

DOI 10.1515/pjvs-2017-0008

Original article

Accuracy of real-time shear wave elastography in the assessment of normal liver tissue in the guinea pig (*cavia porcellus*)

K. Glińska-Suchocka, K. Kubiak, J. Spuzak, M. Jankowski, P. Borusewicz

Department of Internal Diseases with Clinic of Horses, Dogs and Cats, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, pl. Grunwaldzki 47, 50-366 Wrocław, Poland

Abstract

Shear wave elastography is a novel technique enabling real-time measurement of the elasticity of liver tissue. The color map is superimposed on the classic ultrasound image of the assessed tissue, which enables a precise evaluation of the stiffness of the liver tissue. The aim of the study was to assess the stiffness of normal liver tissue in the guinea pig using shear wave elastography. The study was carried out on 36 guinea pigs using the SuperSonic Imagine Aixplorer scanner, and a 1 to 6 MHz convex SC6-1 transducer. An ultrasound guided Try-Cut liver core needle biopsy was carried out in all the studied animals and the collected samples were examined to exclude pathological lesions. The mean liver tissue stiffness ranged from 0.89 to 5.40 kPa. We found that shear wave elastography is an easy, non-invasive technique that can be used to assess the stiffness of liver tissue. The obtained results can be used in future studies to assess the types and changes of liver tissue in the course of various types of liver disease.

Key words: guinea pig, shear wave elastography, liver

Introduction

In recent years, various innovative methods to assess the degree of liver fibrosis and liver damage have been introduced in human medicine. Liver fibrosis is a dynamic process that develops as a result of long-term activity of damaging agents due to an excessive production of connective tissue by stellate cells, which are activated by mediators of inflammation. As the liver fibrosis progresses, the distance between hepatocytes, blood vessels and bile tubules increases, and the liver tissue definition is lost (Bataller and Brenner 2005, Błazik and Durlik 2010, Zaleska-Dorobisz et al. 2015).

The first studies on the clinical utility of elastography were carried out in the 1990s by Ophira et al. (1991). Since then, there has been a rapid development of this technique.

There are three types of elastography which can be divided into two groups: static and dynamic ultrasound elastography (Gennisson et al. 2013).

Static elastography is carried out using an ultrasound transducer, which is applied to the examined tissue causing slight compression. The ultrasound machine analyses the tissue deformation caused by the transducer pressed against the tissue and presents the result in the form of a colour map superimposed on the classic ultrasound image. The repeatability of this

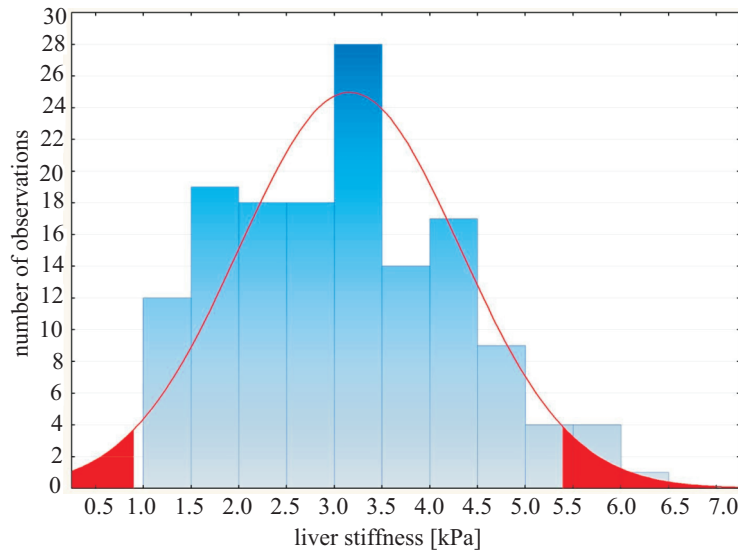


Fig. 1. The graph presents the distribution of stiffness obtained using elastography in healthy liver tissue in guinea pigs.

