

# In-vitro and in-vivo validation of ultrasound shear wave elastography for liver application

Anesa Mulabecirovic

Thesis for the Degree of Philosophiae Doctor (PhD)  
University of Bergen, Norway  
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# **In-vitro and in-vivo validation of ultrasound shear wave elastography for liver application**

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Thesis for the Degree of Philosophiae Doctor (PhD)  
at the University of Bergen

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*“Zwar weiß ich viel, doch möcht' ich alles wissen.”*

Johann Wolfgang von Goethe, 1749-1832

*“I have no special talent. I am only passionately curious.”*

Albert Einstein, 1879-1955

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## Scientific environment

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NCUG was established in 2001 by the National Health Authorities and is a national service of excellent competence for disseminating knowledge and skills in ultrasound (US) among gastroenterologist in Norway and has been acknowledged as a European Learning Centre for Gastrointestinal US by EFSUMB (European Federation of Ultrasound Societies in Medicine and Biology) The main aim of NCUG is to improve ultrasound methods and develop novel examination techniques for patients with diseases within the digestive tract.

Bergen Research Group for Ultrasound in Gastroenterology (BRUSE) at Department of Clinical Medicine, University of Bergen is a working to develop and validate novel methods for clinical ultrasound and has been internationally recognized as a pioneer with a broad collaboration in transabdominal and endoscopic ultrasound.

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National Centre for Ultrasound in Gastroenterology  
Haukeland University Hospital, Bergen, Norway



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Albert Einstein, 1879-1955

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## List of abbreviations

AIH:	Autoimmune hepatitis
ARFI:	Acoustic radiation force impulse
BMI:	Body mass index
B-mode:	Brightness mode
CV:	Coefficient of variation
HCV:	Hepatitis C Virus
IQR:	Interquartile range
kPa:	Kilo Pascals
LSM:	Liver stiffness measurement
MRE:	Magnetic resonance elastography
m/s:	Meters per second
PSC:	Primary sclerosing cholangitis
pSWE:	Point shear wave elastography
ROI:	Region of interest
SE:	Strain elastography
SEM:	Spleen elastography measurements
SSI:	Supersonic Shear Imaging
SWE:	Shear wave elastography
SWSE:	Shear wave speed elastography

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SWSI:	Shear wave speed imaging
SWSM:	Shear wave speed measurement
TE:	Transient elastography
US:	Ultrasound
VTq:	Virtual touch quantification

## Abstract

### Background and aims

Ultrasound (US) elastography is a noninvasive method that is used to investigate tissue elasticity in several organs. In chronic liver disease, the predominant approach is quantitative. By measuring liver stiffness, one could possibly follow the development of fibrosis in chronic liver diseases. The spectrum of US elastography methods has been expanding, however, there is limited validation of several of the new methods. Validation is needed for the methods to be established as tools in clinical practice. The overall aim of this thesis was to validate several US shear wave elastography (SWE) methods, including point shear wave elastography (pSWE) and 2D-SWE, *in vitro* and *in vivo* aiming at liver as the primary organ. In the first study the main aim was to assess and validate the repeatability, reproducibility and interobserver agreement of several US SWE methods. This was approached *in vitro* using liver fibrosis phantoms with known Young's modulus. In the second and third study we assessed *in vivo*; in livers of an adult healthy cohort and a cohort of patients with primary sclerosing cholangitis (PSC). Furthermore, we aimed to define normal liver elasticity, assess number of repeated measurements needed to achieve a representative median value and explore the assessment of fibrosis.

### Methods

Methods to estimate tissue elasticity are usually integrated in US scanners. In the first study we used transient elastography (TE) and methods integrated in GE Logiq E9 (2D-SWE), Hitachi Ascendus (pSWE), Philips iU22 (pSWE) and Samsung RS80A

with prestige (pSWE). Two investigators performed non-continued measurements in parallel on four individual tissue-mimicking liver fibrosis phantoms. In the second study we obtained liver stiffness measurements (LSM) in a healthy cohort of 50 men and 50 women using TE and methods integrated in GE Logiq E9 (2D-SWE) and Samsung TS80A (pSWE). Prior to the LSM all 100 subjects underwent lab tests and US examination in B-mode. Inter- and intraobservation between two examiners were assessed in a subgroup of 24 subjects. In the third study we used the pSWE method integrated in Philips iU22 and included 55 non-transplant PSC patients and 24 matched controls. All subjects underwent US examination and lab tests were performed on patients with PSC. We evaluated inter- and intraobserver variability of the spleen and liver elasticity measurements between two examiners in 19 healthy subjects.

### **Main results**

In the first study we found that all four US SWE methods could differentiate the four individual liver fibrosis phantoms. The methods had high repeatability and reproducibility. The inter- and intraobserver agreement was excellent and there was no significant difference in mean elasticity for all the US SWE methods. Furthermore, the study demonstrated that the difference in elastography measurements acquired with US SWE was larger for the harder phantoms with higher Young's modulus compared to the softer ones. In the second study we found that the reproducibility and repeatability of LSM in healthy livers was high, furthermore, our results showed that the mean liver elasticity in a healthy adult cohort was higher when acquired with the 2D-SWE method, than with non-imaging SWE methods such

as Samsung pSWE or TE. We also found that males had higher liver elasticity than females. In addition, we demonstrated that five consecutive acquisitions may be sufficient for reliable LSM results. In the third study, we found good intra- and interobservation agreement assessing Philips iU22 pSWE measurements of the right liver lobe in the healthy subjects. We also found that the PSC patients had higher LSM than the healthy controls when measuring the right liver lobe, whereas the LSM of the left liver and spleen elasticity measurements were indifferent between PSC patients and healthy controls.

### **Conclusions**

US SWE methods used in our studies demonstrated excellent in vitro and good in vivo repeatability and interobserver agreement. Mean LSM in our healthy cohort was significantly higher when obtained with 2D-SWE, and in male participants. We found no difference across age groups 20-70 years or among non-obese BMI-groups 18-30 kg/m<sup>2</sup>. Our results indicated that five LSM may be sufficient to obtain a reliable result in healthy livers. Furthermore, we showed that PSC patients displayed higher levels of LSM compared to the healthy controls. However, the range of LSM of PSC patients was wide, which could suggest increasing stages of fibrosis through the disease development, making SWE a possible method for prospective studies evaluating SWE as a prognostic tool.

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## List of Publications

Mulabecirovic A., Mjelle A. B., Vesterhus M., Gilja O. H., Havre R. F. Repeatability of shear wave elastography in liver fibrosis phantoms – evaluation of five different systems, PLoS One 2018

Mulabecirovic A., Mjelle A. B., Vesterhus M., Gilja O. H., Havre R. F. Liver elasticity in healthy individuals by two novel shear-wave elastography systems - comparison by age, gender, BMI and number of measurements, PLoS One 2018

Mjelle A. B., Mulabecirovic A. Hausken T., Havre R. F., Gilja O. H., Vesterhus M. Ultrasound and point shear- wave elastography in livers of patients with primary sclerosing cholangitis, Ultrasound in Medicine and Biology 2016



**Related papers (*not included in the Thesis presentation*)**

Mulabecirovic A., Vesterhus M., Gilja O. H., Havre R. F. Reproducibility of quantitative elastography in vitro in five different elastography systems, *Ultrasound in Medicine and Biology* 2016

Bende F., Mulabecirovic A., Sporea I, Popescu A., Sirli R., Gilja O.H., Vesterhus M., Havre R.F. Assessing liver stiffness by 2D-shear wave elastography in a healthy cohort, *Ultrasound in Medicine and Biology* 2018

Roald Flesland Havre, Jo Erling Riise Waage, Anesa Mulabecirovic, Odd Helge Gilja, Lars Birger Nesje. Strain ratio as a quantification tool in strain imaging, *Applied Science* 2018

Mjelle A. B., Mulabecirovic A., Havre R. F., Olafsdottir E., Gilja O.H., Vesterhus M. Liver stiffness in 196 healthy children comparing two shear wave elastography systems and transient elastography, *J Pediatr Gastroenterol Nutr.* 2019

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# 1 Introduction

## 1.1 Preface

For several decades, liver biopsies have been considered the gold standard in diagnostics of liver fibrosis, and used to assess staging and grading by clinicians throughout the world. It is well appreciated that this way of assessing liver fibrosis is far from optimal. The overall performance of grading of liver fibrosis is mainly reliant on the quality of the sample itself. To acquire the liver biopsy, the patients go through an invasive procedure with a risk of serious complications. The incident of these is low, however, but not zero. Furthermore, sampling variability and intra- and interobserver variability reduce the accuracy of liver biopsies (1-3). Evaluation of novel methods requires a comparison to the reference method where the specificity and sensitivity is assumed to be 100% (4). If the reference method is not perfect, such as liver biopsy with its limitations, it causes challenges when investigating new methods as the estimates of novel diagnostic tests are false (4).

In the past two decades novel non-invasive methods to assess liver fibrosis have emerged. Since 2000 there has been published several studies emphasizing the usefulness of novel non-invasive methods to assess liver fibrosis and with potential to be used as a clinical tool. These include serological tests and imaging methods; an example of the latter is US elastography. FibroTest, a patented biochemical test (5-7), and FibroScan, an ultrasound-based elastography method without visualization, are to this date the most validated (8). However, non-invasive techniques such as the serum models based on algorithms may be affected by factors unrelated to the liver as some

of the algorithms contain markers that may be elevated for other reasons, and when measured in serum they may reflect disease progression in other organs (9, 10). As the field of ultrasound-based elastography methods and techniques has expanded, and new sub methods have been introduced, validation of these methods is warranted. This thesis has adds to the knowledge of LSM in idealized settings, in healthy livers and in chronic liver disease.

## **1.2 Historical aspect and background of ultrasound elastography field**

According to the Oxford English dictionary palpation is the feeling of touch (11). In 1822 and 1872 two ancient medical documents were purchased, the Papyrus Edwin Smith and Papyrus Ebers (12). The content dates back to about 1500 BC and contains the first documentation of palpation being performed in ancient Egyptian medicine. In Western medicine it is said that the practice of palpation was not applied reputedly before the 1930s (13). When clinicians palpate patients, they use their hands and generate manual pressure to investigate the examined organ's structure, mobility and elasticity. The information that the clinicians receives through palpation may provide some information about abnormalities, i.e. malignant tumours are usually harder than normal tissue. However, the interpretation of the palpation and perception of elasticity is highly subjective.

In the 17<sup>th</sup> century Sir William Petty described elasticity as the power of recovering the figure, upon removal of force (14). At the beginning of the 1950s, the first

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evidence of what would set the landmark for the evolution of elastography was published by Oestreicher and von Gierke and colleagues. Through studying the physics of vibration in soft tissue they demonstrated that there was correlation between the impedance of tissue and audio frequency (15). Nearly 30 years later, in the 1980s, experiments to differentiate soft and hard tissue by US were performed. Towards the end of the last century, in 1991, Ophir et al. introduced the term elastography as a quantitative US method of imaging biological tissue through strain and Young's modulus (16). In the years to follow, tracking shear waves with US emerged as new elastography method. The proliferation of elastography techniques kept emerging and in 2001 the first prototype of one dimensional transient elastography (TE) using a thumper to induce a shear wave into liver tissue and a single crystal US probe to track the speed of this wave- the Fibroscan, was born (17). In 2004 *Supersonic Shear Imaging* (SSI) was presented, a system that provided shear wave elastography over a larger area through an ultra-high frame rate (18), and throughout the years several similar new methods were introduced in commercial US scanners. Several studies, including from our lab (19-28), were published where elastography methods were tested in clinical studies by clinicians and the potential of US elastography as an investigative clinical tool to assess tissue elasticity for improved diagnostics was anticipated to become powerful.

### 1.3 Ultrasound elastography methods used for evaluating liver fibrosis

The challenge in assessing US elastography to provide information related to the examined tissue's stiffness is that the different methods are not standardized to a common use and they differ in technique; display of strain, of displacement and of shear wave speed (29).



**Figure 1.** An illustration of a liver in US B-mode, demonstrating the challenge in evaluating liver stiffness solely by US B-mode. *Image: O.H. Gilja*

The most common US elastography method for assessment of liver stiffness are the techniques that display shear wave speed, whereas displacement imaging is most common in assessment of lesions in the liver (30). However, when the assumptions used to derive the elastography images and calculation of measurements are not coherent with the examined tissue behaviour, the elastography methods and techniques will most likely differ (10, 31). The practical procedure of liver

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elastography measurements is common for several methods: fasting of minimum 4 hours prior to the investigation is advised as food intake may cause increased liver stiffness values (32-36), and patients are positioned laying with the right arm abducted and elevated above the head.

### ***1.3.1 Strain elastography***

Strain-based elastography (SE), measures deformation of tissue, and is useful for imaging of focal lesions with a tissue stiffness different from the surrounding tissue. It is also called quasi-static elastography, as the echo signals are recorded in overlapping reading-frames several times during a rather slow compression- or decompression phase, and can be applied manually and freehand by the examiner (29). Initially SE displayed solely a qualitative image, where the relative tissue elasticity was shown as a colour overlay on the conventional B-mode. Later, the method has been featured with an integrated quantitative approach to compare strain in two, or more, user selected areas, such as the area one wants to measure and the reference area. This is called Strain Ratio (SR) and if the measurement of the selected area is harder than the reference area, the SR will be greater than 1, meaning that a higher SR value represents an increased tissue stiffness relative to the reference area (37, 38). In order to deliver a reliable result, both lesion and reference tissue should be subject to similar amounts of stress.

$$\text{Strain ratio (SR)} = \frac{\text{Mean strain in the reference area}}{\text{Mean strain in the selected area}}$$

As a general rule strain-based methods provide images that are composed by higher spatial resolution compared to shear wave-based methods, that provide higher elastography image contrast (31). SE is mainly used clinically in assessment of focal lesions in the breast, thyroid and prostate (39). Recently, methods to objectively assess strain are being developed, but currently they cannot be recommended in clinical assessment of liver stiffness measurements (40).

### ***1.3.2 Shear wave speed based elastography (SWSE)***

Elastography methods based on shear waves quantitatively measure the speed of shear waves that travel in the tissue. In shear wave speed based elastography (SWSE), the shear waves can either be generated externally, as in TE, by US radiation through elasticity measurement within a non-adjustable region of interest, or by an US generated 2D image where elasticity is shown within a larger region that may be adjusted (31).

The stiffness of tissue elasticity is obtained by measuring the shear wave velocity ( $c_{sw}$ ) and is given in meters per second (m/s). The tissue stiffness can also be expressed in Shear modulus ( $\mu$ ) in kilo Pascals (kPa). The Shear modulus is linearly proportional to the squared shear wave speed multiplied by the density of tissue ( $\rho$ ). (Equation 1). There are two assumptions made in the calculation of Shear modulus. One assumption is made for the calculation is that the density ( $\rho$ ) of the examined tissue is homogeneous and expressed in kg/m<sup>3</sup> (41), for healthy tissue it is assumed to be relatively constant and similar to density of water 1000 kg/m<sup>3</sup>.

$$\text{Shear modulus } (\mu) = \rho (c_{sw})^2 \quad (1)$$

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A second assumption is made to calculate the Elastic Modulus, or Young's Modulus (E), is that soft tissue behaves in the same manner as an incompressible material with a Poisson ratio ( $\nu$ ) of approximately 0.5 (Equation 2)

$$\text{Shear modulus } (\mu) = \frac{\text{Elastic modulus (E)}}{2(1 + \text{Poisson ratio } (\nu))} \quad (2)$$

By making these two assumptions, we are able to link the Shear modulus ( $\mu$ ) and Young's modulus (E) by equations 1 and 2, and thereby tissue stiffness can be expressed as Elasticity in kPa based on measurements of shear wave velocity ( $c_{sw}$ ), reported in kilo Pascals (kPa) or in m/s (41-47).

$$\text{Young's modulus (E)} \approx 3\mu$$

$$\text{Expected shear wave velocity } (c_{sw}) = \sqrt{\frac{E}{3\rho}}$$

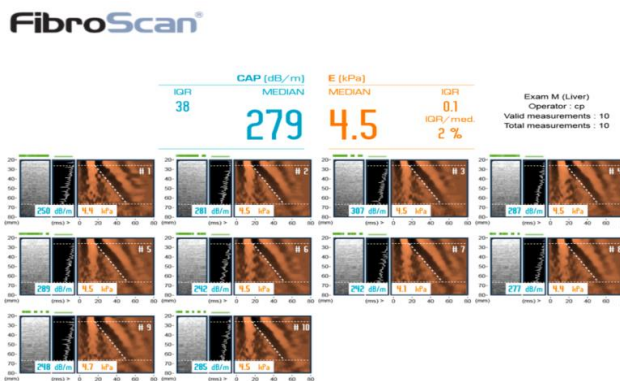
Commercially available US scanners that have elastography methods integrated allow the user to choose if the results of shear wave velocity measurements are to be reported in either kPa or m/s, or both, in contrary to TE where the results are reported in kPa. As the expected, shear wave velocity  $c_{sw}$  has m/s as unit that is measured by the US scanner, and a conversion to kPa (Young's modulus, E) is thus based on the assumptions and equations given above. The European federation of ultrasound in medicine and biology (40) advises to report LSM in m/s rather than the elastic modulus (kPa), however, the LSM obtained with methods integrated in US scanners have frequently been reported in both m/s and kPa in the published literature.



## *Transient elastography (TE)*

Transient elastography (TE) is performed with specially designed US probes with a thumper, designed to create shear waves that propagate through the skin into the liver tissue, while being tracked by US. Three different piston probes are available; S-, M- and XL-probe. The first is aimed at assessment of liver stiffness in children, the second in adults and third in overweight patients.

The measurement of the liver stiffness is shown qualitatively in an M-mode, without direct anatomical visualization in B-mode, and quantified in kPa. If a measurement is not considered valid by the software the instrument does not return a value. The manufacturer recommends that the interquartile range and median measurement ratio (IQR/M) should be less than 30% for the evaluation to be considered valid.



**Figure 2.** An illustration of the result chart, provided by the manufacturer EchoSens, after liver stiffness measurements of a healthy subject have been acquired with M-probe using Transient elastography (TE), Fibroscan® 204 (EchoSens, Paris, France).

*Images: A. Mulabecirovic*

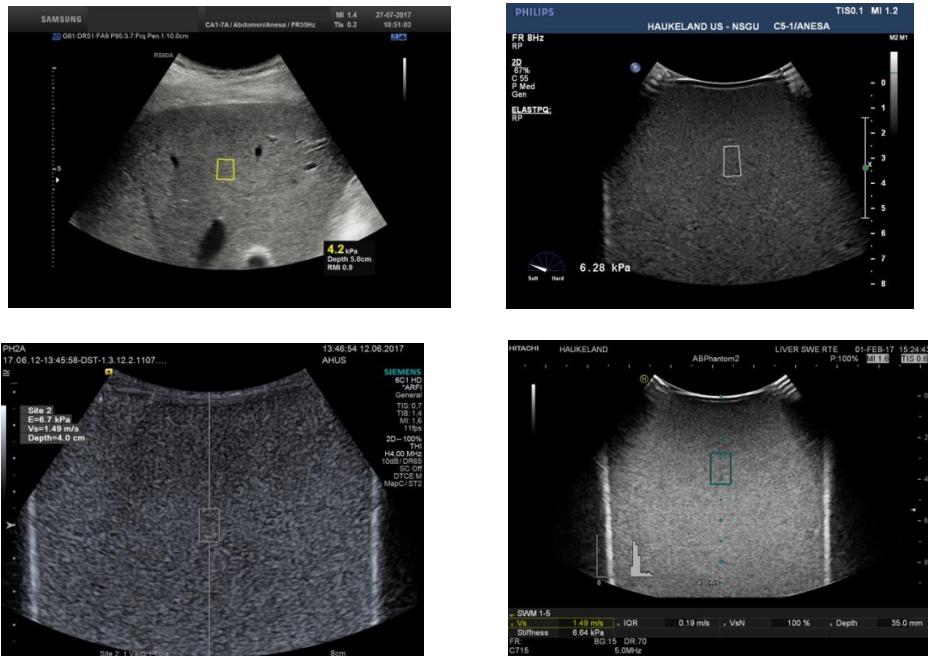
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A limitation of the method is that TE cannot be performed in patients with ascites, moreover, the applicability in patients with obesity or with narrow intercostal spacing is limited (48). Due to the lacking B-mode visualization, evaluation of factors such as recent food intake, gallbladder size, presence of cirrhosis or cholestasis cannot be performed. TE is a user-friendly method, easy to learn, does not demand knowledge in ultrasonography and is currently the most used and validated liver elasticity assessment method among the noninvasive methods to assess liver fibrosis (49-52). Although TE is capable to detect cirrhosis with high accuracy (F2 vs. F4), TE does not accurately distinguish between intermediate stages of fibrosis (F1- F4) (10, 40). It was introduced in Europe in 2003 and was approved by the American Food and Drug Administration (FDA) in 2013, TE, Fibroscan (EchoSens, Paris, France) is used in more than 70 countries to measure liver stiffness. However, the interpretation of the results should be performed by a clinician and with knowledge about the patient's disease and biochemical status (53-55).

