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● *Original Contribution*

MEASUREMENT OF LIVER STIFFNESS USING SHEAR WAVE ELASTOGRAPHY IN A RAT MODEL: FACTORS IMPACTING STIFFNESS MEASUREMENT WITH MULTIPLE- AND SINGLE-TRACKING-LOCATION TECHNIQUES

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Abstract—The clinical use of elastography for monitoring fibrosis progression is challenged by the subtle changes in liver stiffness associated with early-stage fibrosis and the comparatively large variance in stiffness estimates provided by elastography. Single-tracking-location (STL) shear wave elasticity imaging (SWEI) is an ultrasound elastography technique previously found to provide improved estimate precision compared with multiple-tracking-location (MTL) SWEI. Because of the improved precision, it is reasonable to expect that STL-SWEI would provide improved ability to differentiate liver fibrosis stage compared with MTL-SWEI. However, this expectation has not been previously challenged rigorously. In this work, the performance of STL- and MTL-SWEI in the setting of a rat model of liver fibrosis is characterized, and the advantages of STL-SWEI in staging fibrosis are explored. The purpose of this study was to determine what advantages, if any, arise from using STL-SWEI instead of MTL-SWEI in the characterization of fibrotic liver. Thus, the ability of STL-SWEI to differentiate livers at various METAVIR fibrosis scores, for *ex vivo* postmortem measurements, is explored. In addition, we examined the effect of the common confounding factor of fluid versus solid boundary conditions in SWEI experiments. Sprague-Dawley rats were treated with carbon tetrachloride over several weeks to produce liver disease of varying severity. STL and MTL stiffness measurements were performed *ex vivo* and compared with the METAVIR scores from histological analysis and the duration of treatment. A strong association was observed between liver stiffness and weeks of treatment with the liver toxin carbon tetrachloride. Direct comparison of STL- and MTL-SWEI measurements revealed no significant difference in ability to differentiate fibrosis stages based on SWEI mean values. However, image interquartile range was greatly improved in the case of STL-SWEI, compared with MTL-SWEI, at small beam spacing. (E-mail: jhlangdon@urmc.rochester.edu) **© 2017 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound elastography, Shear wave elasticity imaging, Liver fibrosis, Signal processing, Scholte waves.**

INTRODUCTION

Transient elastography (TE, FibroScan) has been established as a clinical tool for evaluation of liver fibrosis (Foucher et al. 2006). Liver stiffness estimates provided by TE have been reported to correlate not only with liver fibrosis, but also with hepatic inflammation and central venous pressure (Georges et al. 2007; Millonig et al. 2010). Additionally, steatosis has been found to alter the measured viscosity of liver independent of the fibrosis for magnetic resonance elastography and crawling wave elastography (Barry et al. 2012; Salameh et al. 2009). However, because TE does not account for the viscosity, steatosis also confounds the measurement of fibrosis. Collagen deposition in fibrosis is still considered to be a major contributor to stiffness, but these confounding factors must be considered when assessing the stiffness of the liver measured by elastography. Along these lines, several groups have investigated the application of alternative elastography techniques to fibrosis staging in animal and human studies (Chen et al. 2013a, 2013b; D'Onofrio et al. 2013; Palmeri et al. 2008; Salameh et al. 2009)

Single-tracking-location (STL) shear wave elasticity imaging (SWEI) is a technique used to estimate the shear stiffness of materials; acoustic radiation force (ARF) is used to generate shear waves that are tracked by pulse-echo imaging along a single tracking location (McAleavey et al. 2007, 2009). The utility of STL-SWEI in the setting of liver fibrosis in a rat model is

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unclear. We previously reported that STL-SWEI provides better precision than similar multiple-tracking-location (MTL)-SWEI based techniques (Elegbe and McAleavey 2013), and this has been verified independently by Hollender et al. (2015). However, it is unknown whether this offers any advantage to STL-SWEI in the setting of estimating fibrosis score. Thus, we sought to compare these two methods in the setting of a liver fibrosis model.