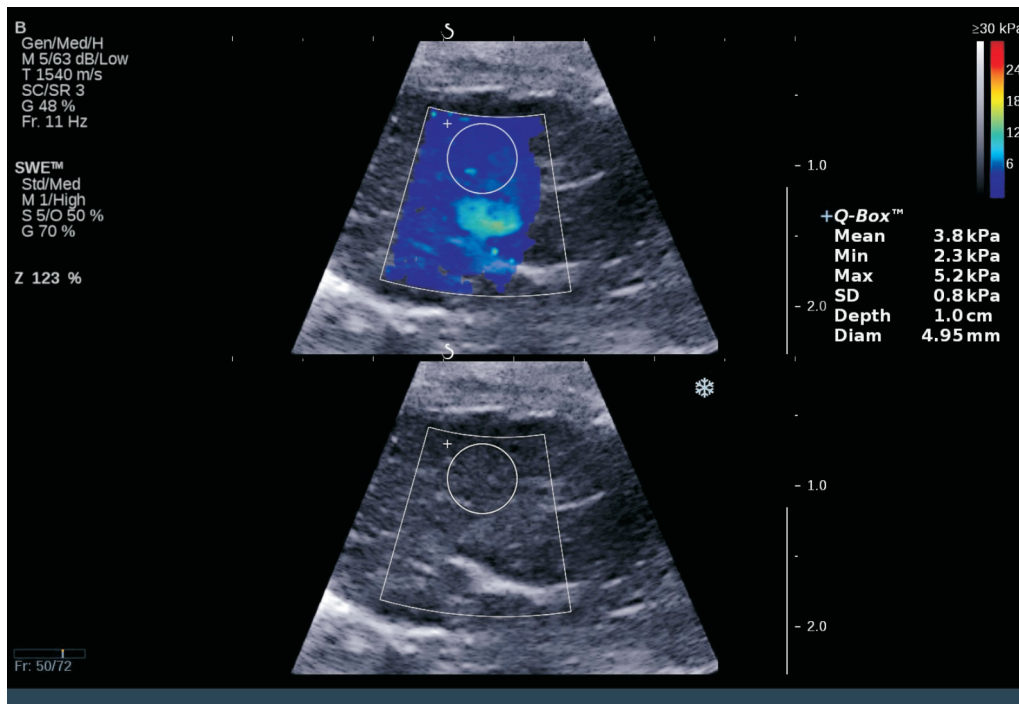


Fig. 2. Right liver lobe elasticity map obtained by using shear wave ultrasonographic elastography. The elasticity score was 3.8 kPa.

method depends on the pressure exerted by the investigator on the tissues using the transducer (Sebastiani 2005).

Dynamic elastography is used to assess tissue elasticity based on the speed of propagation of transverse waves through those tissues. There is an increase in the speed of propagation in stiffer tissues (Bhatia et al. 2010).

Transient elastography (TE), acoustic radiation force impulses (ARFI) and shear wave elastography

(SWE) are the most common methods used to assess liver fibrosis (Cosgrove et al. 2013).

SWE enables real-time assessment of the stiffness of liver tissue. The obtained results are presented as kPa. In addition, a colour map of the tissue stiffness is superimposed on a classic ultrasound image which enables the investigator to choose the site of the assessment of liver tissue stiffness (Pol et al. 2014).

The aim of the study was to assess the stiffness of normal liver tissue in the guinea pig using shear wave elastography.