***Shear wave speed elastography (SWSE) with direct visualization in US B-mode: Shear wave speed measurement (SWSM) and Shear wave speed imaging (SWSI)***

Shear wave speed elastography (SWSE) methods integrated in conventional US scanners are performed with direct and real-time visualization in B-mode using an US probe. Shear wave speed measurement (SWSM) measures the liver stiffness without imaging the elasticity, whereas shear wave speed imaging (SWSI) additionally generates a 2D image where the elasticity is visualized by a colour map within a larger and adjustable region.

SWSM may be known as point shear wave elastography (pSWE) or imaging with Acoustic radiation force impulse (ARFI) and is available in commercial scanners (56). The first method commercially available by Siemens is known under the name “Virtual touch quantification” (VTq). Not long after, Phillips introduced “ElastPQ” followed by several other manufacturers that released their pSWE methods (10, 40). These methods are probably different by the number of shear waves deposited and tracking algorithms, but the details of this is proprietary information to the manufacturers. When pSWE is applied the assessment of shear wave speed is made at one point, whereas when ARFI is applied shear wave speed is assessed using several ARFI lines, and a local average of shear wave speed is determined within a set region of interest (ROI), which is approximately 1cm<sup>2</sup> in size (57, 58). In this thesis we will refer to them as point shear wave elastography (pSWE). With SWSM, the ROI cannot be adjusted in size by the examiner, and the elasticity itself is not directly visualized. It is recommended that the liver stiffness measurement is obtained by placing the ROI 1.5-3.0 cm beneath the liver capsule to avoid artefacts and subcapsular stiffness, avoiding vessels while the patient holds the breath without deep inspiration (10, 40). With direct anatomical visualization in B-mode, the measurement of liver stiffness is the calculated median value of ten valid measurements, and the results are reported in kPa or m/s (56). If a measurement is not considered valid, the method does not return a value. A reliable measurement is when IQR/Median is less than 30% (59).



**Figure 3.** An illustration of commercially available SWS methods, here demonstrated in vivo on a healthy subject with pSWE with SWE Samsung RS80A with Prestige (Samsung Medison Co. Ltd., Seoul, Korea) in the upper left corner. In vitro on a liver fibrosis phantom with known Young's modulus of  $11.5 \text{ kPa} \pm 0.57$ , Model 039, manufactured by Computerized Imaging Reference Systems (CIRS Inc. Virginia, USA) with pSWE Philips iU22 (Eindhoven, Netherlands) in the right upper corner, with SWE Hitachi HI VISION Ascendus (Hitachi Medical corporation, Tokyo, Japan) in the lower right corner and with SWE Siemens Acuson S3000, Virtual Touch™ tissue quantification (VTq), (Siemens Medical Solutions, Mountain View, CA, USA). *Images: A. Mulabecirovic*

SWSI is often referred to as 2D- Shear wave elastography (2D-SWE) as it gives a real time visualisation of soft tissue elasticity properties. Supersonic Shear Imaging

(SSI) was the first SWSI method introduced. By depositing several series of acoustic signals with focus at different depths of the tissue, creating an “acoustic cone”, combined with an ultra-high frame rate, SSI is able to capture in real time, the transient propagation of shear waves. After SSI, other manufacturers have released 2D-SWE methods that are similar, however, these do differ by e.g. the deposition of acoustic signal and sampling frequency (40, 60). 2D-SWE has the ability to produce a 2D image where the tissue stiffness is displayed in real time within a colour map superimposed on a B-mode image. The colour map may be referred to as elastogram where the ROI of measurements can be freely placed and adjusted in size. The colour indicates the stiffness of the tissue. Some commercial scanners allow the users to choose colour definition of soft and hard tissue, for example blue colour indicating soft tissue and red indicating hard tissue. With direct anatomical visualization in B-mode, the analysis box should be set to preferably 15 mm or more and the ROI should be placed in an isoechoic area without vessels. It is recommended that a minimum of three measurements should be obtained and the results of LSM are expressed in either m/s, kPa or both (40). If a measurement is not considered valid, the method doesn't return a valued image. A reliable measurement is when IQR/M is less than 30%, and it is suggested to follow manufacturers advice for acquisition (61).



**Figure 4.** An illustration of commercially available SWSI methods, demonstrated in vitro on a tissue-mimicking inclusion phantom (Model 049 Elasticity QA Phantom, Computerized Imaging Reference Systems Company [CIRS], Norfolk, VA, USA), with 2D-SWE, SSI (Aixplorer, Aix-en-Provence, France) to the left, and in vivo on a healthy subject with 2D-SWE, LOGIQ E9 (GE Healthcare, Milwaukee, Wisconsin, USA) to the right. *Images: A. Mulabecirovic*

## **2 Hypothesis and aims of the thesis**

### **2.1 Hypothesis**

Our prime hypothesis was that US elastography of liver tissue is feasible and reproducible in liver mimicking -phantoms as well as in healthy volunteers and in patients with chronic liver disease. Secondly, we hypothesized that when elastography is obtained in softer tissue in vitro and in vivo, produce lower values with better reproducibility than harder tissue. LSM in healthy livers are higher in men than women and that age, body weight and body mass index (BMI) may influence LSM. There is no difference in variability or reproducibility when performing LSM based on five consecutive LSM instead of 10 in healthy livers. Lastly, we hypothesized that US elastography can be assessed in evaluating fibrosis in patients with PSC. That the LSM obtained in livers of PSC patients would be higher than LSM of healthy livers, and that the feasibility was good and LSM the same of the right and left liver lobe.

### **2.2 Aims**

#### *2.2.1 Main aim*

The main aim of this thesis was to investigate and validate different elastography methods in vitro, in vivo on healthy subjects and non-healthy. Furthermore, to explore if LSM obtained by US methods can be used as a clinical tool and predictor in follow-up of patients with liver disease and establish normal values and variability in a healthy cohort in different age groups. The aims related to each of the studies are mentioned under each study.

### 2.2.2 *Specific aims*

*Study I:* To assess and validate the repeatability of US elastography measurements in four separate liver tissue mimicking phantoms with known elasticity using five different elastography methods.

*Study II:* Establish and define a normal material for liver elasticity using selected methods for elasticity imaging for different age and gender segments and variation width measurements in healthy livers. Assess the intra- and interobserver variability and reproducibility and investigate the difference between five and ten consecutive liver elasticity measurements.

*Study III:* To explore US elastography in patients with PSC using one elasticity imaging US shear wave method; specifically: To investigate whether the elasticity measurements in patients with PSC differed between the left and right liver lobe, whether elasticity measurements in liver and spleen were different in PSC compared to healthy controls, and whether liver elasticity measurements were associated with clinical, B-mode or laboratory signs of fibrosis. Assess the intra- and interobserver agreement.



## 3 Materials and methods

### 3.1 Study object and study populations

#### 3.1.1 Study I

To ideally perform elastography measurements *in vitro* the material measured should mimic properties of healthy and non-healthy soft tissue and be congruous with US SWE modalities. We chose a set of four individual liver fibrosis phantoms (Model 039), manufactured by Computerized Imaging Reference Systems (CIRS Inc. Virginia, USA), as the objects of examination. In contrast to biological tissue which has both elastic and viscoelastic properties, the phantoms consisted of elastic material, Zerdine®, a patented synthetic polymer, contained in a 11.6 cm wide and 14 cm tall cylinder surfaced with a Saran-base. The mechanical and acoustic properties of the phantoms had been individually quality-assessed by the manufacturer.

#### 3.1.2 Study II

The main aim of this study was to define normal liver elasticity in a healthy cohort. 110 healthy subjects were included in the study, these were recruited through the workplace and social network. 10 subjects were excluded from the final analysis due to excessive weekly alcohol consumption (n=2), abnormal laboratory tests (n=3), evidence of malignancy on B-mode US (n=1) and BMI > 30 kg/m<sup>2</sup> (n=4). 50 males and 50 females were included in the final analysis, 10 of each gender in age groups 20-30, 31-40, 41-50, 51-60 and 61-70 years. The subjects were divided into two BMI groups; 18.0 - 25 kg/m<sup>2</sup> (n = 73) and 25.1 - 30 kg/m<sup>2</sup> (n = 27).

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### 3.1.3 Study III

64 patients from a known cohort of non-transplanted PSC patients in Western Norway were to invited at Haukeland University Hospital to participate in the study. 55 patients were included in the study, 49 with PSC, 1 with Small duct PSC and 5 with PSC-AIH overlap syndrome. Patients had a mean age of  $46.4 \pm 16.2$  years (38 males and 17 females). Healthy controls (n=24) included had a mean age of  $40.6 \pm 13.8$  years (8 males and 8 females).

## 3.2 Ethical considerations

Oral and written information was given to all invited subjects, and informed written consent for subjects included in the study II and III. The studies were performed in compliance with the Declaration of Helsinki (2002) and Good Clinical Practice guidelines. The regional ethics committee of Western Norway has approved all of our studies (REC west no. 2012/2214).

## 3.3 Ultrasound elastography

In this thesis we have through study I-III used six different elastography platforms, where five of them were integrated in commercial US scanners. The elastography methods applied in the first study were 2D-SWE Version 2.0 from the system of LOGIQ E9 (GE Healthcare, Milwaukee, Wisconsin, USA), SWE Samsung RS80A with Prestige (Samsung Medison Co. Ltd., Seoul, Korea), SWE Hitachi HI VISION Ascendus (Hitachi Medical corporation, Tokyo, Japan), pSWE Philips iU22

(Eindhoven, Netherlands) and Fibroscan® 204 (EchoSens, Paris, France) with M-probe. In the second study we applied 2D-SWE from GE Logiq S8 (GE Healthcare, Milwaukee, Wisconsin, USA) Version R 4.1.2, SWE from Samsung RS80A with Prestige SWE Version 3.00.03.0824 and Transient elastography (TE, Fibroscan, EchoSens, Paris, France) integrated in the GE Logiq S8 US scanner was applied using the M-probe. In the third study elastography measurements of the liver and spleen were performed using pSWE from Philips (ElastPQ, Version 6.3.2.2, iU22, Philips Healthcare, Andover, MA, USA).

### **3.4 Ultrasound examination and elastography measurements**

All US examinations and elastography measurements were performed by me in study I and II, and by Mette Vesterhus in study III. Anders Batman Mjelle performed elastography measurements for interobserver validation in study I, II and III.

#### *3.4.1 Ultrasound examination in B-mode*

Using a standardized scanning protocol all subjects in study II and III were examined by US in B-mode, and examinations were conducted after a minimum of four hour fasting. In study II, I used Samsung RS80A with Prestige US equipment (Samsung Medison Co. Ltd., Seoul, Korea) and all subjects underwent an US examination of the liver, gallbladder, spleen and kidneys. In study III the liver and spleen were examined using Philips iU22 (Philips Healthcare, Andover, MA, USA).

### 3.4.2 Elastography measurements *in vitro*

In study I the elastography measurements were performed *in vitro* on liver fibrosis phantoms with four different US elastography methods using convex arrayed probes. The placement of the elastography measurement was standardized and placed 2-3 cm below the surface of the liver fibrosis phantoms.



**Figure 5.** An illustration of acquiring LSM live with US SWE, using 2D-SWE from GE Logiq S8 (GE Healthcare, Milwaukee, Wisconsin, USA) Version R 4.1.2, pSWE from Samsung RS80A with Prestige SWE Version 3.00.03.0824 and Transient elastography (TE, Fibroscan, EchoSens, Paris, France) integrated in the GE Logiq S8 US scanner applied using the M-probe. *Images: A. Mulabecirovic. The person on the image above has consented to appear on the photos in this thesis.*

### 3.4.3 Elastography measurements *in vivo*

Study II and III was *in vivo* on healthy and non-healthy subjects. All subjects were examined with their right arm abducted and the elastography measurements were performed immediately after a full US examination of the liver and biliary system. In

study II all US elastography measurements were obtained intercostal in the right liver lobe in a relaxed mid-breath, minimum 2 cm below the Glisson capsule, avoiding vessels and bile ducts. Elastography measurements of the liver were performed using convex arrayed probes, and LSM were given in kPa in study II and m/s in study III.

### **3.5 Statistical analysis**

The Statistical Package for Social Sciences Software versions 22-24 (IBM, Armonk, New York, USA) and MedCalc version 12.7.0.0 was used for data management and statistical analysis. In study I and II all data was plotted manually by me and then proof-read by me on two occasions to ensure accuracy, and I performed all statistical analyses in study I and II. In study III, I contributed to the statistical analyses.

In study I the elastography measurements are presented as median (range), in study II and III data are presented as mean (SD) when normally distributed, and median (range) when not. Reliable measurements were defined as the median value of 10 valid elastography measurements with a ratio of the number of successful acquisitions divided by the total number of acquisitions above 60% and an interquartile range interval less than 30%. In study I and II Pearson's coefficient of correlation was used to assess the interobserver agreement. In study I, II and III Limits of agreement (Bland Altman) was used to assess differences between individual measurements and detect biases for each elastography method, interclass correlation coefficient was calculated to assess the interobserver reliability and the coefficient of variation was calculated to assess the intraobserver variability of elastography measurements.

In study I we used one-way ANOVA and Turkey's test to test the overall significance, the threshold for statistical significance was set to  $p < 0.01$  as multiple testing of methods was performed on phantoms. In study II and III normal distribution was tested using Shapiro-Wilk test and the threshold for statistical significance was set to  $< 0.05$ . Student's t-test and Mann-Whitney U test was assessed according to data distribution. In study II multivariable regression analysis was performed to identify covariables of liver elastography measurements. Only variables with a p-value  $< 0.01$  in a univariable analysis were included in a multivariable regression analysis. In study III correlations between variables were tested by Spearman's rank order correlation coefficient ( $\rho$ ).

## 4 Summary of main results

### 4.1 Results study I

Each of the four liver fibrosis phantoms had different Young's modulus (kPa) and had been batch tested by the producer. The expected elasticities were given in measured shear wave velocity (m/s) and calculated Young's modulus (kPa), the producer provided values with 5% SD. The expected elasticities were  $2.7 \pm 0.14$  kPa ( $1.62 \pm 0.08$  m/s) for phantom 1,  $11.5 \pm 0.57$  kPa ( $3.34 \pm 0.17$  m/s) for phantom 2,  $24.8 \pm 1.24$  kPa ( $4.91 \pm 0.25$  m/s) for phantom 3 and  $46.3 \pm 2.32$  kPa ( $6.70 \pm 0.34$  m/s) for phantom 4. Each of the elastography methods differentiated the four phantoms ( $p < 0.001$ ), and showed more variability in elastography measurements for the harder phantoms (3 and 4) compared to the softer (1 and 2). Phantom 3 was softer than phantom 4, and we found that the oldest of the US elastography method applied (pSWE from Philips iU22 (Eindhoven, Netherlands)) had higher elasticity measurements for phantom 3 ( $p < 0.0001$ ) and highest CV (0.21). No significant difference in measurements, nor in variability, could be demonstrated for any of the other elastography methods and no significant difference in correlation between the observers ( $p = 0.157 - 0.660$ ). For phantom 4, TE measured higher elasticity compared to the methods integrated in an US scanner and showed significant difference when two observers obtained elastography measurements ( $p < 0.0001$ ). Whereas we found no significant difference when comparing two observers' elastography measurements for any of the elastography methods integrated in US scanners ( $p = 0.043 - 1.000$ ). The interobserver correlation (0.991-1.000) and intraobserver correlation (0.987-1.000) was excellent.

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## 4.2 Results study II

The main aim of this study was to establish and define a normal liver elasticity in different age and gender segments using three elastography methods (pSWE, 2D-SWE and TE). We found that the mean LSM for all 100 healthy subjects ranged from 2.0-6.8 kPa. There was no significant difference in obtained LSM measurements between pSWE ( $4.1 \pm 0.8$  kPa) and TE ( $4.2 \pm 1.1$  kPa) ( $p = 0.110$ ), however LSM with the 2D-SWE method ( $4.5 \pm 0.8$  kPa) were significantly higher compared with pSWE and TE ( $p < 0.001$ ). We did not find a difference in mean LSM when obtaining 5 compared to 10 measurements for either 2D-SWE or pSWE. CV was lower for 2D-SWE ( $p < 0.001$ ) and pSWE ( $p = 0.005$ ) than for TE. Interobserver analysis demonstrated no difference in LSM for pSWE ( $p = 0.42$ ), whereas we found a difference for 2D-SWE ( $p = 0.009$ ). The interobserver reliability was good for the US elastography methods with a good correlation between observers for pSWE ( $r = 0.74$ ,  $p < 0.001$ ) and 2D-SWE ( $r = 0.65$ ,  $p < 0.001$ ). The variation of elastography measurements was small with an intraclass correlation for pSWE of 0.85 and 0.78 for 2D-SWE, and we found no observer bias using limits of agreement analysis. The mean LSM for female subjects ( $n = 50$ ) was lower than for males for TE ( $3.9 \pm 1.1$  kPa vs.  $4.5 \pm 1.0$  kPa,  $p = 0.006$ ) and 2D-SWE ( $4.3 \pm 0.7$  kPa vs.  $4.7 \pm 0.7$  kPa,  $p = 0.006$ ), whereas a similar trend was not statistically significant for pSWE ( $3.9 \pm 0.9$  kPa vs.  $4.2 \pm 0.7$  kPa,  $p = 0.063$ ). However, in subjects consuming less than 5 alcohol units or less per week, the difference was significant for all systems. There was no difference in LSM across the age groups (20-30, 31-40, 41-50, 51-60 and 61-70), nor



did we find any difference between subjects with BMI 18.0-25.0 kg/m<sup>2</sup> (n= 73) and BMI 25.1–30.0 kg/ m<sup>2</sup> (n= 29) when comparing the three elastography methods or looking at them combined.

### **4.3 Results study III**

55 non-transplant PSC patients (38 males and 17 females) and 24 healthy controls (8 males and 8 females) with a mean age of  $46.4 \pm 16.2$  and years and  $40.6 \pm 13.8$  years, respectively, participated in the study. Three patients had signs of biochemical significant cholestasis or hepatitis (bilirubin >30 (n=2) or ALT or AST > 5 x ULN (n=1). Signs of advanced liver fibrosis (liver capsule irregularity, periductal fibrosis and coarse liver parenchyma) was identified in 21 patients, 19 patients had splenomegaly, 25 had bile duct dilatation and 2 patients had ascites. Using pSWE (ElastPQ by Philips iU22), we found that when measuring the right liver lobe, PSC patients had higher median LSM compared to the healthy controls (SWV 1.26 [0.73–2.57] m/s vs. 1.09 [0.88– 1.25] m/s,  $p < 0.001$ .) The discrimination between patients and healthy controls was fairly good with AUROC of 0.775 (95 % CI [0.67-0,86) and the optimal cut-off for LSM of 1.24 m/s with a sensitivity of 56.4 and specificity of 95.8. The LSM was higher in patients with signs of advanced liver fibrosis (liver capsule irregularity,  $p=0.001$ ; periductal fibrosis  $p=0.049$ ; coarse liver parenchyma  $p=0.002$ ) than in patients with normal findings on B-mode US. Furthermore, we found that the LSM correlated with patients' fibrosis scores APRI ( $\rho$  0.494,  $P=0.001$ ) and FIB-4 scores ( $\rho$  0.368,  $P=0.017$ ). The LSM of the right liver did not differ between patients with and without splenomegaly ( $p=0.11$ ), nor in patients with

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or without bile duct dilatation ( $p=0.61$ ) and it did not correlate with Mayo risk, BMI, age or PSC duration. Valid LSM of the left liver could only be obtained in 36 patients, and we found no difference ( $P=0.11$ ) in LSM between patients (1.46 [0.59–3.68] m/s) and healthy controls (1.13 [0.91–1.24] m/s), nor between left LSM and BMI, age or PSC duration. When we compared left and right LSM, the LSM did not correlate ( $\rho=0.233$ ,  $P=0.17$ ). We obtained valid elasticity measurements of the spleen in 37 patients (1.47 [0.79–3.13] m/s) and found no difference ( $p=0.83$ ) compared to the healthy controls (1.48 [1.17–1.80] m/s.) Patients with splenomegaly had a tendency of higher spleen elastography measurements (SEM) (1.71 m/s [0.89–2.71]) compared to patients without (1.39 m/s [0.79–3.13]), however this difference was not statistically significant ( $p=0.05$ ). There was no correlation between SEM and LSM of the right or left liver, nor between SEM and BMI, age or PSC duration. We found good inter-and intraobserver agreement for LSM of the right liver in healthy controls.

