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# Model-dependent and model-independent approaches for evaluating hepatic fibrosis in rat liver using shearwave dispersion ultrasound vibrometry

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ABSTRACT

This study assesses gradations of hepatic fibrosis in rat livers using both model-dependent and model-independent approaches. Liver fibrosis was induced in 37 rats using carbon tetrachloride ($CCl_4$); 6 rats served as the controls. Shear wave velocity as a function of frequency, referred to as velocity dispersion, was measured in vitro by an ultrasound elastography method called shearwave dispersion ultrasound vibrometry (SDUV). For the model-dependent approach, the velocity dispersion data were fit to the Voigt model to solve the viscoelastic modulus. For the model-independent approach, the pattern of the velocity dispersion data was analyzed by linear regression to extract the slope and intercept features. The parameters obtained by both approaches were evaluated separately using a receiver operating characteristic (ROC) curve analysis. The results show that, of all the parameters for differentiating between grade F0–F1 and grade F2–F4 fibrosis, the intercept had the greatest value for the area under the ROC curve. This finding suggests that the model-independent approach may provide an alternative method to the model-dependent approach for staging liver fibrosis.



## 1. Introduction

Liver fibrosis results from chronic damage to the liver in conjunction with excessive accumulation of extracellular matrix protein. The main causes of liver fibrosis include many types of chronic liver diseases, such as hepatitis virus infection and alcoholic and non-alcoholic fatty liver disease [1]. The gold standard for assessing the degree of fibrosis is biopsy. However, liver biopsy is an invasive procedure with potential complications such as bleeding and pain [2]. In addition, sampling errors may occur because an extremely small portion of the liver is sampled and liver fibrosis is heterogeneously distributed [3]. Therefore, reliable, simple and non-invasive methods for assessing liver fibrosis are needed [4,5].

Recently, a number of ultrasound-based elastography techniques, including strain elastography [6], transient elastography (TE) [7,8], acoustic radiation force impulse (ARFI) imaging [9], supersonic shear imaging (SSI) [10], and shearwave dispersion ultrasound vibrometry (SDUV) [11], have been applied to non-invasively measure the biomechanical properties of the liver for evaluating fibrosis. These techniques usually apply an external force or acoustic radiation force to induce a deformation or displacement in the soft tissue and detect the dynamic response of the soft tissue to these forces, which relates qualitatively or quantitatively to the mechanical properties of the soft tissue. Some recent review papers have summarized various current commercially available elastographic techniques and discussed their characteristics, limitations and suitability for specific clinical applications [12–14]. Of these methods, TE and ARFI have been widely used in clinical practice for the evaluation of liver fibrosis and have been validated in large cohorts of patients with chronic hepatitis B and C, and non-alcoholic fatty liver disease (NAFLD) [15–22]. For the patients with viral hepatitis, the detection of significant fibrosis (METAVIR score $\geq$ F2) is clinically important because it indicates that patients should receive antiviral treatment [23]. It is regarded that both TE and ARFI can provide reliable measurements of liver stiffness for assessing significant fibrosis and can help to reduce the use of liver biopsies [24].

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**Table 1**
Model-dependent and model-independent parameters at different fibrosis stages. Numbers in parentheses are normalized standard deviations (SD/mean).

| Model/non-model | Parameter | Fibrosis stage | | | | |
|---|---|---|---|---|---|---|
| | | F0 | F1 | F2 | F3 | F4 |
| Voigt | $\mu$ (kPa) | 0.81 (0.14) | 1.42 (0.13) | 1.91 (0.18) | 2.35 (0.17) | 3.53 (0.14) |
| | $\eta$ (Pa s) | 1.07 (0.12) | 1.22 (0.25) | 1.61 (0.17) | 1.64 (0.11) | 1.61 (0.21) |
| | Slope (mm) | 3.60 (0.15) | 3.00 (0.26) | 3.26 (0.19) | 3.19 (0.20) | 2.23 (0.13) |
| | Intercept (kPa) | 0.51 (0.24) | 1.02 (0.14) | 1.48 (0.21) | 1.61 (0.24) | 2.86 (0.15) |

**Table 2**
The AUROC, sensitivity, and specificity values of elasticity, viscosity, intercept and slope for staging significant fibrosis (METAVIR score $\geq$ F2).

| Parameter | AUROC | Sensitivity | Specificity |
|---|---|---|---|
| Elasticity | 0.98 | 0.90 | 1.00 |
| Viscosity | 0.89 | 1.00 | 0.64 |
| Intercept | 0.99 | 0.90 | 1.00 |
| Slope | 0.42 | 0.10 | 0.93 |