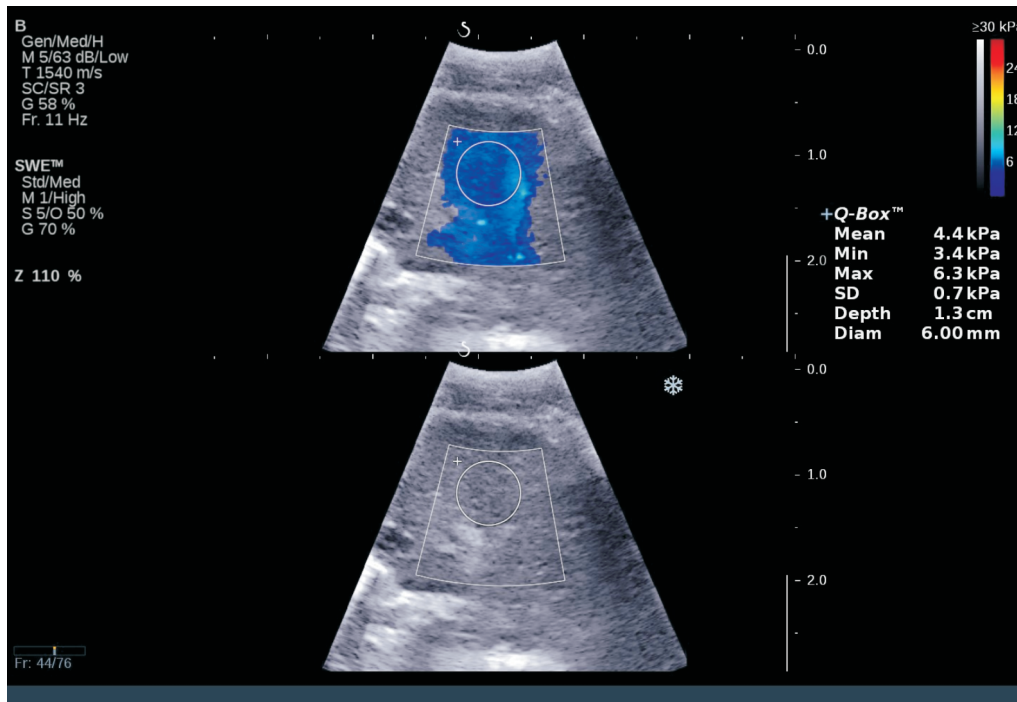


Fig. 3. Right liver lobe elasticity map obtained by using shear wave ultrasonographic elastography. The elasticity score was 4,4 kPa.

Materials and Methods

The study was carried out on 36 guinea pigs of both sexes (2 males, 34 females), between 5 and 6 months old. The ultrasound examination was carried out using the SuperSonic Imagine Aixplorer scanner, and a 1 to 6 MH convex SC6-1 transducer. Next, the site of the B-mode elastographic assessment was chosen. The elastographic study was carried out four times in accordance with elastographic study guidelines (Janczewska et al. 2015). The examination site was carefully shaved, and the examination was carried out by a trained investigator. An appropriate amount of ultrasound gel was applied to the operating field of each animal. The ultrasound transducer was placed at the right intercostal space parallel to it, which minimised tissue compression. The investigator placed the “Region of Interest” (ROI, SWE-box) in an area of homogenous echotexture. The SWE-box did not include large vessels or other structures that could influence the measurements. The measurements were taken approximately 1 cm from the liver capsule. The measured area (Q-box) was placed at the centre of the SWE-box and had a diameter of approximately 4.5-6 mm. The stiffness of the area was determined, and a mean value was calculated.

An ultrasound-guided core needle liver biopsy was carried out in all the animals using a Tru-Cut needle under general anaesthesia. 0.5-1 cm biopsy specimens

were obtained from the right hepatic lobe. The specimens were fixed in a 10% buffered formalin solution and underwent a histopathological analysis to assess the degree of inflammation and fibrosis. The specimens were stained with hematoxylin and eosin and Van Gieson.

The analysis of the elastographic study was carried out using test based on χ^2 statistics.

Institutional and national guidelines for the care and use of animals were followed and all experimental procedures were approved by the II Local Ethics Committee of Poland, no. 99/2014.

Results

Based on the results of the liver elastography, we found that the data were distributed normally ($p=0,178$). A $< \mu -1,96*\sigma; \mu+1,96*\sigma >$ interval was chosen (where μ and σ signify the mean and standard deviation, respectively) with 95% coverage, with values ranging from 0.89 to 5.40 in order to eliminate 5% of the least likely values (Fig. 1). Hence, 5% of those values were smaller than 0.89 or exceeded 5.40. Figures 2 and 3 are elastographic images of the guinea pig liver.

The histopathologic analysis of the liver specimens ruled out inflammatory lesions and liver fibrosis (Fig. 4, 5).