## **5 Discussion**

### **5.1 Methodological considerations**

Guidelines and recommendation of clinical assessment of US elastography has been provided by the European Federation of Societies for Ultrasound in medicine and Biology (EFSUMB) and later by The World Federation for Ultrasound in medicine and Biology (WFUMB) for the liver, as well as other organs (10, 29, 31, 40). In chronic liver diseases, increasing fibrosis occurs and the liver becomes stiffer, which can be monitored noninvasively by shear wave elastography (62, 63). In recent years, an expanding spectrum of US elastography methods from commercially manufacturers has emerged. Because the elastography methods and algorithms used to determine tissue stiffness may vary in different commercially available US systems, the estimates of the liver stiffness within the same liver may be different when liver stiffness measurements are obtained by different methods (40). Hence, analysis of performance and comparative head-to-head studies that investigate, validate and address the agreement as well as the repeatability in vitro- and in vivo are needed, as some of these systems are already being employed in the clinical follow up of patients with chronic liver diseases.

To adequately test the novel elastography methods in vivo and in vitro, a gold standard is needed for comparison. Ideally, a gold standard should have a specificity and sensitivity of 100%, if not the estimates of new methods are false (4). However, in the assessment of liver fibrosis, liver biopsy has traditionally been considered the gold standard, even though it has 35% false-positive and false-negative rate for fibrosis stage in comparison to large surgical biopsies (64). Several studies have

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emphasized the usefulness of non-invasive methods to assess liver fibrosis, the most validated non-invasive method being the one-dimensional shear wave elastography method of Fibroscan, with accuracies around 80% when liver biopsy is taken as a reference and standardized according to staging (8). Shear wave elastography is the most common for assessment of liver fibrosis, whereas strain elastography methods have not yet been developed to assess strain quantitatively, it is currently not recommended for assessment of liver stiffness in clinical practice (40). This has been demonstrated in a number of studies (65). However, also SWE have some limitations in assessment of LSM as several factors influence LSM: the respiration phase, occurrence of non-fasting state, reverberations from the liver capsule, presence of steatosis and obesity, cholestasis, right heart failure, parenchymal inflammation of the liver with elevated transaminases  $>5-10 \times \text{ULN}$  (10, 40).

Prognosis, as well as management of chronic liver disease depend on the amount and progression of liver fibrosis. Presence of significant fibrosis may indicate antiviral treatment (66, 67), especially in chronic viral hepatitis, and presence of cirrhosis is an indication for specific monitoring of possible complications such as increased risk of hepatocellular carcinoma and portal hypertension (68). However, even though the elastography methods assess the stiffness of liver tissue as a proxy for fibrosis, the pathological process that initiate and maintain increased LSM may include both inflammation and neoplastic disease (69).

In this work, there are of course some limitations. Firstly, the phantoms applied did not mimic the viscoelastic properties of human liver, however, the phantoms used in the study spanned the relevant elasticities and had been used in similar studies of

other elastography methods. Furthermore, we did not apply the same elastography methods throughout all three studies, nor did we use liver biopsy and TE as the reference method in our in vivo studies. Ideally, we would have used the same elastography methods in all three studies, including liver biopsy as reference in the last study. This could have rendered even more validation for the methods, and given a direct comparison, in addition to validation- from ideal circumstances with known stiffness in vitro on liver fibrosis phantoms, to in vivo healthy subjects in different age groups and the sick cohort. However, in the second study liver biopsy is not ethically feasible in a healthy group and in the third study liver biopsies are not indicated in patients with diagnosed PSC, and were therefore also considered unethical for study purposes. Also, the number of healthy volunteers and patients could have been much higher, although at a higher cost. The power calculation I performed in study II, did however claim that the number of 100 healthy volunteers was sufficient for the analysis performed. The number of patients included in the third study could have been higher and perhaps performed in a multi-center environment, however, due to PSC being a rare disease it was difficult to obtain a higher number of patients for a single-center study. Furthermore, as study III was performed before study I and II, we did not have access to other quantitative elastography methods, and at the time being the number of patients included in our study is consistent with similar studies for PSC. Further methodological considerations are also discussed in each of the following paragraphs related to each study.

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## 5.2 Discussion of the main results

### 5.2.1 Discussion study I

This phantom study has shown that all five US shear wave elastography methods used, were highly repeatable and had a good agreement, for both one and two observers, when assessed on liver fibrosis phantoms with stiffness levels ranging from healthy liver tissue to cirrhosis. The in vitro design of the study was aimed to evaluate US elastography methods dependencies of stiffness measurements assessed in the non-invasive staging of liver fibrosis using commercially available liver fibrosis phantoms. The phantoms were purely elastic and did not accurately imitate the viscoelastic properties of human liver tissue, however the acoustic properties were comparable to live soft tissue. Because of the many factors that affect liver stiffness measurements in vivo, the study design represents an idealized and simplified situation, where only variability in the US systems and the free-hand scanning is assessed, while the objects examined are limited. Liver fibrosis phantoms do not have the limitation of subcutaneous fat that may affect the measurement depth, narrow intercostal spacing that may affect the applicability of the SWSE methods, we did not have to rely on patient cooperation that in vivo could affect the results, nor did we have factors such as possible ascites, hepatic inflammation or levels of cholestasis to evaluate. However, these factors allowed us to evaluate the SWSE methods under “ideal” circumstances on objects with known stiffness. Under standardized default settings we were able to assess all SWSE methods, without adjusting factors that may affect performance, resulting in a “fair” head-to-head comparison between the SWSE methods. Our results demonstrated a nearly perfect agreement between observers for

all the SWSE methods, and we found no significant difference in mean elasticity measurements between the observers for the SWSI methods. These results are in line with results in literature (70-74), and one recent study investigated six SWSE methods in vivo and found a good to excellent agreement between LSMs performed with the different systems (75). The Ultrasound Shear Wave Speed Committee (SWS), of the Radiological Society of North America (RSNA), Quantitative Imaging Biomarker Alliance (QIBA) conducted a multicentre study comparing several SWSE methods using elastic phantoms, with similar properties as the phantoms used in our study, and found a statistically significant difference in shear wave speed between methods and not related to stiffness, whilst no significant difference was found between observers using the same or equivalent SWSE methods (76). However, due to the study design it is not known which commercially available SWSE methods were applied, nor if the systematic error was due to using equivalent, however, non-identical phantoms. The same group later conducted a study on viscoelastic phantoms, mimicking real liver tissue, and found similar results demonstrating a consistent inter-system variability in viscoelastic phantoms, however, despite the variability the imaging, SWSE methods used in the study were able to differentiate the viscoelastic properties of the phantoms that span healthy to fibrotic liver (77), in line with our results. Furthermore, they found that an increased depth (3 cm, 4.5 cm and 7 cm) of elasticity measurements yielded increased intersystem variability. It has previously been demonstrated that the depth related differences (1- 4 cm) may affect repeatability of elastography measurements in vitro (78), thus to further strengthen

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the evidence all elasticity measurements were acquired approximately 3.5 cm below the transducer surface.

In our study, we found that the elasticity measurement made with TE were higher for the hardest phantom and for both observers. Even though the liver fibrosis phantoms were compatible with shear wave modalities, including TE, most of the phantom elasticity measurements obtained by the SWSE methods were lower than the stiffness values provided by the phantom manufacturer. This is well in line with the literature, where it has been discussed that this might be caused partly by lower frequency and smaller source of vibration in TE compared to shear wave speed measurement and imaging methods, which leads to increased diffraction and thereby induced overestimation of stiffness (79). We evaluated only one SWSI method in our study, and found that it had the lowest variation in elasticity measurements with no difference between observers for either soft or hard phantoms. We assumed that the tendency towards higher repeatability than other SWSE methods in the study, may have been influenced by a different measurement procedure that applies for 2D-SWE, as multiple frames could be acquired allowing several measurements within the same US probe position of the phantom. Even though SWMI is considered to be more precise and less operator dependent, one study has shown that tissue compression by the force extended on the US probe can significantly affect the elasticity measurement results (80). However, the phantom used in the study was made purely of gelatine and placed on a digital scale during measurements where US probe pressure applied was equivalent to grams. Furthermore, 2D-SWE from SSI was performed to obtain elasticity measurements and a linear transducer was applied,



whereas we applied the convex probe. The reasoning for applying the convex probe was that we were measuring liver fibrosis phantoms, and in a clinical setting we would use the same convex probes for real liver scanning. We also questioned if the size of ROI for the measurement could affect the repeatability. For a high quality 2D-SWE elastogram in the setting of chronic liver diseases it has been recommended to use a large ROI, more or equal to 1.8 cm in diameter, in addition to and low standard deviation and optimal depth to ensure low variability and high reliability (61). However, as our study was performed in vitro, we standardized the ROI in our study to 1 cm for 2D-SWE.

### *5.2.2 Discussion study II*

In the second study of this thesis, we used three SWSE methods, TE, one SWSM method and on SWSI method with 2D-SWE, to investigate normal liver stiffness values in a healthy Norwegian cohort of 100 participant with and equal number of men and women in five different age groups. Because SWSE within the normal range can rule out significant liver fibrosis when in agreement with clinical and laboratory background (40), it is pivotal to establish normal values of liver stiffness of novel SWSE methods for the method to be implemented as a reliable method in assessment and follow-up of chronic liver diseases (81).

One problem with TE is that the operator experience significantly influences the reliability of LSM acquired with TE (82, 83). However, it can be fairly easily learned and requires minimal operator training (84, 85). An operator that has performed more

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than 500 examinations with TE is considered an experienced TE operator (40). In our study, only one investigator performed TE, and was considered experienced, and we used TE as the reference standard in direct comparison with the novel two SWSE method.

The novel SWSE methods had good intraobserver and interobserver agreement, this is in concordance with previous studies investigating SWSM methods (71, 72, 74, 75, 86, 87). However, we found that there was a difference between operators when using the SWSI to obtain LSM. In our interobserver analysis, one operator had more than 3 years' experience and the other observer had only 1 years' experience. There is no consensus per today of what constitutes an experienced operator for SWSM and SWSI methods, however, it has been suggested that an experienced operator should have performed at least 300 abdominal US examinations and more than 50 supervised 2D-SWE examinations (40). Previous studies have emphasized the learning curve for 2D-SWE (73, 88). In our study, only one of the operators in our study was experienced. Furthermore, the SWSI method allowed the operators to set the measurement ROI freely within the elastogram avoiding incongruent signals when obtaining LSM, whereas the SWSM method measured the LS without visualizing the stiffness.

Previous reports have found that LSM obtained by TE vary between 4.4 and 5.5 kPa (89-91), the European Association of Liver Diseases (EASL) state in their guidelines that cut-off values vary considerably, however LSM values greater than 6.8 and 7.6 kPa indicate a higher probability of significant fibrosis on liver biopsy (92). A recent

meta-analysis found that the mean LS obtained by TE for truly healthy non-obese individuals was 4.7 kPa (93), in our cohort the mean LSM obtained with TE was 4.2 kPa, 4.0-4.5 kPa 95% CI. When comparing the mean LSM obtained with the two novel SWSE methods, we found that the SWSI method obtained higher values than TE and the SWSM method, whereas the SWSM method did not differ from TE. Previous studies have shown similar results for 2D-SWE and healthy LSM in the range between 4.5 and 5.5 kPa (73, 94-96), whereas our cohort had a LSM with 95% CI between 4.3 and 4.7 kPa for the SWSI method. We chose to report the LSM in kPa for the SWSM method, as the method opted for this and we wanted to perform a head to head comparison between the methods, and found the mean LSM to be 4.1 kPa, whereas previous normal values found using SWSM methods report the LSM in the range between 1.07 and 1.16 m/s (72, 74, 97, 98).

One of the strengths in our study was the comprehensive exclusion of liver disease in a cohort with equal number of male and female participants in the different age groups. Factors affecting LSM have been extensively discussed in the literature. In our study we found that LSM was higher in male participants using SWSI, however, not when using SWSM. Similar results have been demonstrated previously for SWSI (94, 96), whereas for SWSM previous reports have showed inconsistent results of gender affecting LS (99-101). One study has demonstrated that males have higher LS than females when using SWSM (102), however the number of participants were unequal, whereas we had an equal number of male and female participants. Using TE it has been shown that males had higher LSM than females (89, 90), in line with our results. However, a large meta-analysis including 26 healthy cohorts with a minimum

of at least 50 participants did not find that gender was associated with a statistically significant influence on LSM (103). Regarding the influence of gender and conflicting study reports for the different SWSE methods, it may indicate that there is a need to define separate cut-off values for normal LSM for males and females in healthy subjects, and possibly also in patients with liver fibrosis. One study has proposed that different hormone levels between the genders in rats may suggest a possible explanation for the differences in LSM between the genders (104). Another study has demonstrated results that might indicate that differences between genders occur in the age group 12-17 years (105). Accordingly, possible gender differences need to be investigated further in humans.

Factors such as age and BMI have been discussed to be a variable that may influence LSM in normal subjects. Many studies have been published on this matter; however, the results have been inconsistent. In our study, all participants were carefully interviewed, information regarding alcohol consumption was collected, the subjects had normal liver biochemical analyses, normal findings on B-mode US examination and no history of chronic or present hepatic disease in addition to BMI less than 30 kg/m<sup>2</sup>. Inclusion and exclusion criterions in healthy studies may contribute to, and perhaps partly explain, differences between our findings and other reports. We categorized the subjects in two BMI groups, first group with BMI 18.0–25.0 kg/m<sup>2</sup> (n=73) and second with BMI 25–30 kg/m<sup>2</sup> (n=27), and could not find that BMI affected LSM. However, some studies have demonstrated that BMI and age affect LSM with conflicting results (95, 96, 106). Compared to subjects with normal weight, higher LSM has been demonstrated in subjects with BMI below 18.5 kg/m<sup>2</sup> and

above 30 kg/m<sup>2</sup> (107). However, in our study patients with BMI below 18.0 kg/m<sup>2</sup> and above 30 kg/m<sup>2</sup> were not considered healthy and therefore excluded from the study. Furthermore, we did not use the XL-probe, which was developed for obese patients and differs from the M-probe as it uses a lower frequency, has a deeper focal length and a lower depth, making it suitable for patients with large skin to liver capsule distance (108). It has been addressed in the literature that an increased waist circumference is associated with an increased failure rate of obtaining LSM with TE (82, 100, 107, 109, 110), but whether the waist circumference influences the normal range of LSM is unclear as most studies have focused on BMI rather than waist circumference and with varying results (111). We recorded skin to liver capsule distance and waist circumference for all subjects, however, we did not include these data in our analysis. In retrospect, we acknowledge that these analyses could have strengthened the study and contributed to utterly to specify our results.

It has also been investigated if age is a confounding factor of LSM, and similar to BMI the results have been inconsistent, demonstrating no difference across different age groups, higher LSM in higher age and higher LSM in lower age (89, 90, 102, 112). In our cohort we found no difference in LSM for any of the methods between the five age groups, ranging from 20 to 70 years and including 10 male and 10 female subjects in each group. One study did demonstrate an association of higher LSM in subjects aged over 40 years using SWSI (94), however, the study design was not identical to ours and the biochemical analyses were not present for all subjects.

Whether factors such as comorbidity i.e. steatosis and heart failure, might be confounders in the analyses of age effect on LSM remains to be answered.

One of the interesting findings in our study is that we demonstrated no difference between acquiring 5 instead of 10 LSM for either the SWSI nor the SWSM method. There is an ongoing discussion of how many measurements must be acquired as a minimum for the LSM to be representative, and currently EFSUMB recommends 10 measurements for SWSM and TE, whilst only 3 measurements with SWSI (40, 113). Using SWSI methods, a previous report has demonstrated excellent intraobserver reproducibility and suggesting 6 LSM to be optimal number of measurements (114), whereas another report concluded that 10 measurements should be acquired when assessing SWSM methods (115). We found no significant difference in mean LSM of 5 and 10 measurements for either methods, and our results suggest that a reliable LSM may be obtained with fewer SWSE measurements. As a consequence of fewer repetitions of measurements, the examination could be performed in less time. However, our results reflect healthy non-obese subjects without chronic liver disease. Therefore our results are not representative to apply in patients with liver fibrosis, where the variability in measurements maybe higher, as it has been demonstrated that higher variability increases with higher liver stiffness (116).

### *5.2.3 Discussion study III*

According to EASL, primary sclerosing cholangitis (PSC) is a rare chronic inflammatory autoimmune liver disease primarily affecting the bile ducts, leading to biliary fibrosis and progressing to liver cirrhosis over time (117). Nearly half of patients with PSC are asymptomatic at time of diagnosis (118), and the disease is

considered premalignant and being associated with cancers in the hepatobiliary tract and colorectum (119). There is no effective medical therapy to this date, and it has been estimated that PSC accounts for 10-15% of all liver transplants in Europe, which is the only proven life-extending intervention for this patient group (119). In the diagnostic approach of PSC, imaging plays an important role, as the diagnosis of PSC is primarily identified with characteristic bile duct changes on magnetic resonance cholangiopancreatography (MRCP) (120), where typical cholangiographic changes define the diagnosis of PSC (117). Although US is not diagnostic in PSC, it is useful as signs of a thickened bile duct wall, focal dilatations of the bile duct and gallbladder abnormalities can easily be observed in patients with PSC and other reasons for cholestatic disorder and biliary obstruction can be excluded (121, 122), as the goals of management and treatment of PSC are primary prevention of end-stage liver disease and associated symptoms (122). Furthermore, the EASL guidelines recommend that patients with PSC should be considered with annual US examination of gallbladder abnormalities (120).

The last years there has been a vast interest, and focus, on investigating non-invasive clinical tools in the assessment of the disease. Several reports have suggested that a non-invasive approach to evaluate the degree of fibrosis and possible changes will be of significant importance in the follow-up of the patient group. As PSC is characterized by a patchy distribution of fibrosis that disturbs the homogeneity of liver tissue, the use of US elastography is even stronger indicated (27, 123). The aim of our study was to explore US SWSE as a non-invasive method, as it covers larger

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areas of the liver and allows direct visualization of liver, to evaluate liver fibrosis in a Norwegian PSC cohort.

We detected signs of liver fibrosis on abdominal US in 21 patients and LSM obtained in this group were higher. Furthermore, in applying SWSM we identified another 12 patients with higher LSM but without visual signs of fibrosis on B-mode. The SWSM method used in our study had been designed to measure LSM in patients with viral hepatitis (124-127) and proven as a non-invasive and repeatable method for LSM. We found that the feasibility of the SWSM method applied, as well as intraobserver and interobserver agreement was excellent for the right liver lobe, in line with previous reports using the same SWSM method (102). Furthermore, we observed that liver fibrosis can easily be evaluated by both SWSE and traditional US B-mode during the same procedure.

