hepatic diffuse excessive deposition of the extracellular matrix (Friedman 2008; Lee and Friedman 2011). The non-invasive diagnosis of liver fibrosis stage has important clinical applications for determining both disease prognosis and treatment (Castera et al. 2005; Ngo et al. 2006; Park et al. 2013; Wong et al. 2010b). Furthermore, it is also crucial for monitoring treatment response (Andersen et al. 2011; Fung et al. 2011). In the past 10 y, ultrasound elastography has gained unanimous recognition in the field of liver diseases (Andersen et al. 2011; Castera et al. 2005; Fung et al. 2011). To date, several ultrasound elastography techniques have been applied to the diagnosis of chronic liver disease, including transient elastography (TE), acoustic radiation force impulse imaging (ARFI) and shear wave elastography (SWE) (Chen et al. 2012; Grgurevic et al. 2015; Wong et al. 2010a). A meta-analysis that included 18 studies with a total of 2772 patients revealed that TE had excellent diagnostic accuracy in quantifying liver fibrosis in patients with chronic hepatitis B. Chon et al. (2012) reported that the mean areas under the receiver operating characteristic curve (AUROCs) for the diagnosis of significant fibrosis (F2), severe fibrosis (F3) and cirrhosis (F4) was 0.859 (95% confidence interval [CI], 0.857–0.860), 0.887 (95% CI, 0.886–0.887) and 0.929 (95% CI, 0.928–0.929), respectively.

Liver stiffness (LS), however, depends on many factors. The first and main factor is collagen deposition in the extracellular matrix. Another important factor is pressure in the liver, which is either hydrostatic or osmotic, as hepatic lobules are covered with the non-extensible Glisson's capsule of connective tissue. In cases of liver congestion, hydrostatic pressure is high, and therefore, LS increases (Millonig et al. 2010). During inflammatory episodes, the accumulation of interstitial liquid and inflammatory infiltrate increases osmotic pressure and, consequently, increases LS (Chang et al. 2009; Sagir et al. 2008).

Extensive research on the use of ultrasound elastography in chronic liver disease allows us to conclude that splenic elasticity parameters positively correlate with the severity of liver cirrhosis and presence of esophageal

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varices caused by changes in portal vein pressure (Colecchia et al. 2012; Fraquelli et al. 2014). In addition, previous studies have established that together with the increase in liver fibrosis stage, there is also a trend toward an increase in spleen stiffness (SS) (Grgurevic et al. 2015; Leung et al. 2013). Moreover, the diagnostic accuracy of TE imaging of the spleen for staging liver fibrosis is similar to that for the liver (Hu et al. 2014; Stefanescu et al. 2011). Furthermore, there was no significant difference between the diagnostic accuracy of spleen SWE and liver TE in any stage of fibrosis (Leung et al. 2013). On the other hand, the AUROCs of SS were reported to be lower than those of LS for distinguishing the different stages of liver fibrosis (Grgurevic et al. 2015).

Therefore, the exact cause of changes in splenic elasticity during liver fibrosis remains unknown. Splenic pulp pressure (SPP), an index of portal venous pressure, was found to be increased in murine CCl_4-induced liver fibrosis (Theodorakis et al. 2009). We hypothesized that portal venous pressure might be responsible for the changes in splenic elasticity. The aim of this study was to evaluate the value of SS in estimating liver fibrosis, thereby verifying our hypothesis.

METHODS

Ethics statement

This study was performed according to the *Guide for the Care and Use of Laboratory Animals*, and all procedures involving animals were approved by the Committee for Institutional Animal Care and Use of our hospital (IACUC Protocol 12-5001).

Animal model and experimental design

Sprague-Dawley rats (200–220 g) were obtained from Huafukang Biotechnology (Beijing, China) and housed in sterile isolated cages with a 12-h light/dark cycle at room temperature (20 ± 2°C) with a relative humidity of 30%–50%. Animals were fed sterilized food and had access to water. All animals used in this study were allowed to acclimate to the animal research facility for 1 wk before the beginning of the experiment.

Fifty Sprague-Dawley rats were divided into two groups: 40 rats were used for the experimental model of liver fibrosis, and 10 rats were used for the control group. All stages of liver fibrosis (S1–S4) were induced in the rats at different times after CCl_4 treatment. Fifty percent CCl_4 dissolved in olive oil was injected by oral gavage twice a week at a dose of 0.2 mL/100 g rat weight in the fibrosis group, whereas olive oil was injected by oral gavage at the same dose in the control group. At the second, fourth, sixth and eighth weeks after the beginning of the experiment model, 10 treatment rats and 2 or 3 control rats were randomly selected for ultrasonography. After ultrasonography and portal pressure measurement, rats were sacrificed, and right liver lobes were fixed in 10% buffered formalin for histologic assessment.

Contrast-enhanced ultrasound

Contrast-enhanced ultrasound (CEUS) was performed using an IU22 system (Philips Healthcare, Amsterdam, Netherlands) and a broadband linear array transducer with a frequency of 5–12 MHz (element number: 256, center frequency: 7.5 MHz). Real-time harmonic contrast imaging with a mechanical index of 0.08 was employed. Scanning depth was maintained at 2.5 cm. A single focus point was placed at the level of the lower edge of the spleen, and all parameters were consistent for all animals.

The second-generation contrast agent SonoVue (Bracco Diagnostic, Milan, Italy), containing sulfur hexafluoride-filled microbubbles, was used for CEUS imaging. After being fasted for 8 h, rats were anesthetized with pentobarbital sodium (40 mg/kg). After conventional ultrasonography, the probe was placed in the subcostal position to display the spleen section. A bolus of SonoVue (0.1 mL/kg) was then administered to each rat through a 22-gauge (22G) tail vein catheter. The bolus of contrast agent was immediately followed by an injection of normal saline (0.3 mL). Real-time contrast imaging clips of 180 s were stored for later measurement and analysis.

Time–intensity curves (TICs) of CEUS for the spleen parenchyma were generated with QLab quantification software on the IU22 system. Regions of interest (ROIs) were carefully placed on each section of the spleen to avoid intra-splenic large vessels. Arrival time (AT) was the time the contrast agent arrived at the spleen parenchyma. The time between the peak and the start of contrast enhancement of the spleen parenchyma was defined as the time to peak (TTP). Peak intensity (PI) was the maximum intensity of the TIC in the spleen parenchyma. The slope from PI to ½PI was defined as the slope of decrease (SOD). We used the formula

$$\mathrm{SOD} = 1/2\mathrm{PI(db)}/\Delta T\mathrm{(s)}\times 100\% \quad (1)$$

where ΔT is the time from PI to ½PI during washout of the contrast agent (Fig. 1).

Supersonic shear wave imaging

Supersonic shear wave imaging (SSWI) is an ultrasound-based technique for real-time visualization of soft tissue elasticity properties. In this technique, shear waves are generated by focused ultrasound, and speeds of the shear waves (V_S) are detected in real time with ultrafast ultrasound imaging (up to 20,000 fps). This