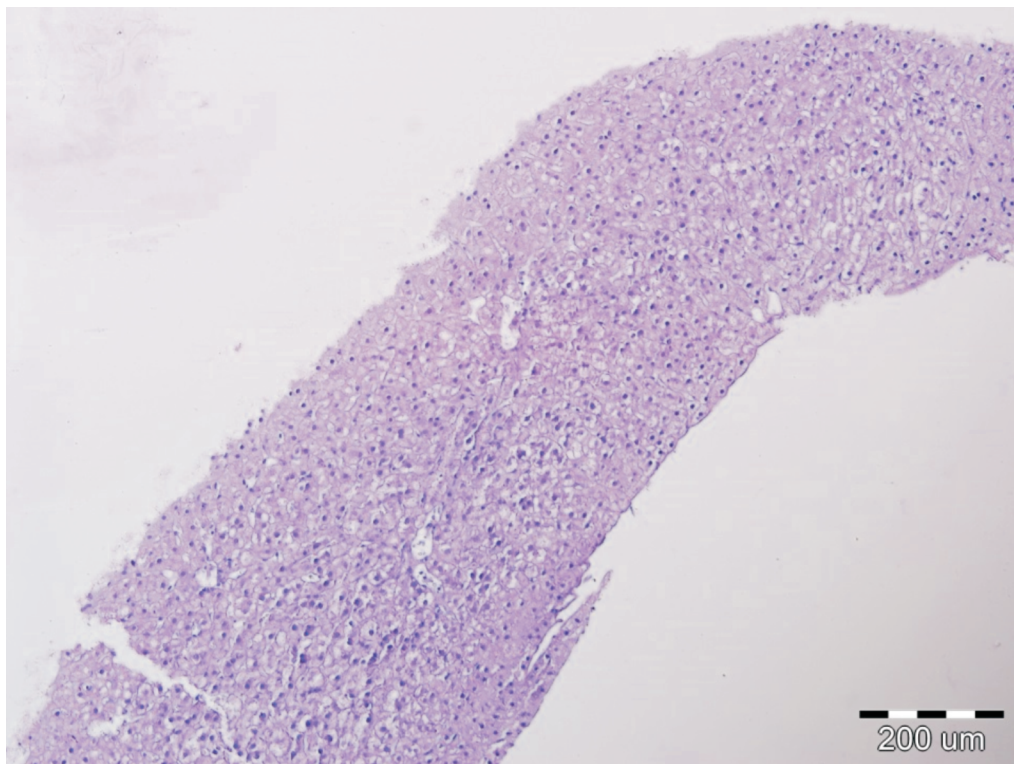


Fig. 4. A histopathological examination of a liver tissue specimen – microscopic image. HE staining, x200.