In our study we found that patients with PSC have higher LSM, compared to the healthy controls, when performed with SWSM. These results were in line with literature (128). In addition, it has been demonstrated that the SWSM method used in our study, has good to excellent correlation with histological evaluation of fibrosis (124, 128). Previous studies have found similar results using TE (123, 129-131), and TE is considered an accurate marker of liver fibrosis in PSC (132). One study included 28 patients with PSC and 78 with PBC and showed that TE was very specific and sensitive for predicting advanced fibrosis (129). Notably, TE is most suitable at the extremes of histological stages and it is established in the literature that cholestasis affects the accuracy of LSM in non-invasive assessment of liver fibrosis (133). Considering that PSC is a cholestatic disease, it might complicate the



evaluation of LSM obtained by SWSE methods. In this respect, applying TE solely for LSM presents a limitation because the method does not enable visualization of the gallbladder or the presence of intrahepatic cholestasis, that can be done with B-mode US. We only used one SWSE method in our study, and did not have access to use TE as a reference method. In a recent publication reviewing liver elastography guidelines (134), the author concluded that TE and ARFI-based methods, as the one used in our study, have the same accuracy in liver stiffness assessment, and emphasized that some studies have suggested that ARFI-based methods are more accurate than TE. One study used ARFI to obtain LSM, and demonstrated that LSM is higher in patients with obstructive cholestasis, suggesting that the presence of biliary obstruction should be an exclusion criterion in the baseline LSM (135). In our study, only two patients had bilirubin  $> 30 \mu\text{mol/L}$ , and 47% of the patients had some degree of bile duct dilatation on B-mode US. However, we could not demonstrate any difference in LSM in these patients compared to patients lacking signs of bile duct dilatation on B-mode.

We found a low success rate and high variability of LSM of the left liver lobe, and there was a larger range in LSM of the left liver lobe compared to LSM of the right liver lobe. Anatomical localization might be a possible explanation, as the cardiac and respiratory movements cause more motion and thereby affect reliability of LSM obtained (102, 115, 136). Several studies have investigated differences of LSM in left and right liver lobe with varying conclusions, some have concluded that that LSM was higher when obtained in the left lobe (115, 137), whereas others found no

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difference (138). Interestingly, in our study population, we found that the LSM of the left liver lobe correlated with APRI in those 66% valid LSM obtained.

In our analyses we found that a cut-off value for LSM of 1.24 m/s had good ability (AUC of 0.775) to discriminate between PSC patients and healthy controls. Previous findings have suggested a similar cut-off of 1.23 m/s (98), however, the patient population in this study included different aetiologies and none PSC patients. From a clinical point of view, we hesitate to say if 1.24 m/s is representative as an ideal cut-off for all PSC patients. If the aim is to identify early diagnosis of liver fibrosis, sensitivity is favoured, and by assessing LSM our results suggest an increased sensitivity in identifying liver fibrosis PSC patients. However, if the aim is to identify patients in a high-risk group who need follow-up, specificity is favoured over sensitivity. Therefore, longitudinal studies are necessary to evaluate if SWSM methods can be used to estimate progression during follow-up of PSC patients. A recent study concluded that the same SWSM method can evaluate fibrosis in AIH and PSC with good accuracy for detecting hepatic fibrosis, however, the authors suggest to be cautious when interpreting LSM in early fibrosis stages (139). Moreover, it is well known from previous reports that the cut-off values for significant fibrosis and cirrhosis vary with the aetiology of liver disease e.g. compared to alcoholic liver disease, suggested cut-off values are lower for cystic fibrosis liver disease, which has a similar fibrotic distribution as PSC (124, 140-142).

The lack of histological correlation is an obvious limitation of our study.

Furthermore, we did not include TE as a reference method, nor did we perform a head to head comparison with TE or other elastography methods. Liver biopsy is in

general not indicated in patients with PSC as it may result in sampling error and variability, due to the patchy distribution of fibrosis (143). Similar to PSC, primary biliary sclerosis (PBC) has a patchy distribution of fibrosis (27), and one study demonstrated that only 10 of 50 explant PBC livers had a consistent stage throughout the liver (144). Although we did not have histological correlation in our study, we found that LSM correlated with aspartate aminotransferase (AST)-to-platelet ratio index (APRI) and Fibrosis index based on four factors (FIB-4), two suitable serum-based scores with moderate sensitivity and accuracy for detecting liver fibrosis stage (145). Our findings indicate that by adding LSM to an US evaluation, increased sensitivity for identifying fibrosis in PSC patients could be implied.

Using magnetic resonance elastography (MRE) an entire elastography map of the liver is generated, enabling a visualisation of the whole liver. Some recent publications have shown that the method may be used to predict liver decompensation and that it correlates with different stages of liver fibrosis (146-148). However, compared to US elastography methods, MRE does have the limitation of being much more expensive, time-consuming and not widely available.

## 6 Conclusions

We have demonstrated the feasibility and validated the reliability of several SWSE methods, from in vitro on liver fibrosis phantoms, to healthy subjects to patients with PSC. We have also provided evidence for the reproducibility of SWSE methods and suggested normal LSM values for two novel SWSE methods and our results indicate that obtaining 5 LSM may be sufficient to obtain reliable results. Lastly, we have suggested a cut-off LSM value to discriminate between PSC patients with fibrosis and healthy liver subjects. Our studies have contributed to further open the door to possibilities in the clinical and diagnostic implementation of SWSE assessment in liver diseases. Although larger studies are necessary in order to bring together different SWSE methods to establish a reference base, we believe that our work provides clinicians and researchers with specific advice on how to apply these methods with guidance on cut-off values when assessing LSM with SWSE methods.

## 7 Future perspectives

We would like to use the same novel methods applied in our second study, where we have established normal values for a Norwegian cohort, in a longitudinal and prospective study where we follow up patients with established chronic liver disease before, under and after they start treatment for Hepatitis C Virus (HCV). Using the same software, implemented in the same methods as in our healthy study and TE as a reference we would like to investigate whether SWSE can be used, not only to define the indication for treatment through LSM, but also to investigate whether fibrosis may be reversible in treated patients and investigate what role frequency in the follow up of patients with HCV plays. In this respect, we have an ongoing project where we have included 70 patients. In addition, we are analysing results from a study where we conducted elastography measurements and assessed the controlled attenuation parameter (CAP), integrated in Fibroscan, in an apparently healthy cohort. We hope that these results may contribute in the further research of non-alcoholic fatty liver disease (NAFLD), where elastography has shown promising results. Furthermore, we are performing a longitudinal and prospective follow-up of patients with PSC with the same SWSE method assessed in study three. We are observing that US elastography is frequently applied in daily routine when scanning patients with liver diseases. In the future, elastography holds great promise as a clinical tool, also by adding prognostic information in diseases such as PSC.

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# Paper I

I



RESEARCH ARTICLE

# Repeatability of shear wave elastography in liver fibrosis phantoms—Evaluation of five different systems

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## Abstract

This study aimed to assess and validate the repeatability and agreement of quantitative elastography of novel shear wave methods on four individual tissue-mimicking liver fibrosis phantoms with different known Young's modulus. We used GE Logiq E9 2D-SWE, Philips iU22 ARFI (pSWE), Samsung TS80A SWE (pSWE), Hitachi Ascendus (SWM) and Transient Elastography (TE). Two individual investigators performed all measurements non-continued and in parallel. The methods were evaluated for inter- and intraobserver variability by intraclass correlation, coefficient of variation and limits of agreement using the median elastography value. All systems used in this study provided high repeatability in quantitative measurements in a liver fibrosis phantom and excellent inter- and intraclass correlations. All four elastography platforms showed excellent intra- and interobserver agreement (interclass correlation 0.981–1.000 and intraclass correlation 0.987–1.000) and no significant difference in mean elasticity measurements for all systems, except for TE on phantom 4. All four liver fibrosis phantoms could be differentiated by quantitative elastography, by all platforms ( $p < 0.001$ ). In the Bland-Altman analysis the differences in measurements were larger for the phantoms with higher Young's modulus. All platforms had a coefficient of variation in the range 0.00–0.21 for all four phantoms, equivalent to low variance and high repeatability.

## OPEN ACCESS

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## Introduction

Elastography is a non-invasive imaging technique that aims to assess tissue elasticity in several organs through quantitative or semi-quantitative measurements. In the last years, several manufacturers have introduced new elastography methods, offering shear-wave based elasticity mapping or measurement integrated in high-end scanners. The methods are aimed to be used as a clinical tool in several fields of medicine, however the use of shear wave elastography

(SWE) methods has predominantly focused on application in chronic liver diseases. The elastography methods that are implemented vary by technique, reported parameter, application and are not standardized to a common use. The different manufacturers apply propriety patented calculation modes, which might result in different values. [1, 2] This has been addressed in previous studies where liver elasticity has been assessed, and several papers have confirmed that the different technologies have different cut-off values. [1, 3, 4] However, it is important that comparative studies address the repeatability and agreement of the emerging technologies in vitro as well as in vivo in healthy and non-healthy patients. So far there is not enough scientific evidence in the literature to validate the most recent technologies.

All elastography methods are based on that the tissue elasticity is measured by Young's modulus as pressure in kilopascals (kPa). The relationship between the applied stress and resulting strain is defined by Young's modulus and quantifies tissue elasticity. This means that the harder the tissue elasticity is, the higher Young's modulus (elasticity) will be.

The SWE methods use an acoustic pulse to create shear waves that travel perpendicularly to, and much slower than the longitudinal ultrasound (US) waves, making it possible to track and measure them within a limited distance. [5, 6] The velocity of the propagating shear waves is faster in harder than in softer tissue, making it a useful method in the evaluation of soft tissue. The main elastography technologies can be divided into strain imaging, shear wave speed measurement and shear wave speed imaging (2D-SWE). The technologies differ by the type of force applied, the visual representation of tissue elasticity and possibility to perform quantitative assessment of recorded tissue elasticity. [7] The elasticity measurements, using SWE or 2D SWE, may be expressed as either shear wave velocity (m/s) or Young's modulus (kPa).

Most SWE methods integrated into US scanners provide real-time visualization (Brightness mode/B-mode) allowing the examiner to position the specific area of interest for elasticity measurements. This is of great clinical value as it gives the ability to evaluate the liver tissue, and perform elastography measurements whilst avoiding vessels and choosing the region of interest at the right depth from the liver capsule. Elasticity itself is often not visualized (Point shear wave elastography; VTQ and ElastPQ); however, some 2D-shear wave elastography (2D-SWE) methods, including GE 2D-SWE and Supersonic SWE, offer real time visualization of elasticity by a color map within the measurement area and a numerical calculation of shear wave speeds or elasticity. One exception is TE, which was one of the first elastography technologies available. [8–10] While well validated in the literature, TE lacks ultrasound visualization and cannot be applied in patients with perihepatic ascites. [7]

The aim of this study was to compare and assess the agreement and repeatability of three novel elastography technologies and compare their results to one established shear wave method on liver fibrosis phantoms.

## Material and methods

### Study design

We used five different elastography systems, which were all commercially available and approved for medical use in diagnostic ultrasound. The systems reported the tissue elasticity in meters per second (m/s) or kilopascals (kPa), as Young's modulus. Two individual observers (A.M. and A.B.M.) obtained data from all elastography methods individually, blinded to each other's results. Each observer (A and B) performed free-hand scanning of the four, separate, tissue-mimicking phantoms and made ten separate measurements of each phantom using the same elastography imaging settings. Both observers had more than 2 years' experience in ultrasound and elastography. Only one observer was certified for Fibroscan. The curvilinear probes were applied for imaging and elastography on the ultrasound scanners, whilst the M-probe

**Table 1. Expected measurements and acoustic properties for the liver fibrosis phantoms represented with  $\pm 5\%$  SD.**

Phantom	Young's modulus (kPa)	Density (g/cm <sup>3</sup> )	Speed of sound (m/s)	Expected shear wave velocity (m/s)
1	2.7 $\pm$ 0.14	1.03	1533	1.62 $\pm$ 0.08
2	11.5 $\pm$ 0.57	1.03	1536	3.34 $\pm$ 0.17
3	24.8 $\pm$ 1.24	1.03	1531	4.91 $\pm$ 0.25
4	46.3 $\pm$ 2.32	1.03	1530	6.70 $\pm$ 0.34

<https://doi.org/10.1371/journal.pone.0189671.t001>

was applied for TE. The region of interest (ROI) was standardized; for 2D-SWE 1 cm circle, for S-Shearwave Elastography and Acoustic Radiation Force Impulse (ARFI) a standardized box 1x0.5 cm and for Shear Wave Measurement (SWM) 1x1.5 cm and fixed in size. The ROI was placed 2–3 cm under the liver fibrosis phantom surface. The elastography systems were evaluated for inter- and intraobserver variability by, coefficient of variation, interclass correlation and limits of agreement using the median value. Each image was recorded to the hard drive of the scanners and stored to an external storage device. Software versions and default settings are provided in the Appendix.

### The objects of examination

The object of examination were liver fibrosis phantoms manufactured by Computerized Imaging Reference Systems (CIRS Inc. Virginia, USA). The model 039 consisted of four separate phantoms of varying stiffness (Table 1). Each phantom was 10 cm deep and made with Zerdine®, a patented synthetic polymer, housed in a 14 cm tall and 11.6 cm wide cylinder with a Saran-based scan surface and a scanning well. The phantom was compatible with the ultrasound shear wave modalities including Fibroscan Transient Elastography and ARFI. It had standard configuration with the following nominal acoustic properties: Attenuation: 0.5dB/cm/MHz, Contrast: 0 dB with respect to CIRS liver reference. The actual acoustic and mechanical properties of each phantom had been batch tested by the manufacturer by an external method, and the measured and calculated values are provided in Table 1. Similar cylindrical Zerdine phantoms from CIRS have been determined to be adequately homogeneous based on testing performed by the Nightingale Laboratory at Duke University and QIBA (Dept. of Biomedical Engineering). [11, 12]

### Elasticity imaging and SWE platforms applied

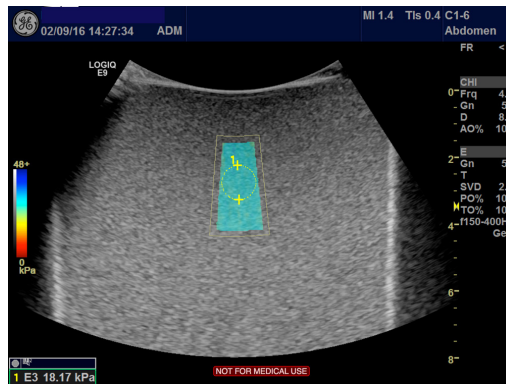
#### GE 2D-Shear wave elastography (SWE)

The elastography method of 2D-SWE was applied from the system of LOGIQ E9 (GE Healthcare, Milwaukee, Wisconsin, USA) Version 2.0, using the C1-6 probe. The method generates shear wave velocity through an acoustic push pulse, creating a color mapped elastogram. The color indicated the stiffness of the tissue, where red was soft and blue hard. Within the color map, the operator could place a region of interest (ROI) and adjust the size of the ROI. After placing the ROI, under default scanner settings, the elasticity measurements were automatically acquired by the system. (Fig 1) In our study we standardized the size of our ROI to 1 cm and the measurement was obtained at least 2 cm inferior of the liver fibrosis phantom surface. [13] The measurements were expressed in kPa.

#### Samsung S-Shearwave Elastography (S-SWE)

Using the RS80A with Prestige ultrasound equipment (Samsung Medison Co. Ltd., Seoul, Korea) we assessed the S-Shear wave Elastography (Version 2.0). Within the brightness mode (B-mode) window, using default scanner settings, the ROI could be placed freely and had a

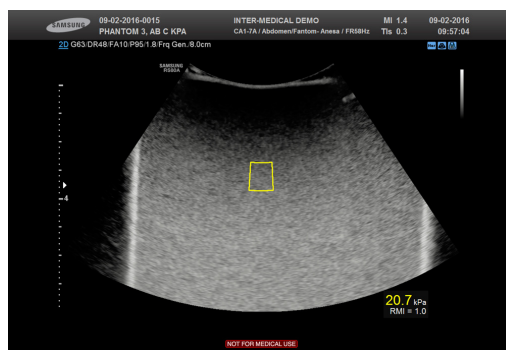




**Fig 1. GE 2D-SWE.** The figure illustrates the method of 2D-SWE by GE performed on liver fibrosis phantom 3 with Young’s modulus 24.8 kPa  $\pm$ 5%. The color box (centre) represents the elastogram, and the circle represents the ROI where the elasticity measurement is acquired. The blue color indicates harder tissue, as semi-quantitatively presented by the color scale to the left.

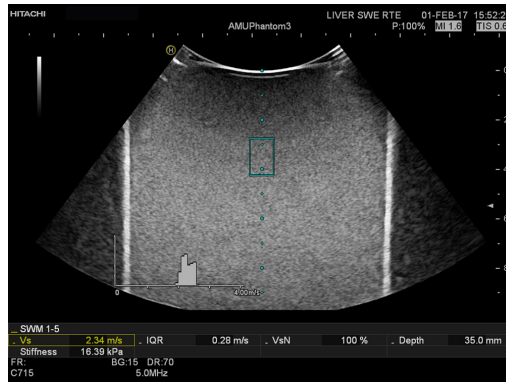
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fixed height of 1 cm. (Fig 2) The width was automatically adjusted depending of the measurement depth. If the ROI was placed in an area where measurements could not be obtained, for example at 7 cm depth, the color of the box changed to orange, symbolizing an invalid position. We placed the ROI at least 2 cm inferior of the phantom surface. The measurements were expressed in m/s and kPa simultaneously. The method had a unique performance index, “Reliability Measurement Index” (RMI), which is calculated by the weighted sum of the residual of the wave equation and the magnitude of the shear wave. [14] RMI ranging from 0.0–1.0, where 0.4 or higher is considered as acceptable whilst 1.0 is considered a very high value of RMI, and strongly correlates with reproducible measurements, according to the manufacturer. The proposed index is utilized to filter out unreliable measurements and result in performance improvement of shear wave elastography.



**Fig 2. Samsung (S-SWE).** Samsung S-Shearwave Elastography assessed on liver fibrosis phantom 3. The yellow box (centre) represents the shear-wave measurement area. ROI and the RMI (Reliability Measurement Index) is expressed below the obtained elasticity measurement of 20.7 kPa.

<https://doi.org/10.1371/journal.pone.0189671.g002>



**Fig 3. Hitachi (SWM).** Hitachi SWM was applied on liver fibrosis phantom 3. The ROI is represented by the blue box (centre). The shear wave velocity measurements are presented in the histogram, and the median is given as  $V_s$  in m/s. Stiffness is based on this value expressed in kPa as well as the IQR (m/s), VsN (Reliability Index for shear wave velocity measurement) and the depth of the sample.

<https://doi.org/10.1371/journal.pone.0189671.g003>

### Hitachi Shear Wave Measurement (SWM)

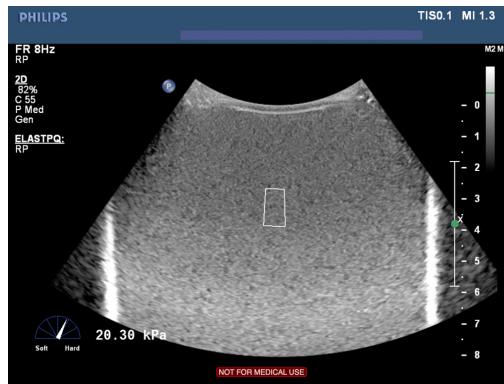
Using a Hitachi HI VISION Ascendus (Hitachi Medical corporation, Tokyo, Japan) scanner SWM was applied using the EUP-C715 probe (1-5MHz). Within one SWM measurement several push track sequences are delivered and the SWM samples the shear wave velocity in multiple positions, at different depths, inside the ROI. This is automatically repeated within a short time (<1second). Per acquired SWM, the system displays a histogram and measurement overview. The distribution of the multiple velocity measurements ( $V_s$ ) were displayed in a histogram, the IQR, depth of sample, the median of  $V_s$  in m/s and transformed to kPa. (Fig 3) The method has a built-in feature, the VsN, which is a reliability index of the  $V_s$  values acquired per measurement and functions as a quality indicator, and ranges from 0–100%. [15] Ten repeated acquisitions were made, using default scanner settings, and the results were given in m/s. The elasticity was also provided in kPa, by calculation of Young’s modulus, configured by Hitachi’s application specialist.