Most of the ultrasound-based elastography techniques use the group velocity of the shear wave to characterize the stiffness of liver. The underlying assumption is that the liver is purely elastic and the dispersion caused by viscosity is neglected. However, many recent studies have observed that dispersion of the shear wave occurs in the liver [25,26], which indicates that a viscoelastic description may be beneficial. Hence, a viscoelastic description of the mechanical behavior is more nearly accurate and physically correct than a purely elastic one. Furthermore, the viscosity as well as the elasticity of the tissue can provide useful information about the pathological state of the tissue [27]. Recently, some studies have examined frequency-dependent shear wave velocity, referred to as velocity dispersion then applied the dispersion to characterize the tissue in a model-dependent or a model-independent manner [28–32]. The model-dependent approach assumes that the behavior of the tissue under investigation accords with a certain physical model and fits the dispersive properties to the model to solve the model parameters [33]. Most of the studies that adopted this approach used the Voigt model to obtain the viscoelasticity of liver. Alternatively, the model-independent approach directly obtains features from the dispersive properties without any rheological model assumptions. Barry et al. used a linear function to fit the dispersion curve of the shear velocity and obtained the slope and intercept of the dispersion for assessing liver viscoelastic properties [30]. They hypothesize that increasing amounts of fat in the normal liver will increase the slope of shear wave dispersion, while slightly reducing the speed of sound [30]. This hypothesis was supported by some studies in phantoms and mouse livers [30,31]. In a recent paper, Parker reviewed the empirical findings across a number of studies and summarized the dispersion (or slope) range in lean and steatotic livers [34].

To date, the model-independent approach has only been used for steatosis. Although Barry et al. suggested increasing collagen content (more elastic) would increase the dispersion intercept [30], they did not confirm the model-independent approach in liver fibrosis. Information about the change of the dispersion pattern during fibrosis development is very limited. Our previous study measured the in vitro viscoelasticity of rat liver with hepatic fibrosis using shear waves induced by an acoustic radiation force [35]. The curve of the velocity dispersion was fitted using the Voigt model to derive the elasticity and viscosity. The objective of the previous study was to evaluate the diagnostic performance of viscoelasticity, as obtained by the model-dependent approach, for staging liver fibrosis. However, we observed several limitations of the model-dependent approach in that study and expected to investigate the feasibility of model-independent approach in the future study. This study is an extension of that previous work. The goal of this current study was to estimate liver fibrosis using both model-dependent and model-independent approaches and to systematically compare the performances of both approaches from a variety of perspectives.

## 2. Materials and methods

### 2.1. Animal model

Liver fibrosis was induced in male Sprague-Dawley (SD) rats weighing 200−220 g (Guangdong Medical Laboratory Animal Center, Guangdong, China). Thirty seven rats were given carbon tetrachloride ($CCl_4$) for different numbers of days to induce different stages of liver fibrosis (F1–F4) and six healthy rats were used as a control group (F0). 50% $CCl_4$ in olive oil was injected subcutaneously twice per week. The dose was 0.6 mL/100 g rat weight the first time and 0.3 mL/100 g the remaining times. Three, five, eight and ten weeks after the first injection, some rats (14, 5, 9, and 9, respectively) were sacrificed and the left lateral lobes of the rat livers were harvested for ultrasound measurements. The other lobes of the rat livers were fixed in 10% buffered formalin for histological assessment. The fibrosis stage was ultimately determined by the histological result, not by the length of time that the rats had been exposed to $CCl_4$. All of the procedures were approved by the Animal Care Committee guidelines of Shenzhen University and the Guangdong Medical Laboratory Animal Center.

### 2.2. Histological assessment

The excised liver tissues were fixed in 10% formalin solution for at least 24 h. After washing and dehydrating, they were embedded in paraffin and sliced to a thickness of 7 $\mu$m. The paraffin slices were stained with Masson's trichrome by histopathology technicians. Two slices from each rat were used for the histological assessment. The slices were analyzed using an Olympus BX41 microscope by pathologists who were blind to the results of the ultrasound measurements. The stage of fibrosis was evaluated according to the METAVIR scoring system [36].

### 2.3. Ultrasound measurements

The rat liver samples were measured in vitro by a custom-made ultrasound system, which can produce a radiation force and track the shear wave propagation using the SDUV technique. The excitation sequence for the radiation force consisted of 10 tone bursts, with each tone burst having a central frequency of