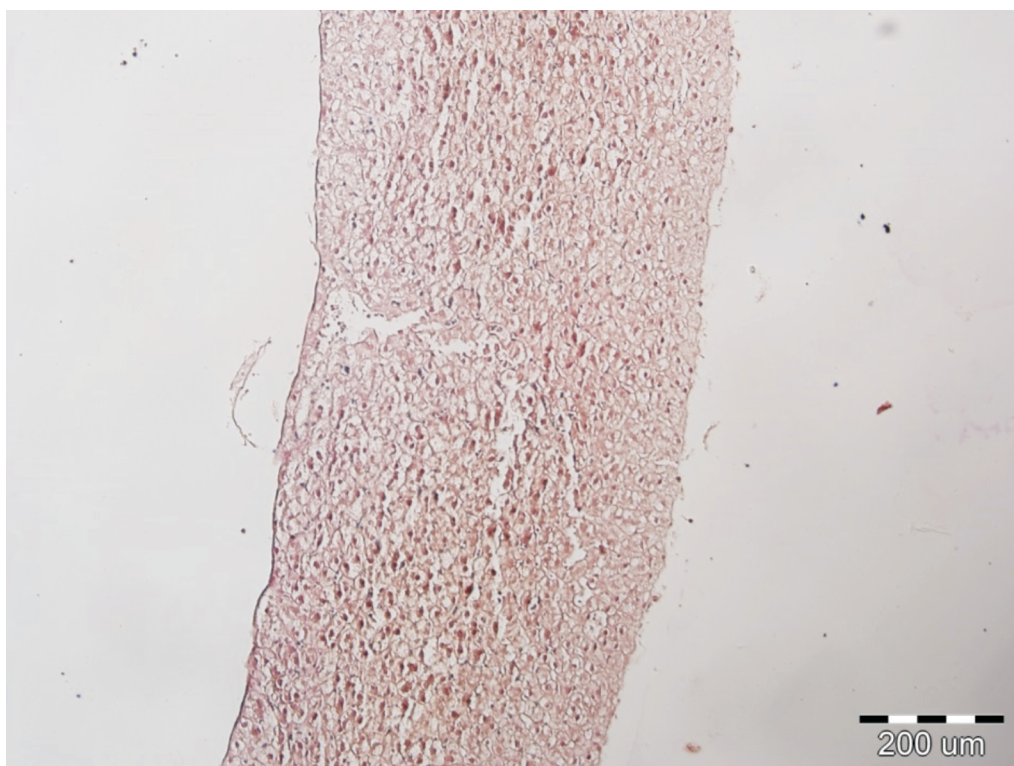


Fig. 5. A histopathological examination of a liver tissue specimen – microscopic image. Van Gieson staining, x200.

Discussion

In human medicine, elastography is a valuable non-invasive technique used to assess liver tissue stiffness (Arda et al. 2013). It enables the determination of the degree of liver fibrosis and facilitates the choice of an appropriate treatment. It is a fast and easy imaging technique that may become a non-invasive alternative to liver biopsies. The method is based on the use of acoustic radiation force impulses, which may be set at various penetration depths at supersonic speed and may be enhanced by forming a Mach cone to increase shear wave propagation. Young's modulus reflects the speed of shear wave propagation and is directly related to tissue elasticity (Sarvazyan et al. 1998). Shear wave elastography makes it possible to obtain operator-independent and repeatable results as well as greater spatial resolution compared to other elastography techniques.

The study carried out by Ferraioli et al. on humans using real-time shear wave elastography revealed that normal liver tissue stiffness ranged from 4.92 kPa to 5.39 kPa (Ferraioli et al. 2012). Similar results were obtained by other authors (Bavu et al. 2011, Arde et al. 2013, Gennisson et al. 2013). Based on numerous human studies, a liver tissue stiffness scale has been created based on the degree of liver fibrosis. This scale differs depending on the elastography technique used. In the case of SWE, liver tissue stiffness at F0 (according to the Metavir scale) can reach 6.5 kPa. At F1, the stiffness ranges from 6.5 to 9.1; F2 from 9.1 to 10.8; F3 from 10.8 to 13.3, and F4 from 13.3 kPa (Bavu et al. 2011).

In veterinary medicine, Holdsworth et al. assessed normal liver tissue elasticity in healthy dogs (Holdsworth et al. 2014). They did not perform histopathological assessment of the studied liver tissue to rule out pathological lesions. They assessed tissue stiffness at three depths (0-2, 2-4 and 4 cm). The study was carried out using a Siemens S2000 scanner and ARFI (acoustic radiation force impulse) elastography. This method is based on the generation of short 2.6 MHz ultrasound impulses by the ultrasound transducer, which causes tissue deformation. Thanks to this technique, it is possible to obtain a transverse wave precisely positioned at a chosen location. The ultrasound machine measures wave propagation in m/s. In the SWE technique used by us, several transverse waves are generated at different tissue depths and interact to form a new cone-shaped transverse wave. Unlike ARFI, SWE produces a two-dimensional colour map of tissue stiffness, which is superimposed on the classic ultrasound image. After freezing the image, the investigator can take measurements from a larger, two-dimensional area of organ tissue.

When using the ARFI, the investigator can take measurements only from a unidimensional area. The results of tissue stiffness were not compared as there were differences in the types of elastography used in the study on dogs (ARFI) and guinea pigs (SWE).

Conclusions

Our study aimed to evaluate liver tissue stiffness in healthy guinea pigs. The obtained results can be used in future studies to assess liver tissue stiffness in the course of various types of liver disease. We focused on the practical application of SWE in veterinary diagnostics. We found that this imaging technique is quick and easy to use in small animals. In addition, SWE is non-invasive, repeatable and does not require anaesthesia. SWE enables real-time ultrasound imaging of the liver, allowing the investigator to choose the best measurement site and providing greater reliability of the results. Our results indicated that the stiffness of healthy liver tissue in the guinea pig ranged from 0.89 to 5.4 kPa. We plan to carry out further elastography studies in order to assess liver tissue stiffness in different stages of liver fibrosis and to correlate those results with the results of the histopathological analysis of the examined liver tissue. Liver disease may be diagnosed based on elastographic studies in animals with contraindications to a liver biopsy. Hence, it would be useful to determine the correlation between tissue stiffness and histopathological results of liver biopsies as well as the liver tissue stiffness in the course of liver fibrosis. Elastography may also be used in routine diagnostics to assess the effectiveness of an applied therapy and to avoid repeated liver biopsies.