### Philips point shear-wave elastography (pSWE)

Using a Philips iU22 (Eindhoven, Netherlands) a point shear wave elastography platform, also known as ARFI quantification, was applied using a C5-1 probe. The method is based on a quantitative measurement of tissue elasticity, as the ultrasound probe produces a dynamic force that is applied through focused radiation force impulse. This generates shear waves that propagate perpendicularly to the push pulse through the tissue, across the ROI where the propagation of shear wave velocity is measured [2, 16]. The measurements were obtained, using default scanner settings, applying minimum pressure to the phantom surface whilst holding the probe still. The ROI, which was standardized and had a fixed area of 0,5x1 cm, was placed within the field of view obtaining an elasticity measurement. (Fig 4) 10 individual measurements were repeated, and the results were displayed as a median and mean with standard deviation (SD) of 10 measurements. The elasticity was expressed in kPa.

### Fibroscan (Transient elastography, TE)

Applying Fibroscan® 204 (EchoSens, Paris, France), we used the standard M-probe with a transducer frequency of 3.5 MHz on all four phantoms. The probe generates a vibration with



**Fig 4. Philips ARFI (pSWE).** Philips pSWE elastography is applied on liver fibrosis phantom 3. The shear wave measurement area is represented by the white box (center). The stiffness is shown in kPa on the left together with the unnumbered scale indicating the stiffness of the tissue, here shown towards hard.

<https://doi.org/10.1371/journal.pone.0189671.g004>

50 Hz frequency and 2 mm amplitude, which induces a shear wave propagation. The velocity of the shear wave is directly calculated by the device and the results are expressed in kilopascals (kPa) without B-mode. [2, 17] Valid measurements were performed. Both observers aimed to fulfill all quality parameters when performing the measurements. (Fig 5) The device displayed median value of ten measurements, number of failed measurements, the IQR and IQR/median. Reliable measurements were defined by the producer: a measurement success rate (SR) of  $\geq 60\%$  and IQR of  $< 30\%$ . [17, 18]

### Statistical analysis

The statistical analysis was performed using SPSS, Version 24.0, IBM Statistics (Armon, New York, NY, USA). Descriptive statistics and one-way analysis of variance was used to analyze



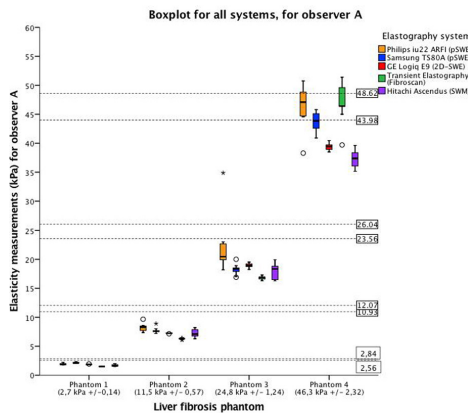
**Fig 5. Fibroscan (Transient Elastography, TE).** This figure illustrates the assessment of transient elastography on liver phantom 3. 10 valid elastography measurements are listed on the right side, where also success rate and invalid measurements are reported. The IQR/median is used as a quality parameter, and aimed to be below 30% while obtaining a success rate of at least 60%.

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the data. The measurements are represented as median values with min-max for 10 measurements of each phantom, and for each system. The interquartile range (IQR) and the dispersion of the measurements is represented in the boxplots. A higher box (IQR) represents a larger spread in measurements, and represents the data between the 25th and 75th percentile, essentially the range of the middle 50% of the data. Reliable measurements were defined as: median value of 10 valid LS measurements with a success rate (ratio of the number of successful acquisitions divided by the total number of acquisitions)  $\geq 60\%$  and an interquartile range interval  $< 30\%$ . IQR/Median (%) is illustrated in the bar charts, and was calculated for both observers individually as well as together and for all systems. [17, 18] We calculated the coefficient of variation (CV) of the intraobserver variability, which is the standard deviation (SD) divided by the mean value. Inter-class correlation coefficients (ICC) were calculated to present the interobserver reliability; ICC near 1.00 indicated high reliability. One-way ANOVA and Tukey’s test was used to test the overall significance, and  $p < 0.01$  was chosen as level of significance because we performed multiple testing by platforms and phantoms. Inter-observer agreement was assessed by correlation plots using Pearson’s coefficient of correlation (r). Limits of agreement were assessed to discover differences between individual measurements for each method. [19, 20]

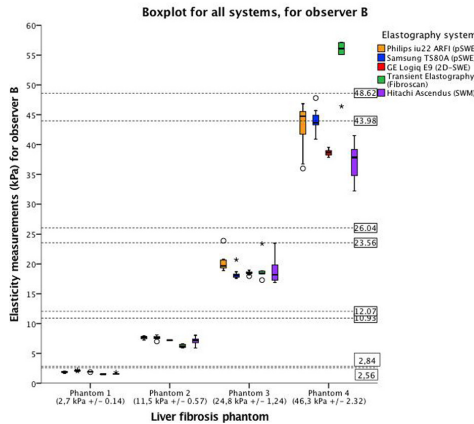
### Results

All liver fibrosis phantoms (1–4) could be significantly differentiated by all elastography methods ( $p < 0.001$ ) as illustrated by the boxplots in Figs 6 and 7. Figs 6 and 7 shows the measurement variability, interquartile range (IQR) and median values, represented by the vertical distribution of the box, which is illustrated in different colors for the respective systems. All systems showed a low variability for the softer phantoms (1 and 2), compared with the harder



**Fig 6. Variation in elasticity measurements for all systems for observer A.** The boxplot displays the median and the interquartile range, whiskers represent the 90th percentile of the measured elasticity by observer A for the four phantoms. The height of the box represents the measurement variability of the single observer for each of the phantoms. The horizontal axis represents the four phantoms with increasing stiffness; phantom 1 (2.7  $\pm$  0.14 kPa), phantom 2 (11.5  $\pm$  0.57 kPa), phantom 3 (24.8  $\pm$  1.24 kPa) and phantom 4 (46.3  $\pm$  2.32 kPa). The range each phantom stiffness is presented by the dotted lines within the figure. The vertical axis represents elasticity measurements (kPa) obtained by observer A. The colors represent the systems applied in the study: yellow, Philips iU22 (pSWE); blue, Samsung TS80A (pSWE); red, GE E9 (2D-SWE); green, Fibroscan (Transient Elastography) and purple, Hitachi Ascendus (SWM).

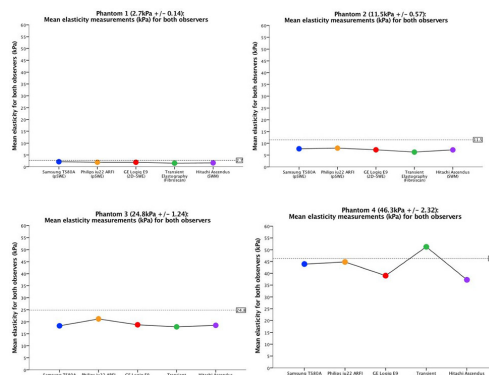
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**Fig 7. Variation in elasticity measurements for all systems for observer B.** The boxplot displays the median and the interquartile range, whiskers represent the 90th percentile of the measured elasticity by observer B for the four phantoms. The height of the box represents the measurement variability of the single observer for each of the phantoms. The horizontal axis represents the four phantoms with increasing stiffness: phantom 1 ( $2.7 \pm 0.14$  kPa), phantom 2 ( $11.5 \pm 0.57$  kPa), phantom 3 ( $24.8 \pm 1.24$  kPa) and phantom 4 ( $46.3 \pm 2.32$  kPa). The range each phantom stiffness is presented by the dotted lines within the figure. The vertical axis represents elasticity measurements (kPa) obtained by observer B. For color representation, we refer to Fig 6.

<https://doi.org/10.1371/journal.pone.0189671.g007>

phantoms (3 and 4). This is confirmed in the correlation analysis and limits of agreement. When acquiring elasticity measurements (mean for both observers) of phantom 3, Philips ARFI showed higher mean and measurement variability compared to all the other systems ( $p < 0.001$ ). Both observers obtained higher elasticity measurements with all the shear wave methods than TE for phantom 1–3, and lower for phantom 4. (Fig 8) Furthermore, we could



**Fig 8. Mean elasticity measurements for both observers.** The figure shows the common mean for both observers within each phantom and for all systems. On the horizontal axis, the systems are listed with name, and on the vertical axis the mean elasticity measurements are expressed in kPa. The dotted line within the graph represents the elasticity of the respective phantom, provided by the producer. For color representation, we refer to Fig 6.

<https://doi.org/10.1371/journal.pone.0189671.g008>

**Table 2. Mean and median of measurements for observer A (Mean/Median A) and B (Mean/Median B) in all liver fibrosis phantoms (1–4) and for all systems.**

	Philips iU22 XM (ARFI)	Samsung RS80A (SWE)	GE Logiq E9 (2D-SWE)	Hitachi Ascendus (SWM)	Fibroscan (TE)
<b>Phantom 1</b>	<b>2.7 kPa ± 0.14</b>				
Mean/Median A	1.94/1.93	2.12/2.10	1.86/1.86	1.67/1.61	1.50/1.50
Mean/Median B	1.87/1.89	2.11/2.10	1.91/1.92	1.59/1.56	1.50/1.50
CV A	0.08	0.04	0.02	0.10	0.00
CV B	0.06	0.03	0.01	0.06	0.00
CV AB	0.07	0.04	0.02	0.08	0.00
<b>Phantom 2</b>	<b>11.5 kPa ± 0.57</b>				
Mean/Median A	8.24/8.29	7.71/7.60	7.24/7.24	7.27/7.11	6.28/6.30
Mean/Median B	7.64/7.73	7.63/7.65	7.20/7.21	7.12/7.21	6.25/6.20
CV A	0.08	0.06	0.01	0.09	0.02
CV B	0.03	0.04	0.00	0.08	0.04
CV AB	0.06	0.05	0.01	0.08	0.03
<b>Phantom 3</b>	<b>24.8 kPa ± 1.24</b>				
Mean/Median A	22.18/20.46	18.27/18.40	18.94/18.92	17.97/18.34	16.80/16.90
Mean/Median B	20.17/19.68	18.31/18.15	18.51/18.47	19.01/18.20	18.91/18.40
CV A	0.21	0.05	0.02	0.07	0.02
CV B	0.07	0.05	0.02	0.12	0.09
CV AB	0.14	0.05	0.02	0.09	0.05
<b>Phantom 4</b>	<b>46.3 kPa ± 2.32</b>				
Mean/Median A	46.64/47.09	43.76/43.85	39.40/39.44	37.31/37.38	47.23/46.40
Mean/Median B	43.03/44.74	44.09/43.65	38.62/38.67	37.22/37.83	55.23/56.10
CV A	0.09	0.04	0.02	0.04	0.07
CV B	0.09	0.04	0.01	0.08	0.06
CV AB	0.09	0.04	0.01	0.06	0.07

Coefficient of variation for observer A (CV A) and B (CV B) and for both observers (CV AB).

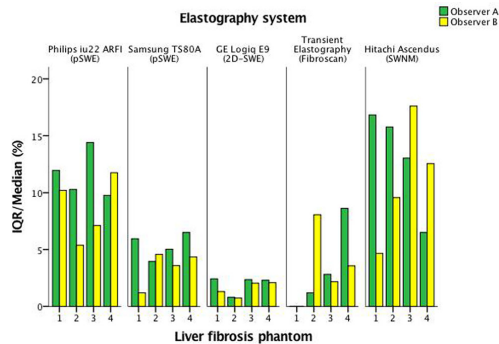
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not demonstrate significant difference in elasticity measurements and variability between Samsung, GE, Fibroscan, Hitachi. For phantom 4, differences in mean elasticity measurements between the systems were statistically significant difference ( $p < 0.001$ ), however, Philips and Samsung ( $p = .869$ ), and GE and Hitachi ( $p = .355$ ), did not demonstrate significant differences, respectively. CV was in the range 0.00–0.21. Philips ARFI showed the highest CV for phantom 3 (observer A: CV = 0,21). The mean values for each observer and CVs are given in Table 2 and illustrated in Figs 6, 7 and 8.

All systems had reliable measurements and an IQR/median <30% when applied in vitro (Figs 9 and 10). GE 2D-SWE and Samsung RS80A (pSWE) showed the lowest variation for all phantoms and both observers individually. Transient elastography did not show any variation for the softest phantom for either of the observers, whilst Hitachi (SWM) and Philips (sSWE) demonstrated slightly higher variation for all phantoms and both observers.

Overall, there was no significant difference between observer A and B's mean elasticity measurements, for most of the systems and all phantoms (1–4) ( $p = 0.043$ – $1.000$ ). However, there was a significant difference between TE in phantom 4 ( $p < 0.00$ ), as shown in Table 3.

The correlation of all systems combined, was excellent ( $r = 0.985$ ). (Fig 11) All systems used in this study provided a high repeatability in quantitative measurements for all liver fibrosis phantoms and excellent correlation between the two observers (Fig 11). Interobservation



**Fig 9. IQR/Median (%) for all systems and both observers.** IQR/Median (%) is presented on the vertical axis for observer A (green) and B (yellow). The phantoms 1–4 are numbered on the horizontal axis.

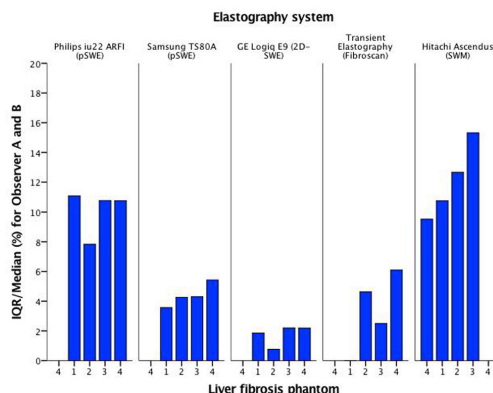
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correlation was 0.981–1 and intraobservation correlation 0.987–1. (Table 3) For all systems, except Philips ( $p = 0.009$ ), no significant difference in correlation was seen between the observers ( $p = 0.157$ – $0.660$ ). Pearson’s coefficient of correlation was excellent and in the range of  $r = 0.981$ – $1.000$ .

The reliability of measurements was demonstrated by the limits of agreement method, based on the difference from a common mean in measurements by observer A and B. Larger deviations of the mean from 0 reflect larger differences between observers, indicating observer bias. For intraobserver variation, we found a higher measurement variability for harder phantoms (phantom 3 and 4) than for the softer (1 and 2). In our study the deviation from the mean was limited for all methods, although all methods illustrated a larger spread in measurements for the harder phantoms (Fig 12).

## Discussion

The expanding spectrum of novel ultrasound elastography techniques demands comparative studies that address the agreement and repeatability of the emerging technologies *in vitro* as



**Fig 10. IQR/Median (%) for all systems and both observers.** IQR/Median (%) is presented on the vertical axis for both observers (blue). The phantoms 1–4 are numbered on the horizontal axis.

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**Table 3. Level of significance for elasticity measurements between observer A and B, for all systems.**

	Philips iU22 XM (ARFI)	Samsung RS80A (SWE)	GE Logiq E9 (2D-SWE)	Hitachi Ascendus (SWM)	Fibroscan (TE)
<b>Phantom 1</b>	<b>2.7 kPa ± 0.14</b>				
Observer A Mean ± SD	1.9±0.2	2.1± 0.1	1.9±0.03	1.7±.2	1.5±0
Observer B Mean ± SD	1.9±0.1	2.1±0.1	1.9±0.03	1.6±0.1	1.5±0
P-value	.686	1.000	.965	.670	1.000
<b>Phantom 2</b>	<b>11.5 kPa ± 0.57</b>				
Observer A Mean ± SD	8.2±0.7	7.7±0.5	7.2±0.04	7.3±0.6	6.3±0.1
Observer B Mean ± SD	7.6±0.2	7.6±0.3	7.2±0.03	7.1±0.6	6.3±0.3
P-value	0.043	1.000	1.000	.998	1.000
<b>Phantom 3</b>	<b>24.8 kPa ± 1.24</b>				
Observer A Mean ± SD	22.2±4.7	18.3±0.9	18.9±0.3	18.0±0.9	16.8±0.4
Observer B Mean ± SD	20.2±1.5	18.3±0.9	18.5±0.3	19.0±2.3	18.9±1.6
P-value	.352	1.000	1.000	.965	.288
<b>Phantom 4</b>	<b>46.3 kPa ± 2.32</b>				
Observer A Mean ± SD	46.6±3.7	43.8±1.6	39.4±0.6	37.3±1.4	47.2±3.5
Observer B Mean ± SD	43.0±3.9	44.1±1.8	38.6±0.5	37.2±2.9	55.2±3.3
P-value*	0.078	1.000	1.000	1.000	<0.00

\*P-value >0.05 indicates that there was no significant difference in mean of elasticity measurements between the observers

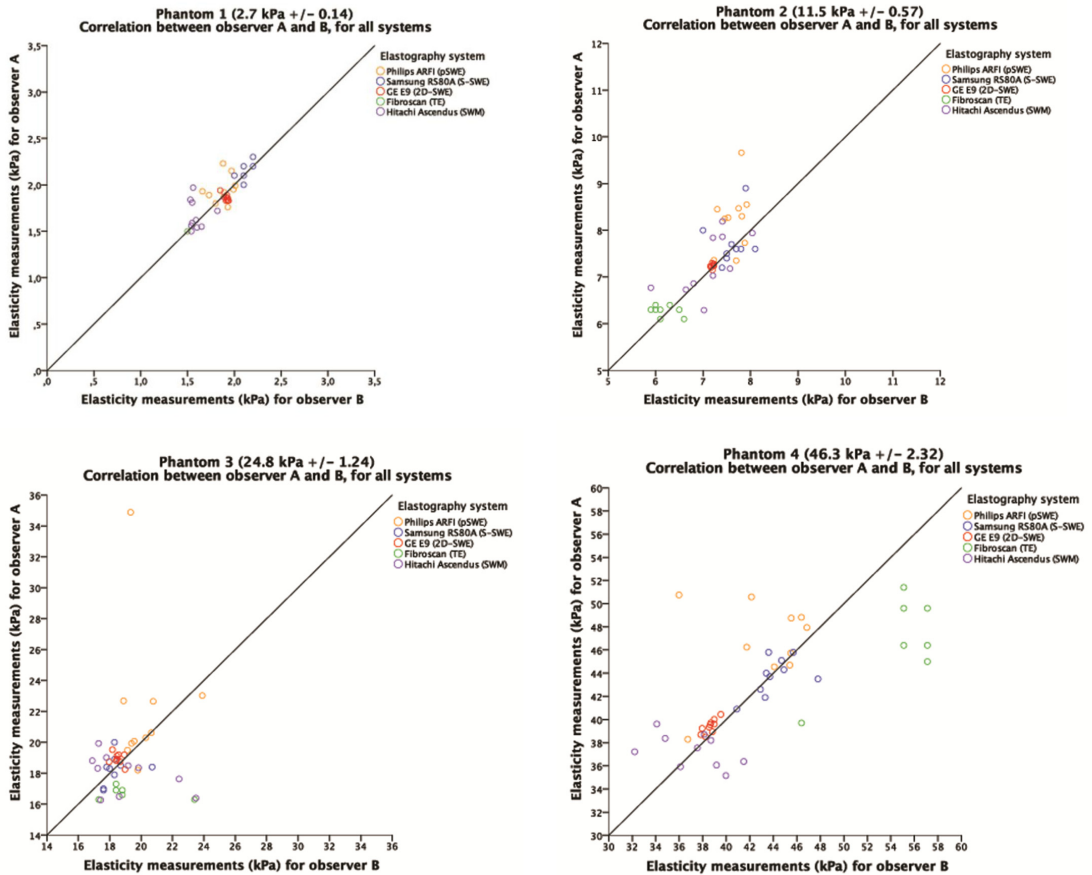
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well as *in vivo* in healthy and non-healthy patients. The shear wave elastography technologies may vary between the systems from different manufacturers; furthermore, similar techniques applied in different systems may result in different values of shear wave speed measurements based on variations related to frequencies and to the algorithms used to determine tissue properties. [1] Analyses of performance as well as head-to-head comparisons between several novel elastography systems which have been introduced in the market for clinical use over recent years, are still scarce and we believe that the present paper addresses a need in this regard as these systems are already beginning to be employed in the clinical follow-up of patients.