In this work, estimates of the liver stiffness are made comparing both STL and MTL measurements using a cross-correlation of the pairs of impulsive shear wave excitations as in McAleavey et al. (2007) and using the GPU-based implementation discussed in Langdon and McAleavey (2015). The resulting measurements are compared with the METAVIR fibrosis scores for the livers as determined by histologic analysis by a board-certified pathologist. Previous shear wave dispersion ultrasound vibrometry (SDUV) studies have also examined the utility of shear wave elastography in rat liver fibrosis models (Chen et al. 2013b; Zhu et al. 2014a). Unlike in previous studies, we did not use a solid–solid boundary condition for our measurements as the time required to set samples in gelatin increases the coagulation following excision and typically requires the sample to cool to room temperature. However, this can lead to the production of a type of surface wave known as a Scholte wave, which we previously examined in the setting of engineered tissue characterization (Mercado et al. 2015). Therefore, the effect of these surface waves on SWEI measurements of *ex vivo* rat livers is also explored below.

METHODS

Animal preparation

Sixty-six male Sprague-Dawley rats, weighing 300–400 g, were acquired for this experiment after protocol approval by the University Committee on Animal Resources. The rats were housed at the University of Rochester School of Medicine and Dentistry vivarium in a 12 h:12 h light:dark cycle room with two rodents per cage. Rats were identified using an ear punch numbering system.

Following the dosing procedures of our previous study, 56 rats were treated with intra-peritoneal injections of a 1 mL/kg, 1:4 carbon tetrachloride:olive oil solution to induce liver fibrosis. Carbon tetrachloride is a well-known model for generating liver fibrosis that approximates alcoholic liver disease (McLean et al. 1969; Tamayo 1983). Injections were performed at the rat's vivarium housing unit three times per week. Our control group consisted of 10 rats that were euthanized and scanned without injection.

Carbon tetrachloride–treated rats were sacrificed at various time points ranging from 0 to 9 weeks with a roughly even distribution. Because the control rats were not treated, they were not sacrificed on a particular schedule. *Post hoc* analysis revealed a METAVIR fibrosis score progression rate of approximately one stage every 1 to 2 weeks. During the study, 3 rats died prematurely from complications of cirrhosis (approximately 5%). Three additional rats were unable to be analyzed because of inadequate histological samples. Finally, 2 other rats were excluded secondary to technical problems in the data acquisition process (introduced gas bubbles and incorrect imaging mode). As a result, 58 rats are included in our analysis.

Animals were euthanized and scanned with ultrasound at our laboratory facilities. All animals were euthanized by anesthetic overdose using either pentobarbital or a ketamine/xylazine mixture. Immediately on cessation of breathing, the animals were shaved, and *in situ* images were acquired. These images are not included in the analysis of this study. Subsequently, the liver was removed, and a series of *ex vivo* scans immediately followed. In the scan series, the left lobe, median lobe and right lobe were scanned in that order. Results for the left and median lobes are presented here. The caudate lobe was not used, as it was too small to scan with our system.

Ultrasound settings and processing

The imaging experiments described here were performed using a Siemens Antares ultrasound scanner and our in-house elasticity software (Langdon and McAleavey 2015). A VF 10-5 linear array transducer operating at 5.7 MHz, focal depth of 2 cm, pulse repetition frequency of 10.5 kHz and an F-number of 3.5 was used throughout the study. SWEI images were acquired using various tracking offsets and beam spacing, as discussed below. Each image was acquired 10 times to allow averaging of the speckle displacement data after windowed normalized cross-correlation of the raw A-line data. This is referred to here as an "averaging set" and requires 2–4 min of acquisition time at 12 s per image. This imaging rate allows for the transducer to cool between acquisitions but was limited mostly by data transfer time. The images were available for review in real time.

Following initial STL-SWEI linear modulus estimation, a region of interest (ROI) was manually segmented in our software by the end user. The segmentation of the SWEI data outlines the area of the B-mode image containing the rat liver. A median value and interquartile range was extracted from the SWEI data within this ROI. Of note, the STL-SWEI measurement will be referred to as the "apparent shear stiffness" because it is not the true shear stiffness caused by the boundary effects, as discussed below.

In post-analysis, the SWEI stiffness estimate means were sorted by fibrosis score. Statistical comparisons were performed using permutation tests on the mean difference between each pairwise fibrosis stage grouping. This type of statistical test is appropriate when the underlying distribution of the groups is not known to be Gaussian; a