Acknowledgements

Project nr: UOD-DEM-1-351/001 was financed by the National Centre for Research and Development and by the European Regional Development Fund under the Innovative Economy Operational Programme.

Publication supported by Wroclaw center of Biotechnology, program "The Leading National Research Center (KNOW)" for years 2014-2018.

References

- Arda K, Ciledag N, Aribas BK, Aktas E, Kose K (2013) Quantitative assessment of the elasticity values of liver with shear wave ultrasonographic elastography. *Indian J Med Res* 137: 911-915.

- Bataller R, Brenner DA (2005) Liver fibrosis. *J Clin Invest* 115: 209-218.
- Bavu E, Gennisson JL, Couade M, Bercoff J, Mallet V, Fink M, Badel A, Vallet-Pichard A, Nalpas B, Tanter M, Pol S (2011) Noninvasive in vivo liver fibrosis evaluation using supersonic shear imaging: a clinical study on 113 hepatitis C virus patients. *Ultrasound Med Biol* 37: 1361-1373.
- Bhatia KS, Tong CS, Cho CC, Yuen EH, Lee YY, Ahuja AT (2010) Shear wave elastography of thyroid nodules in routine clinical practice: preliminary observations and utility for detecting malignancy. *Eur Radiol* 22: 2397-2406.
- Błazik E, Durlak M (2010) Mechanisms of liver fibrosis and methods of its assessment. *Med Sci Rev – Hepatologia* 10: 25-29.
- Cosgrove D, Piscaglia F, Bamber J, Bojunga J, Correas JM, Gilja OH, Klauser AS, Sporea I, Calliada F, Cantisani V, D'Onofrio M, Drakonaki EE, Fink M, Friedrich-Rust M, Fromageau J, Havre RF, Jenssen C, Ohlinger R, Săftoiu A, Schaefer F, Dietrich CF (2013) EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 2: Clinical applications. *Ultraschall Med* 34: 238-253.
- Ferraioli G, Tinelli C, Zicchetti M, Aboe E, Poma G, Di Gregoria M, Filice C (2012) Reproducibility of real-time shear wave elastography in the evaluation of liver elasticity. *Eur J Radiol* 81: 3102-3106.
- Gennisson JL, Deffieux T, Fink M, Tanter M (2013) Ultrasound elastography: principles and techniques. *Diagn Interv Imaging* 94: 487-495.
- Holdsworth A, Bradley K, Birch S, Browne WJ, Barberet V (2014) Elastography of the normal canine liver, spleen and kidneys. *Vet Radiol Ultrasound* 55: 620-627.
- Janczewska E, Pisula A, Simon K (2015) Rekomendacje dotyczące wykonywania badań elastograficznych wątroby. *Przegląd Epidemiologiczny* 69:429-433.
- Ophir J, Céspedes I, Ponnekanti H, Yazdi Y, Li X (1991) Elastography: a quantitative method for imaging the elasticity of biological tissues. *Ultrason Imaging* 13: 111-134.
- Pol S, Gennisson J, Bavu E, Deffieux T, Fink M, Mallet V, Tanter M (2014) Non-invasive staging of liver fibrosis with ShearWave™ elastography imaging. <http://www.sonoworld.com/Client/Centers/WhitePaper.aspx?ContentId=58>.
- Sarvazyan AP, Rudenko OV, Swanson SD, Fowlkes JB, Emelianov SY (1998) Shear wave elasticity imaging: a new ultrasonic technology of medical diagnostics. *Ultrasound Med Biol* 24: 1419-1435.
- Sebastiani G (2009) Non-invasive assessment of liver fibrosis in chronic liver diseases: implementation in clinical practice and decisional algorithms. *World J Gastroenterol* 15: 2190-2203.
- Zaleska-Dorobisz U, Pawluś A, Kucharska M, Inglot M (2015) SWE elastography in assessment of liver fibrosis. *Postepy Hig Med Dos* 69: 221-226.