In the present study, we evaluated the use of four different shear wave elastography methods and transient elastography in a head-to-head design, assessing the reliability of measurement acquisition and repeatability *in vitro* using four individual, quality controlled, commercially available liver fibrosis phantoms which represented elasticities ranging from values found in healthy liver tissue to cirrhosis. We found a high degree of repeatability, both for individual observers and between two observers for all the methods, as reported by CV in Table 2 and level of significance in Table 4.

GE 2D-SWE showed the lowest CV (0.00–0.02), with no significant differences in mean elasticity between observers for either soft or hard phantoms ( $p = .965–1.000$ ). This was the only 2D-SWE method evaluated in this study. The tendency towards higher repeatability compared to the other shear wave methods, may be influenced by a different scanning and measurement procedure compared to the other methods. For GE 2D-SWE, several frames can be acquired within one loop, allowing several measurements in the identical probe position of



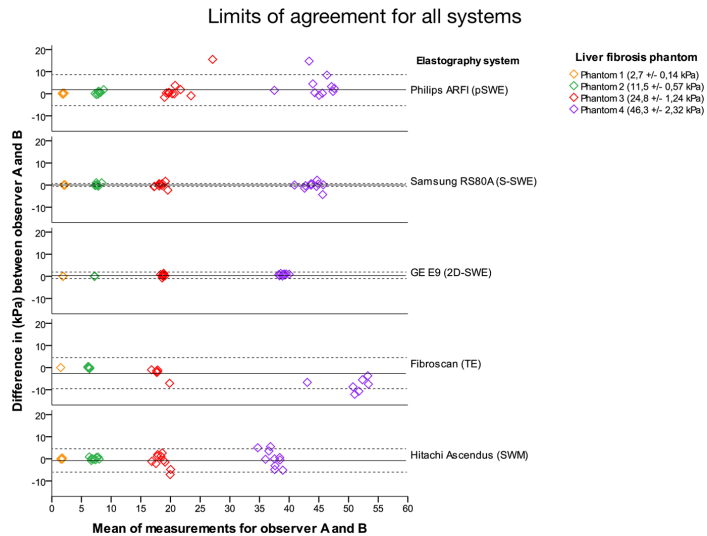


**Fig 11. Correlation between observers in measurement of each phantom for all systems.** The horizontal and vertical axes represent measurements by observer B and A, respectively. The unit measured is kilopascals (kPa). The line in the graph represents the line of unity. For color representation, we refer to Fig 6.

<https://doi.org/10.1371/journal.pone.0189671.g011>

the liver fibrosis phantom. However, in a clinical setting, the ability to keep an organ motionless for the time taken to acquire several measurements in one loop is more limited, necessitating acquisition of several loops which might affect repeatability in vivo. Adjustment of the size of the measurement ROI by the observer might also potentially affect repeatability; however, this was standardized in our study.

We have demonstrated a nearly perfect interobserver agreement (Table 4) for all the four novel systems as well as for TE, and no significant difference in mean elasticity measurements between the observers (Table 3) for the novel systems. This is in line with previous reports from studies assessing the intra- and interobserver reliability in other shear wave systems that have been longer on the market, which have also concluded with a high repeatability for shear



**Fig 12. Limits of agreement.** The elastography system used are identified on the right side of the graph. The phantoms are identified by color, and represented in the upper right corner. The colors represent the four phantoms with increasing stiffness; yellow as phantom 1 ( $2,7 \pm 0,14$  kPa), green as phantom 2 ( $11,5 \pm 0,57$  kPa), red as phantom 3 ( $24,8 \pm 1,24$  kPa) and purple as phantom 4 ( $46,3 \pm 2,32$  kPa). The horizontal axis represents the common mean value of all measurements in both observers while the vertical axis represents the difference between individual measurements and this common mean (kPa), displaying the variability of measurements for the four phantoms. The black line within each system represents the common mean value, the dotted lines represent 95% Confidence Interval. A mean value close to 0 on the vertical axis means that the two observers apply the measurement scale without bias. If it deviates from 0, one of the observers tend to measure higher or lower values systematically compared to the other observer.

<https://doi.org/10.1371/journal.pone.0189671.g012>

wave elastography *in vitro*, as well as *in vivo* in breast masses, healthy liver tissue in adults and children. [21–24]. In our study, the elasticity measurements of TE were to comparatively higher on phantom 4, and significantly present for both observers. A difference in experience with TE, between observer A and B, might be a possible factor. However, previous publications have stated that the slope of the curve for elastography measurements across increasingly hard phantoms or liver tissues may differ between elastography systems and platforms. Oudry et al. demonstrated that vibration-controlled transient elastography (TE) had a steeper slope, and elastography values increased more, between phantoms of increasing hardness compared to a shear wave technique, and discussed that this was in part due to the lower frequency and smaller

**Table 4. Inter- and intraclass correlation for both observers, for all systems.**

System	Probe	Intraobserver correlation (ICC)	Interobserver correlation A+B (ICC)
Philips iU22 XM (ARFI)	C5-1	0.989	0.981
Samsung RS80A (SWE)	C5-1	0.999	0.998
GE Logiq E9 (2D-SWE)	C1-5	1	1
Hitachi Ascendus (SWM)	C175	0.992	0.985
Fibroscan (TE)	M-probe	0.991	0.995

<https://doi.org/10.1371/journal.pone.0189671.t004>

size of the vibration source in TE leading to increased diffraction, an effect which is increased as materials get harder and which is known to induce overestimation of stiffness. [25]

Significant differences in shear wave velocity was demonstrated at different imaging depths (3–7 cm) in one study; and a significant difference in shear wave speed estimated among the systems was shown. However, due to the study design using different phantoms for different depths, the question of whether this systematic error was due to the imaging system or difference in the material properties of the phantom remained unanswered. [12] Another phantom study evaluated the repeatability of two elastography methods at depths 1–4 cm, and found that the depth related differences were small, but significant [26]. In our study, we used the same liver fibrosis phantoms and we subsequently performed all measurements at similar distance, approximately 3.5 cm inferior of the transducer surface.

The liver fibrosis phantoms were compatible with shear wave modalities, including TE and ARFI, however most of the phantom elasticity measurements obtained by the shear wave methods underestimated the elasticity values provided by the manufacturer of the phantom, especially for the softer phantoms. (Fig 4) This was equally observed by both observers, and may be caused by the *in vitro* material, although the phantom material density was  $1.03\text{g/cm}^3$ , which is comparable to live soft tissue. The liver fibrosis phantoms did not provide the same elastic properties as live soft tissue, the acoustic properties are similar and comparable to live soft tissue. It is previously shown that change in attenuation coefficient may affect the penetration results for ultrasound scanners and cause variations not related to the performance of the scanner. However, these changes would not have a significant effect. [27] It was beyond the scope of this paper to evaluate whether similar underestimation will occur when scanning live tissue, and this must be further investigated *in vivo*.

The present results in a homogeneous tissue-mimicking phantom material are promising for the successful clinical application of the novel shear wave methods from Hitachi, GE and Samsung; however, the study has some limitations inherent to its *in vitro* design. In a clinical setting, factors such as fasting status, narrow intercostal spaces, variable amounts of subcutaneous fat (affecting measurement depths) and variable patient cooperation (ability to maintain breath-hold) may affect results, which may also be influenced by variable levels of cholestasis, hepatic inflammation, ascites and other factors. The purely elastic phantoms do not accurately mimic the viscoelastic properties of human liver; however, no viscoelastic phantoms are currently commercially available to our knowledge. The phantoms we employed spanned relevant elasticities and had been subject to rigorous testing and quality control from the producer, and have been employed in similar studies of other systems. The *in vitro* design allowed us to standardize the default settings for phantom scanning, for all methods and both observers, and assess them without adjusting factors that may affect performance, such as depth of measurements. The homogenous and isotropic material of the phantoms is key for repeatability of measurements, but differ from the situation of scanning liver tissue *in vivo*.

## Conclusion

We have demonstrated similar and excellent repeatability and interobserver agreement for four novel SWE systems using liver tissue-mimicking phantoms. Further studies are needed to evaluate the performance of these methods in human liver scanning.

## Supporting information

**S1 Study data.** This excel file includes the recorded raw data obtained by both observers, for each of the systems.  
(XLSX)

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## Author Contributions

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# Paper II

II



RESEARCH ARTICLE

# Liver elasticity in healthy individuals by two novel shear-wave elastography systems—Comparison by age, gender, BMI and number of measurements

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**Data Availability Statement:** Due to ethical restrictions and local regulations on sharing a de-identified or anonymized data set, we are prohibited from sharing our data publicly. Public availability of our data would compromise participant privacy and confidentiality. Contact information for the Regional Committee for Medical and Health Research Ethics (REC West) to whom data requests may be sent is provided below. Please cite the study reference number: REK vest 2012/2214. Postal address: REK Vest,

## Abstract

### Objective

Establishing normal liver stiffness (LS) values in healthy livers is a prerequisite to differentiate normal from pathological LS values. Our aim was to define normal LS using two novel elastography methods head-to-head and to assess the number of measurements, variability and reproducibility.

### Materials and methods

We evaluated shear wave elastography (SWE) methods integrated in Samsung RS80A and GE S8 by obtaining LS measurements (LSM) in 100 healthy subjects (20–70 years). Transient Elastography (TE) was used as reference method. Data were analyzed according to age, sex, BMI and 5 vs. 10 measurements. All subjects underwent B-mode ultrasound examination and lab tests to exclude liver pathology. Interobserver variation was evaluated in a subset ( $n = 24$ ).

### Results

Both methods showed excellent feasibility, measuring LS in all subjects. LSM-mean for GE S8 2D-SWE was higher compared to TE ( $4.5 \pm 0.8$  kPa vs.  $4.2 \pm 1.1$ ,  $p < 0.001$ ) and Samsung RS80A ( $4.1 \pm 0.8$  kPa,  $p < 0.001$ ). Both methods showed low intra- and interobserver variation. LSM-mean was significantly higher in males than females using 2D-SWE, while a similar trend for Samsung SWE did not reach significance. No method demonstrated statistical significant difference in LSM across age and BMI groups nor between LSM-mean based on 5 vs. 10 measurements.

### Conclusion

LSM was performed with high reproducibility in healthy adult livers. LSM-mean was significantly higher for GE S8 2D-SWE compared to Samsung RS80A and TE in healthy livers.



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Males had higher LSM than females. No method demonstrated statistical significant difference in LSM-mean across age- and non-obese BMI groups. Our results indicate that five LSM may be sufficient for reliable results.

## Introduction

Chronic liver disease is one of the leading causes of morbidity and mortality worldwide [1, 2]. Assessment of liver fibrosis is important for chronic liver disease of various aetiologies for outcome prediction, risk stratification and selection for screening programs (e.g. endoscopy for oesophageal varices) as well as therapeutic decisions [2]. Non-invasive methods including ultrasound elastography have emerged within the past decade and are increasingly replacing liver biopsy for liver fibrosis assessment, avoiding the risks and discomforts of this invasive method. Nonetheless, ultrasound elastography encompasses several methods with important technological differences, ranging from vibration-controlled transient elastography (TE, Fibroscan) to methods based on deposition of an acoustic pulse such as point shear wave elastography (pSWE) and more recently 2D-SWE. TE has been extensively validated and is recommended for clinical use by several international guidelines, and an increasing number of studies evaluating the accuracy of various elastography methods have provided evidence for the utility of elastography imaging. However, with the expanding spectrum of ultrasound based elastography systems, it has become increasingly clear that the various technologies and platforms may yield different estimates of liver stiffness (LS) within the same liver. Hence, current guidelines acknowledge a need to establish reference values for normal liver stiffness in healthy livers for each specific equipment model in order to allow accurate diagnosis of pathological liver stiffness [3, 4].

To our knowledge, this is the first study to evaluate liver stiffness measurements (LSM) in healthy liver subjects using 2D-SWE from GE Logiq S8 (GE Healthcare, Milwaukee, WI, USA) as well as SWE from Samsung RS80A (Samsung Medical, Seoul, Korea). Our study primarily aimed to define normal values of liver stiffness (LS) for males and females across adult age groups using these two novel platforms. We applied TE using Fibroscan integrated in the GE Logiq S8 ultrasound scanner (Echosens, Paris, France) as a reference method. Furthermore, we aimed to analyse influencing factors, such as BMI, and to assess the inter- and intraobserver variability and reproducibility, as well as to investigate the difference between obtaining five and ten consecutive liver stiffness measurements in order to calculate a representative median liver stiffness measurement (LSM).

## Material and methods

### Study design and subject population

The study was designed as a single-centre cross-sectional prospective study in selected healthy individuals. The protocol was in accordance with the Declaration of Helsinki for research in medicine and biology, and was approved by the Regional Committee for Medical and Health Research Ethics in Western Norway. All subjects were given oral and written information about the study and were invited to participate. Informed written consent was obtained from each subject enrolled. The study was performed in August and September 2017, at the Department of Gastroenterology, Haukeland University Hospital in Bergen, Norway.

**Table 1. The characteristics of healthy subjects, by age group.**

	20–30 (n = 20)	31–40 (n = 20)	41–50 (n = 20)	51–60 (n = 20)	61–70 (n = 20)
<b>Characteristics</b>					
Age, years <sup>±</sup> (range)	27.8 ± 2 (25–30)	34.1 ± 2.7 (31–40)	44.3 ± 3 (41–50)	55.7 ± 3 (51–60)	64.15 ± 2.5 (61–69)
Gender; Female/Male, n	10/10	10/10	10/10	10/10	10/10
BMI, kg/m <sup>2±</sup> (range)	22.5 ± 2.4 (19.4–27.2)	23.8 ± 2.3 (20.7–28.4)	24.4 ± 3 (18.1–28.7)	24.5 ± 2.3 (20–29.9)	24.7 ± 2.9 (20–29.6)
Weight group <sup>±</sup> , n (%)					
18.0–25 kg/m <sup>2</sup>	17 (17%)	15 (15%)	15 (15%)	13 (13%)	13 (13%)
25.0–30 kg/m <sup>2</sup>	3 (3%)	5 (5%)	5 (5%)	7 (7%)	7 (7%)
Alcohol units per week <sup>±</sup> (range)	4.4 ± 2.5 (0–8)	3.8 ± 2 (0–6)	3.7 ± 2.6 (1–10)	4.4 ± 2.6 (0–10)	4.2 ± 3 (0–10)
<b>Biochemical profile</b>					
Total bilirubin μmol/L* [<19 μmol/L] (IQR; range)	10.5 (8.7–12.5; 4–18)	17 (5.5–15.3; 3–39)	9.5 (7.5–11.4; 4–21)	8.5 (7.1–12.1; 4–28)	8.6 (7.4–9.7; 4–15)
AST, U/L * [15–45 U/L] (IQR; range)	23.5 (21.9–28.6; 17–41)	25.0 (21.6–27.7; 15–45)	22.0 (20.5–26.1; 15–40)	23.0 (21.7–25.7; 16–32)	23.5 (21.5–27.8; 13–37)
ALT, U/L * [10–70 U/L] (IQR; range)	17.0 (16.9–28.4; 11–55)	22.5 (18.7–27.1; 8–45)	23.0 (21.5–30.7; 15–47)	22.5 (20.1–30.7; 14–40)	24.5 (22–28.7; 14–46)
GGT, U/L* [10–115 U/L] (IQR; range)	18.5 (15.5–22.1; 7–42)	15.0 (13.5–19.5; 9–29)	19.0 (17.0–34.4; 5–68)	17.0 (16.7–26.0; 11–48)	22.5 (17.0–35.3; 9–96)
Serum Albumin, g/L* [39–50 g/L] (IQR; range)	48.0 (46.4–49.3; 41–53)	47.0 (45.6–48.2; 43–52)	46.5 (45.2–47.4; 41–51)	46.5 (45.2–49.7; 43–66)	46.0 (45.1–46.8; 43–50)
Platelet counts, 10 <sup>9</sup> /L* [145–387 10 <sup>9</sup> /L] (IQR; range)	237.5 (210.7–255.5; 152–352)	222.5 (210.6–254.8; 155–334)	256.5 (229.9–284.4; 141–400)	244.5 (221.8–273.8; 144–355)	245.5 (221.3–271.8; 165–365)
APRI score* (IQR; range)	0.29 (0.26–0.37; 0.17–0.56)	0.29 (0.26–0.37; 0.15–0.68)	0.26 (0.22–0.31; 0.13–0.45)	0.26 (0.24–0.34; 0.18–0.58)	0.31 (0.25–0.35; 0.10–0.55)
FIB-4 score* (IQR; range)	0.66 (0.59–0.77; 0.4–1.09)	0.79 (0.72–0.93; 0.42–1.41)	0.82 (0.73–1.09; 0.42–1.93)	1.12 (0.99–1.36; 0.61–2.01)	1.38 (1.05–1.56; 0.52–2.38)

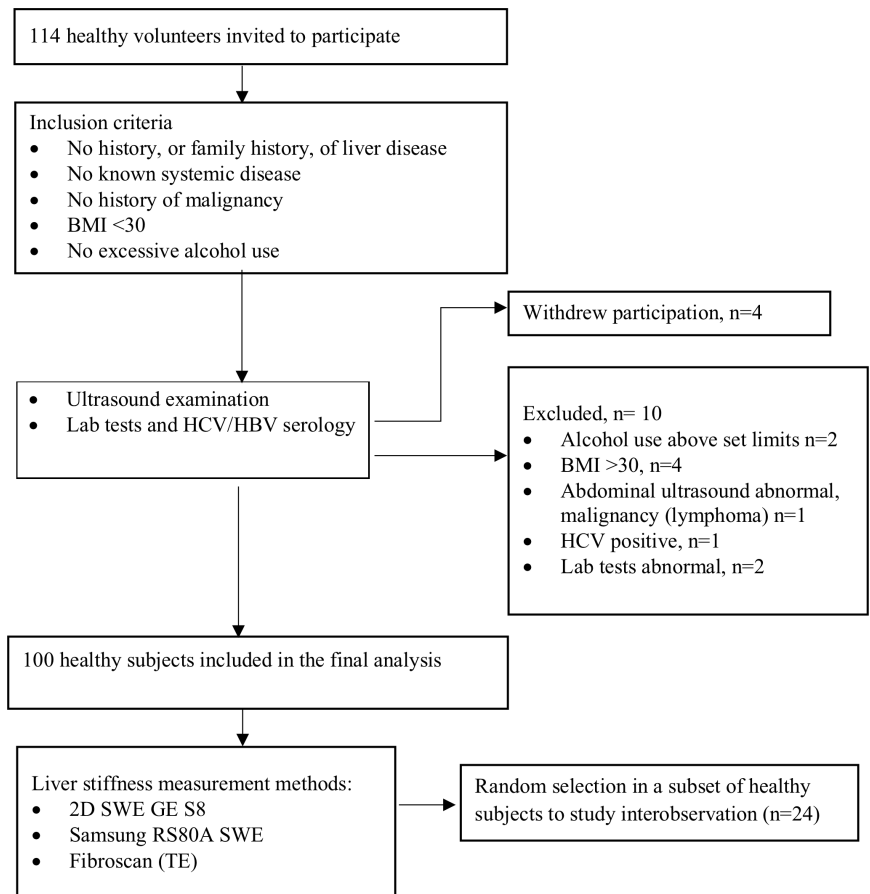
\*Data are presented as median ± SD. Data are presented as mean ± SD. SD, Standard deviation; IQR, Interquartile range (representing upper and lower bound); Range (from minimum value to maximum value). BMI, Body Mass Index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; APRI, AST to Platelet Ratio Index; FIB4, Fibrosis-4. Reference values for our laboratory tests are given in the brackets, normal values cover both genders.

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The characteristics of healthy subjects are shown in Table 1. The subjects consisted of volunteers with various occupational backgrounds recruited amongst staff, their families and social network. Volunteers were recruited into five groups by age, with 10 males and 10 females per group: 20–30, 31–40, 41–50, 51–60 and 61–70 years (Table 1). Liver disease was ruled out as far as possible by patients' history, laboratory tests and negative viral markers. In total ten subjects were excluded, weekly alcohol use extending 10 units for males and 6 units for females (n = 2), abnormal laboratory tests (n = 3) or evidence of malignancy on ultrasound examination (n = 1). Individuals with BMI >30 kg/m<sup>2</sup> were excluded (n = 4). Four subjects withdrew their participation consent (Fig 1). We included 100 healthy subjects in the final analysis. A random subset of subjects (n = 24) were included for assessment of interobserver variability. For analyses regarding the effect of BMI, the subjects were divided into two groups with BMI between 18.0 and 25 kg/m<sup>2</sup> (n = 73) and BMI between 25 and 30 kg/m<sup>2</sup> (n = 27), respectively.

### Laboratory analyses

On the day of ultrasound and elastography, blood was sampled and biochemical analyses were performed using standard routine laboratory protocols. The tests included C-reactive protein (CRP), haemoglobin, leukocytes, platelets, creatinine, total bilirubin, albumin, international



**Fig 1. Method of selection of healthy subjects.** Flow chart of data collection.

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normalization rate (INR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT). The laboratory analyses were performed in our hospital’s laboratory, and reference values were gender specific. Three subjects had a bilirubin value outside the gender specific reference range, but none of these were excluded as the values normalized and diagnostic work-up showed no evidence of liver disease. Viral markers for hepatitis C virus (HCV) and hepatitis B virus (HBV) were also included. APRI and FIB-4 scores of fibrosis were calculated using published algorithms [5, 6].

### B-mode ultrasound examination

All subjects underwent B-mode ultrasound examination of the liver, gallbladder, spleen and kidneys using a Samsung RS80A before SWE examination. All examinations were conducted after a minimum of four hours of fasting, using a standardized scanning protocol and by a

single operator (AM) with >3 years' experience in abdominal ultrasound. Small hepatic capillary haemangiomas were found in 9 subjects; none of these subjects were excluded as the lesions were confirmed by contrast enhanced ultrasound and were considered small and unlikely to influence the liver stiffness.

### Elastography methods and SWE examination

Three shear wave elastography (SWE) methods were assessed in the study and are listed below in chronological order of assessment. The scanner settings were standardized for all systems. All measurements were performed by a single operator (A.M.). In order to evaluate interobserver variation, a subset of subjects ( $n = 24$ ) were examined by two independent observers (A.M. and A.B.M.). Observer A (A.M.) and B (A.B.M.) had >3 and 1 years' experience in ultrasound liver scanning and elastography, respectively. The subjects were fasting (minimum 4 hours) and examined in the supine position with their right arm abducted. All SWE measurements were obtained in the right liver lobe through an intercostal space in relaxed mid-breath hold with minimal transducer pressure being applied; for Samsung RS80A and GE S8 the measurements were acquired in the right lobe about 2 cm beneath the Glisson capsule, perpendicular to the capsule, avoiding large liver vessels, bile ducts and rib shadow in B-mode. Each observer performed first 10, and then 5 separate measurements in the same area with each of the ultrasound based elastography methods. A valid LS assessment was considered as the median value and range of 10 and 5 measurements, acquired in a homogenous area (Samsung RS80A) or in a homogenous elastogram (GE S8 2D-SWE) with an interquartile range (IQR)/median <30% and a success rate (SR)  $\geq 60\%$ .

**Samsung RS80A SWE.** The Prestige ultrasound system (Samsung Medison Co. Ltd., Seoul, Korea) was applied using a CA1-7A convex array probe with a frequency of 1–7 MHz. The software version was 3.00.03.0824. The method measured the average liver elasticity within a region of interest (ROI). Within the brightness mode (B-mode) window, using default scanner settings, the ROI could be placed freely, with a fixed height of 10 mm. The width was automatically adjusted depending on the measurement depth (Fig 2). LSM was expressed in kilopascals (kPa) and meters per second (m/s).

**GE Logiq S8 2D-SWE.** 2D-SWE from the S8 Ultrasound scanner (GE Healthcare, Milwaukee, Wisconsin, USA), Version R4.1.2, was applied using the C1-6 convex array probe with a frequency of 1–6 MHz. Within the elastogram a circular ROI was placed, standardized to 10 mm in our study and under default scanner settings. The elastic modulus of the liver was automatically acquired by the system. The colour 2D-SWE images were captured and 2–3 elasticity frames per breath-hold (3–5 seconds) were recorded. One ROI was placed within each homogeneously coloured elastogram (Fig 3). LSM was expressed in m/s and kPa.

**Transient elastography (TE).** Integrated in the GE Logiq S8 ultrasound scanner, TE (Fibroscan®, EchoSens, Paris, France), was applied using the M-probe with a frequency of 3.5 MHz and used according to the manufacturer's instructions. A reliable and valid measurement acquisition was defined as SR  $\geq 60\%$  and IQR/median <30% [7].

### Statistical analysis

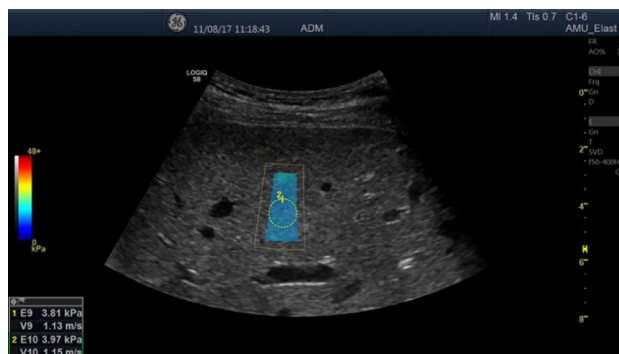
The statistical analysis was performed using SPSS, Version 24.0, IBM Statistics (Armon, New York, NY, USA). We used descriptive statistics for demographic, clinical and laboratory characteristics. Sample size power estimation was performed using a 2-sided comparison of two-means model. Estimating a difference in means of 4.0–4.5 kPa with a standard deviation of 0.5 kPa between the methods, 80% power and type I error of 5% yielded a sample size of 16; we compared groups consisting of 20 individuals or more. Variables were tested for normal



**Fig 2. Samsung RS80A SWE performed on a healthy liver.** The figure illustrates Samsung RS80A SWE method performed on a healthy subject. The yellow box (centre) represents the shear-wave measurement area and is expressed below the obtained elasticity measurement of 3.4 kPa.

<https://doi.org/10.1371/journal.pone.0203486.g002>

distribution by calculation and graphics using the Shapiro Wilk test and Q-Q Plot. Differences between numerical variables with a normal distribution were assessed with parametric tests (t-test), and those with a non-normal distribution, with nonparametric tests (Mann-Whitney). P-values of < 0.05 were considered significant. Data are presented as mean (SD) when the data were normally distributed. We calculated the coefficient of variation (CV) of the intraobserver variability. Inter-class correlation coefficients (ICC) were calculated to present the



**Fig 3. 2D-SWE by GE S8 performed on a healthy liver.** The figure illustrates the method of 2D-SWE by GE performed on a healthy subject. The coloured box (centre) represents the elastogram, and the circle represents the ROI where the elastic modulus (LSM, liver stiffness measurement) of the liver is acquired. The blue colour indicates soft liver tissue, as semi-quantitatively presented by the colour scale to the left.

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interobserver reliability. Inter-observer agreement was classified as poor (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), good (0.61–0.80) and excellent (0.81–1.00) [8]. Correlations were tested by Pearson correlation coefficient. Limits of agreement were assessed according to Bland and Altman to discover differences between individual measurements and to detect possible biases for each method [9, 10]. IQR/Median (%) was calculated for both observers individually as well as together, and for all systems [3, 11].

## Results

A total of 100 healthy subjects were included. LSM was obtained by three different elastography methods (Samsung RS80A, GE S8 2D-SWE and TE). The feasibility of the methods was excellent and successful measurements were obtained in all 100 subjects by all three methods. The characteristics of the healthy subjects are shown in Table 1.

### Measurement variability for the different elastography methods

The overall mean value of the median liver stiffness (LSM-mean) in 100 healthy subjects ranged from 2–6.8 kPa (Table 2).

LSM-mean by GE S8 2D-SWE was significantly higher compared to LSM-mean by TE ( $4.5 \pm 0.8$  kPa vs.  $4.2 \pm 1.1$ , respectively,  $p < 0.001$ ) and Samsung RS80A ( $4.1 \pm 0.8$  kPa,  $p > 0.001$ ), whereas no significant difference was seen between Samsung RS80A and TE ( $p = 0.11$ ) (Fig 4).

The coefficient of variation (CV) ranged from 0.03–0.28 for all systems (0.03–0.28 for Samsung RS80A SWE, 0.05–0.28 for GE S8 2D-SWE and 0.04–0.20 for TE). TE had a significantly higher CV than GE S8 2D-SWE ( $p < 0.001$ ) and Samsung RS80A ( $p = 0.005$ ). Furthermore, between GE S8 2D-SWE and Samsung RS80A we found a small, but significant difference in CV ( $p = 0.03$ ). Interobserver analysis was performed on 24 randomly selected subjects. No significant differences in LSM-mean between two independent observers (A.M. and A.B.M) was demonstrated for Samsung RS80A SWE ( $4.4 \pm 0.8$  kPa vs.  $4.4 \pm 0.8$  kPa, respectively,  $p = 0.42$ ), however, we did find a significant difference between observers for GE S8 2D-SWE ( $4.5 \pm 0.6$  kPa vs.  $5.1 \pm 0.7$  kPa, respectively,  $p = 0.009$ ) (Fig 5).

Interoperator reliability was good for both Samsung RS80A SWE and GE S8 2D-SWE. Pearson’s correlation coefficient between observers was significant for both methods ( $r = 0.74$ ,  $p < 0.001$  vs.  $r = 0.65$ ,  $p < 0.001$ , respectively) (Fig 6).

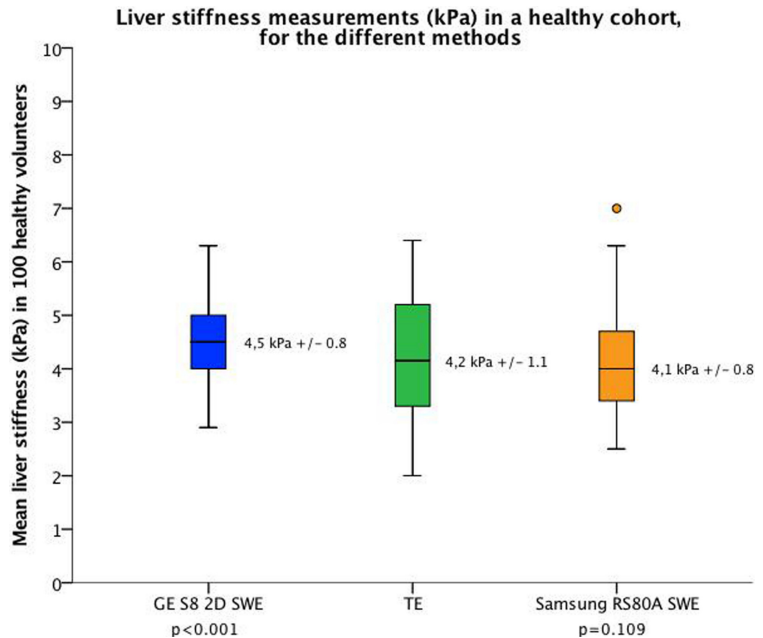
The intraclass correlation coefficient (ICC) was good for both Samsung RS80A and GE S8 2D-SWE (ICC = 0.85 vs. ICC = 0.78, respectively). There was no indication of observer bias for either GE S8 2D-SWE or Samsung RS80A SWE as illustrated by limits of agreement

**Table 2. Liver stiffness values (kPa) for the different methods.**

Method	2D-SWE GE S8	Samsung RS80A SWE	Fibroscan (TE)
Mean LS, kPa	4.5	4.1	4.2
Range	2.9–6.3	2.5–6.8	2.0–6.4
SD	0.8	0.8	1.1
95% CI	4.37–4.67	3.91–4.23	4.0–4.5
CV	0.17	0.21	0.27
CV [range]	0.05–0.28	0.03–0.28	0.04–0.20

Liver stiffness (LS) values (kPa) for 2D-SWE GE, Samsung RS80A and TE. Data are presented as mean with 95% Confidence Interval (CI) and standard deviation (SD), coefficient of variation (CV) and the range of CV for the respective methods.

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**Fig 4. Liver stiffness (kPa) in a healthy cohort for the different methods.** This boxplot figure displays the median and the interquartile range for LSM for each system. Whiskers represent the 90% percentile of the measured liver stiffness. The height of the box represents the variability in LSM between the healthy study subjects for each of the following three systems: blue, GE S8 2D-SWE; green, Transient Elastography (TE, Fibroscan) and orange, Samsung RS80A SWE. P-values indicate if there is a significant difference between the novel systems (Samsung RS80A or GE S8) and TE.

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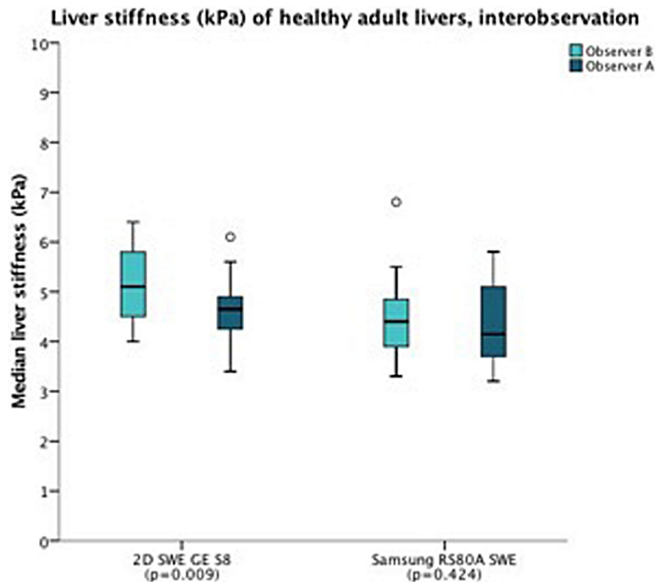
analysis; however, GE S8 2D-SWE showed a trend of a slightly larger deviation of the mean than Samsung RS80A SWE (Figs 7 and 8).

### Difference in liver elasticity by gender, age and BMI

LSM-mean was significantly higher in males compared to females for TE ( $4.5 \pm 1.0$  kPa vs.  $3.9 \pm 1.1$  kPa, respectively,  $p = 0.006$ ) and GE S8 2D-SWE ( $4.7 \pm 0.7$  kPa vs.  $4.3 \pm 0.7$  kPa, respectively,  $p = 0.006$ ). A similar trend for Samsung RS80A SWE did not reach significance ( $4.2 \pm 0.7$  kPa vs.  $3.9 \pm 0.9$  kPa, respectively,  $p = 0.063$ ) (Fig 9, Table 3). In a post hoc analysis of subjects consuming 5 alcohol units or less per week ( $n = 69$ ) we found significant differences in LSM-mean between males ( $n = 33$ ) and females ( $n = 36$ ) for all systems; for GE S8 ( $4.8 \pm 0.7$  kPa vs.  $4.2 \pm 0.8$  kPa,  $p = 0.003$ ), TE ( $4.7 \pm 1.0$  kPa vs.  $3.8 \pm 1.1$  kPa,  $p = 0.001$ ) and Samsung ( $4.2 \pm 0.7$  kPa vs.  $3.8 \pm 0.7$  kPa,  $p = 0.006$ ).

None of the systems demonstrated statistical significant difference in LSM across age groups (Table 4).

LSM-mean showed no significant difference between subjects with BMI  $25\text{--}30$  kg/m<sup>2</sup> and BMI  $18.0\text{--}25.0$  kg/m<sup>2</sup> for any individual system (GE S8 2D-SWE ( $4.5 \pm 0.8$  kPa vs.  $4.4 \pm 0.8$  kPa, respectively,  $p = 0.49$ ), TE ( $4.3 \pm 1.1$  kPa vs.  $4.1 \pm 1.1$  kPa, respectively,  $p = 0.36$ ), Samsung RS80A SWE ( $4.1 \pm 0.9$  kPa vs.  $3.9 \pm 0.6$  kPa, respectively,  $p = 0.28$ ) or all systems combined ( $4.1 \pm 0.9$  kPa vs.  $4.3 \pm 0.9$  kPa,  $p = 0.128$ ) (Fig 10).



**Fig 5. Liver stiffness (kPa) of healthy adult livers, interobservation.** The boxplot shows interobservation between observer A (dark blue) and B (light blue). The horizontal axis represents the systems Samsung RS80A SWE and GE S8 2D-SWE and the p-value is given. For boxplot interpretation, we refer to Fig 4.

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### Difference in variability and reproducibility of LSM when using 5 measurements instead of 10

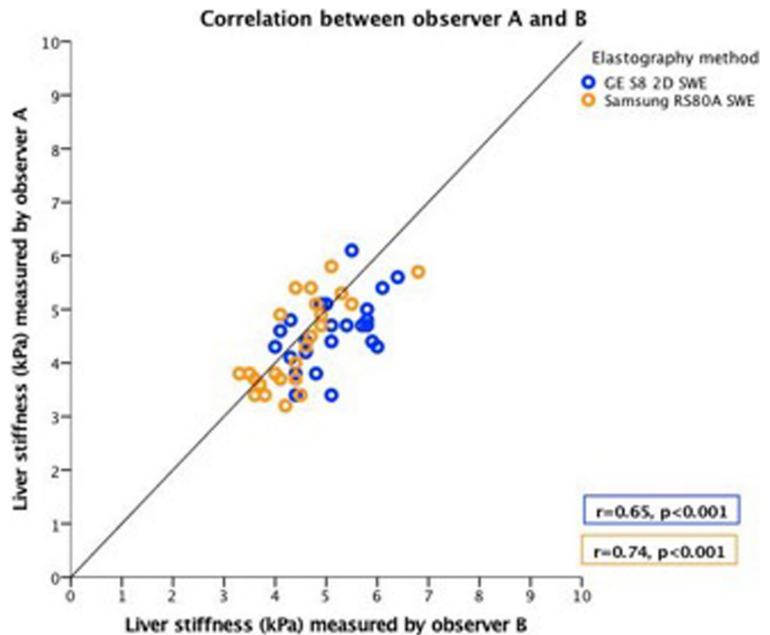
There was no significant difference in LSM-mean using 5 or 10 measurements for the ultrasound based SWE methods (GE S8 2D-SWE  $4.4 \pm 0.66$  kPa vs.  $4.5 \pm 0.76$  kPa, respectively,  $p = 0.05$ ; and Samsung RS80A SWE:  $4.1 \pm 0.86$  kPa vs.  $4.1 \pm 0.81$  kPa, respectively,  $p = 0.08$ ) (Fig 11).

### Discussion

To the best of our knowledge, this is the first study to investigate normal LSM values by two new elastography techniques (Samsung RS80A SWE and GE S8 2D-SWE) compared head-to-head and with TE as reference, in a healthy cohort. The comprehensive exclusion of liver disease as well as the direct comparison to TE as a reference standard represent strengths of our study. Data regarding normal values in liver stiffness for each of the new elastography techniques are needed to establish standardized reference bases [12], which are pivotal for clinical implementation of novel elastography systems as reliable methods for diagnostics, staging and assessment of disease progression in chronic liver diseases.

We found a mean LSM of  $4.3 \text{ kPa} \pm 0.8$  across the two methods, confirming that on average LSM for 2D-SWE GE S8 ( $4.5 \pm 0.8$  kPa) and Samsung RS80A ( $4.1 \pm 0.8$  kPa) were in the same range as other elastography systems [13–15]. In this head-to-head comparison between elastography systems, 2D-SWE GE S8 demonstrated slightly higher values than both Samsung RS80A and TE, while measurements made with Samsung RS80A were not significantly different from the reference method. There was also a small, but significant difference in the



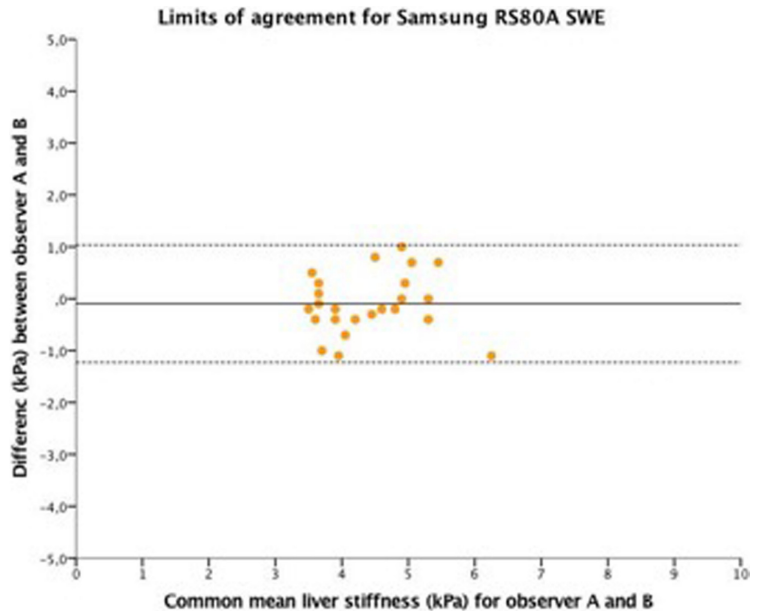


**Fig 6. Correlation between observer A and B.** The horizontal and vertical axes represent measurements by observer B and A, respectively. The unit measured is kilopascals (kPa). The line in the graph represents the line of unity. The Pearson correlation coefficient ( $r$ ) and significance ( $p$ ) for each system is given in the lower right corner. For colour representation, we refer to [Fig 4](#).

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coefficient of variation between the two novel methods. Previous studies have shown similar results for 2D-SWE from Aixplorer [16, 17]. Our results confirm that LSM levels are significantly different depending on the method applied. We found differences both between SWE methods and TE as the reference method, and between the two different SWE systems. In clinical practice LSM greater than 6.8–7.6 kPa indicates a higher probability of significant fibrosis ( $F \geq 2$ ) on liver biopsy; however, the EASL clinical practice guidelines state that cut-off values vary considerably and ranging 5.2–9.6 kPa for different systems. For predicting cirrhosis (F4), the optimal cut-off ranges from 11 to 15 kPa [18]. In that context, a net difference of 0.3 kPa is probably too small to represent a clinically significant difference, however, it underscores the need to compare methods also in fibrotic livers, where the differences may be more expressed, as we know that variability increases with higher liver stiffness [19].

Both methods showed good interobserver reliability and intraclass correlation. Similarly, previous studies have shown excellent interobserver agreement ranging from  $r = 0.80$ – $0.97$  for pSWE methods [20, 21]. However, we observed a significant difference in LS measurements between the two observers for 2D-SWE GE S8 but not for Samsung RS80A. One possible explanation for this discrepancy may be that 2D-SWE allows the examiner to place the measurement ROI within the elastogram and avoid incongruent signals, while Samsung SWE performs several automated SWE speed measurements within the elasticity measurement area without visualisation of the stiffness. The 2D-SWE method is slightly more user dependent and may acquire a longer learning curve. Previous studies on 2D-SWE measurements of liver

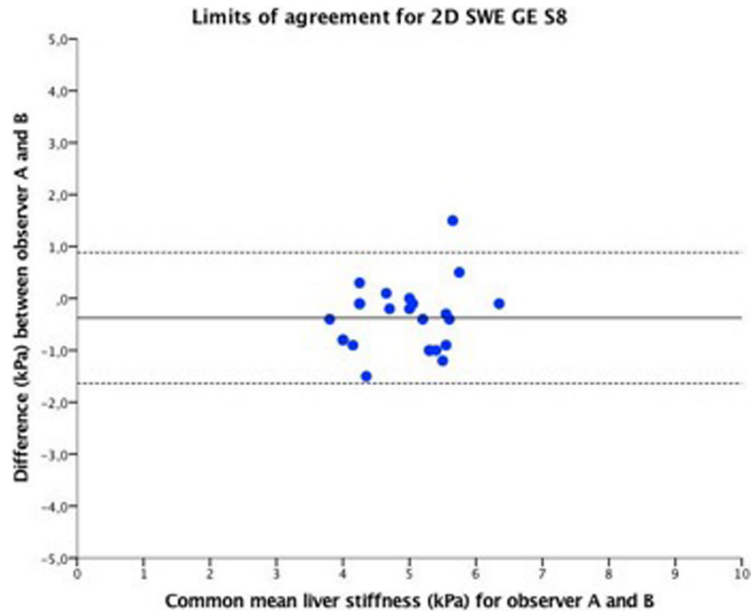


**Fig 7. Limits of agreement for Samsung RS80A SWE.** The figure presents the limits of agreement for Samsung RS80A. The horizontal axis represents the common mean value of all measurements in both observers for, while the vertical axis represents the difference between individual measurements and this common mean (kPa), displaying the variability of measurements. The black line within each system represents the common mean value, the dotted lines represent the 95% confidence intervals. A mean value close to 0 on the vertical axis means that the two observers apply the measurement scale without bias.

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elasticity have demonstrated a learning curve for this method, but with similar reproducibility [16, 22]. Evaluating the intra- and interobserver agreement for 2D-SWE from GE and SWE from Samsung, we demonstrated a good interobserver and better intraobserver agreement for both systems compared to the results reported for Aixplorer 2D-SWE (Figs 7 and 8).

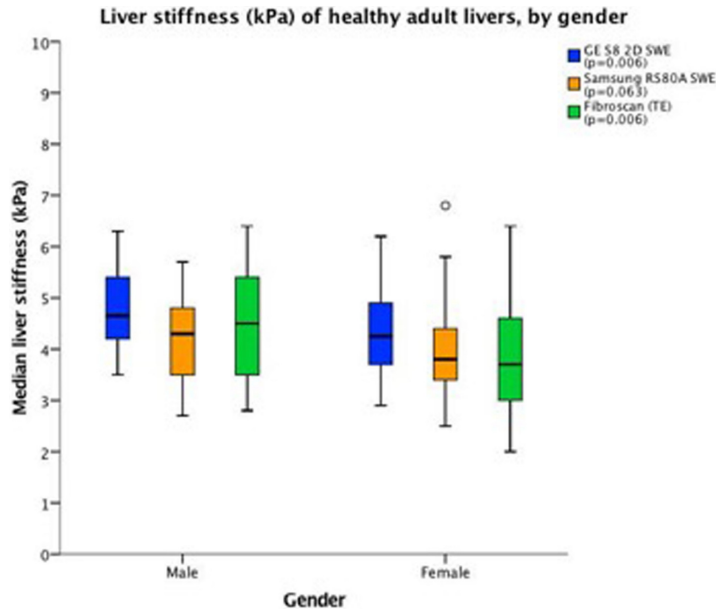
We found a significantly higher LSM in adult male subjects for TE and GE S8 2D-SWE, whereas a similar trend for Samsung RS80A SWE did not reach significance. This is an important finding, indicating that it may be necessary to define separate cut-off values for normal liver and possibly also for levels of liver fibrosis for male and female patients. Previous studies have shown inconsistent results regarding the effect of gender on LSM [23, 24]. Using pSWE, Ling et al. demonstrated that males had 8% higher LSM than females; however, the study had more than twice as many female participants compared to male participants [14]. In contrast, using ARFI, one study found no significant difference between genders in 137 subjects [25] in line with our results for Samsung RS80A. Two studies conducting reliable LSM with TE in 1190 subjects over 45 years, and in 746 healthy subjects, found that male gender was associated with higher liver stiffness [26, 27] and our results for TE confirmed this. Using Aixplorer 2D-SWE from Supersonic Imagine, it has been suggested that males may have higher LSM than females [13]. A study of LSM in healthy children, using the same system, did not demonstrate significant difference between genders [28]. The lack of significant gender difference for LSM in healthy liver tissue for Samsung SWE in the present study may be due to different



**Fig 8. Limits of agreement for 2D-SWE GE S8.** Limits of agreement for 2D-SWE GE S8, for legend we refer to Fig 7. <https://doi.org/10.1371/journal.pone.0203486.g008>

technology and signal processing compared to the two other scanners. Despite that we found a significant difference between genders for all systems in our post hoc analysis ( $n = 69$ ), the study may be underpowered considering the observed SD of 0.8 kPa for the Samsung SWE compared to our power estimation anticipating 0.5 kPa as SD. Furthermore, different hormone levels have been proposed as an explanation of LSM differences between genders, and should be investigated further in *in vivo* studies [29].

In our study, LSMs were not significantly affected by age or BMI. Multiple studies have addressed age as a variable of influencing liver stiffness in normal subjects, and the results have been inconsistent, reporting no difference across age groups [14], higher LSM in older [26] or younger [27] age. We did not demonstrate significant differences in LSM between the five age groups for any of the methods. Possibly, analyses of the effect of age on LSM is confounded by other factors such as steatosis and heart failure that are more prevalent in older populations. One study investigating GE E9 Logic 2D-SWE in healthy subjects reported an LSM-mean of  $5.1 \text{ kPa} \pm 1.3$ , with higher LSM values compared to TE, similar to our findings [17]. In contrast to our results, they reported that age over 40 years was associated with higher LSM, but did not find significant difference in LSM between genders (21 males and 58 females). In the present study, we included only healthy volunteers, carefully interviewed all subjects regarding alcohol consumption, and performed full biochemical analyses and B-mode ultrasound examination of all in contrast to some other studies [12], and in our view, less strict inclusion criteria and missing data regarding liver enzymes in the other study may contribute to these differences. Higher LSM values have been reported in healthy subjects with low BMI ( $< 18.5 \text{ kg/m}^2$ ) as well as in obese subjects compared with normal-weight subjects [30]. We did not demonstrate a difference in LSM between subjects with BMI  $18.0\text{--}25.0 \text{ kg/m}^2$  compared to BMI  $25\text{--}30 \text{ kg/m}^2$ ;



**Fig 9. Liver stiffness (kPa) of healthy adult livers, by gender.** The boxplot shows the liver stiffness by gender. The horizontal axis represents the gender; males and females. The colour interpretation for each system and the level of significance is given in the upper right corner. For legend interpretation, we refer to Fig 4.

<https://doi.org/10.1371/journal.pone.0203486.g009>

however, obese patients with BMI>30 were not included in this study. Normal values for LS in underweight and obese subjects, as well as technical feasibility of Samsung RS80A SWE and GE S8 2D-SWE, should be further investigated and established.

There is an ongoing discussion of the minimum number of measurements needed when acquiring LSM with SWE. The EFSUMB guidelines recommend at least 10 measurements for pSWE and TE, and a minimum of 3 measurements when using 2D-SWE, to obtain consistent results [31, 32]. One study reported excellent intraobserver reproducibility based on 6

**Table 3. Liver stiffness values (kPa) of healthy adult livers, by gender.**

Gender	Female (n = 50)	Male (n = 50)	p-value
<b>GE S8 2D-SWE</b>			
Mean ± SD (kPa)	4.3 ± 0.7	4.7 ± 0.7	p = 0.006
95% CI	4.1–4.5	4.5–4.9	
<b>Samsung RS80A SWE</b>			
Mean ± SD (kPa)	3.9 ± 0.9	4.2 ± 0.7	p = 0.063
95% CI	3.7–4.2	4.0–4.4	
<b>Fibroscan (TE)</b>			
Mean ± SD (kPa)	3.9 ± 1.1	4.5 ± 1.0	p = 0.006
95% CI	3.6–4.2	4.2–4.8	

Liver stiffness values (kPa) for 2D-SWE GE, Samsung RS80A and TE, by gender. Data are presented as mean ± standard deviation with 95% Confidence interval (CI).

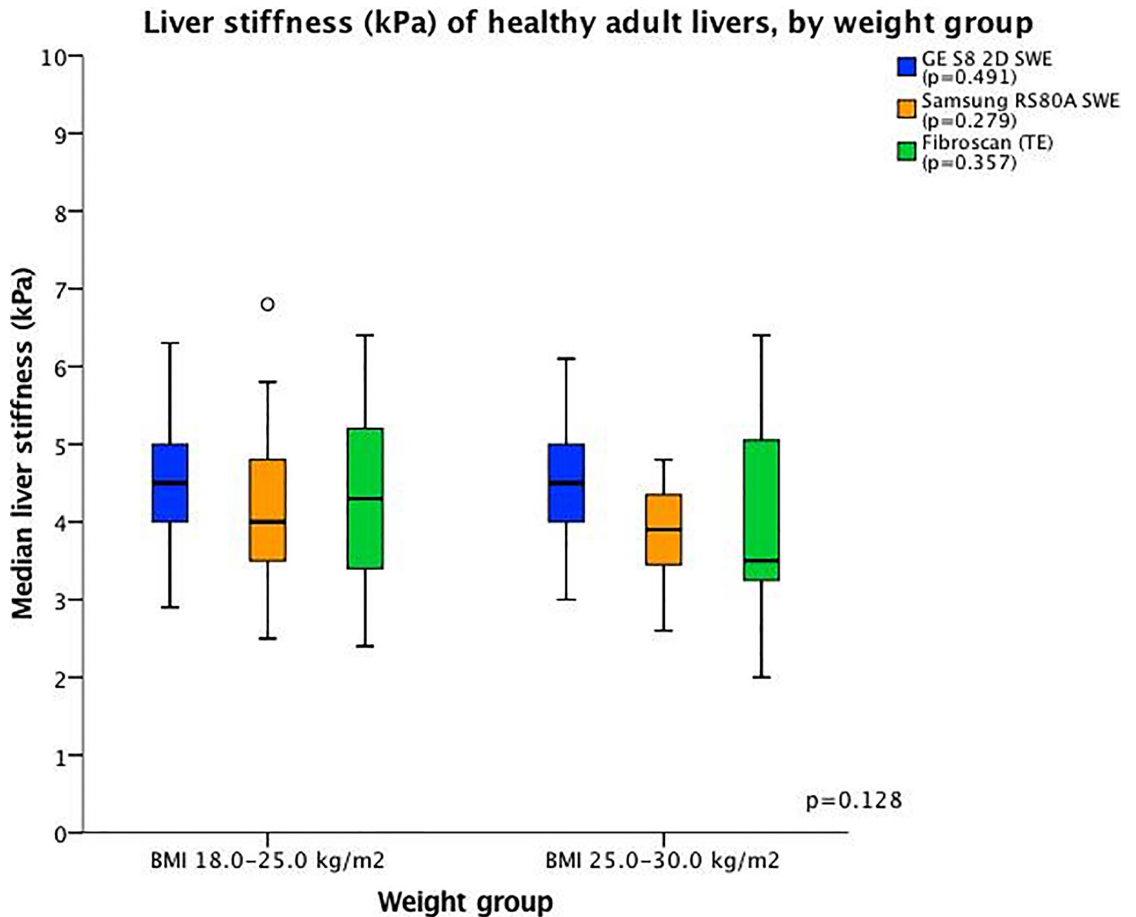
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**Table 4. Liver stiffness values (kPa) of healthy adult livers, by age group.**

Age group	20–30 (n = 20)	31–40 (n = 20)	41–50 (n = 20)	51–60 (n = 20)	61–70 (n = 20)	p-value
<b>GE S8 2D-SWE</b>						
Mean ± SD (kPa)	4.5 ± 0.9	4.7 ± 0.8	4.5 ± 0.7	4.4 ± 0.7	4.5 ± 0.7	p = 0.843
95% CI	4.1–4.9	4.3–5.1	4.1–4.8	4.1–4.8	4.2–4.9	
<b>Samsung RS80A SWE</b>						
Mean ± SD (kPa)	4.3 ± 0.9	4.2 ± 0.8	4.0 ± 0.8	4.1 ± 0.6	3.9 ± 1.0	p = 0.630
95% CI	3.9–4.7	3.8–4.5	3.6–4.4	3.8–4.3	3.4–4.4	
<b>Fibroscan (TE)</b>						
Mean ± SD (kPa)	4.4 ± 1.1	4.3 ± 1.3	4.2 ± 1.0	4.2 ± 1.1	4.1 ± 1.1	p = 0.630
95% CI	3.9–4.9	3.7–5	3.7–4.7	3.7–4.7	3.6–4.7	

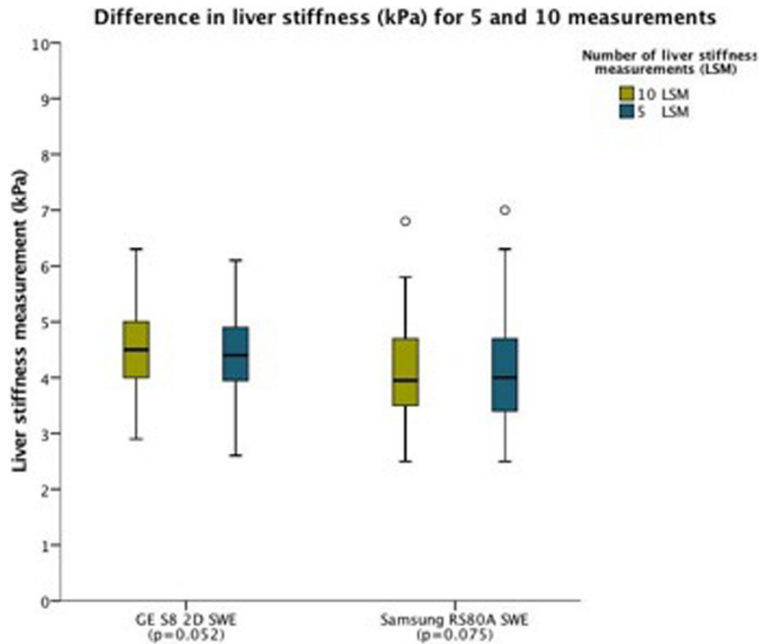
Liver stiffness values (kPa) for 2D-SWE GE, Samsung RS80A and TE, by age group. Data are presented as mean ± standard deviation with 95% Confidence interval (CI).

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**Fig 10. Liver stiffness (kPa) of healthy adult livers, by weight group.** The boxplot shows the liver stiffness by weight group. The horizontal axis represents the BMI group. The colour interpretation for each system and the level of significance is given in the upper right corner. For boxplot interpretation, we refer to Fig 4.

<https://doi.org/10.1371/journal.pone.0203486.g010>



**Fig 11. Difference in liver stiffness (kPa) for 5 and 10 measurements.** The boxplots show difference in liver stiffness for 5 and 10 measurements. The horizontal axis represents the systems, and the vertical axis the liver stiffness measured. The colour interpretation for 5 and 10 measurements (green and blue, respectively) is given in the upper right corner. For boxplot interpretation, we refer to Fig 4.

<https://doi.org/10.1371/journal.pone.0203486.g011>

measurements, concluding that the optimal minimum number of measurements with 2D-SWE was 6 [15]. For pSWE (ARFI), one study concluded that 10 measurements instead of 5 should be performed to obtain a reliable estimation [33]. To the best of our knowledge there are no studies that have directly investigated the difference in mean LS between 5 and 10 separate measurements for several ultrasound SWE methods. Our results did not show significant difference in median LS for 5 versus 10 measurements. This suggests that a reliable median LSM can be obtained with fewer measurements than ten for both 2D-SWE GE S8 and Samsung RS80A in healthy livers. This is important as it indicates that adequate measurements can be made by fewer repetitions and in less time, however our results in healthy livers may not apply in patients with higher degree of liver fibrosis where measurement variability may be higher.

The main limitation of the study is the lack of liver biopsies as a reference method, which is not ethically feasible in a healthy group. Our study design included 100 healthy participants, excluding unknown liver disease by imaging, blood tests and anamnesis.

## Conclusion

All methods were successfully applied in our cohort of 100 healthy subjects. The mean of median LSM for the two new elastography methods (GE S8 and Samsung RS80A) showed a slight difference. Our study shows a significantly higher liver stiffness in males compared to

females, however we found no significant difference in LS between BMI groups 18–30 kg/m<sup>2</sup> or between the age groups 20–70 years. Furthermore, our findings indicate that five acquisitions are sufficient to obtain a reliable LSM using Samsung RS80A or GE S8 2D-SWE in healthy subjects.

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## Author Contributions

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**Data curation:** Anesa Mulabecirovic.

**Formal analysis:** Anesa Mulabecirovic.

**Investigation:** Anesa Mulabecirovic, Anders Batman Mjelle.

**Methodology:** Anesa Mulabecirovic, Mette Vesterhus, Roald Flesland Havre.

**Project administration:** Anesa Mulabecirovic.

**Resources:** Anesa Mulabecirovic, Roald Flesland Havre.

**Software:** Anesa Mulabecirovic.

**Supervision:** Anesa Mulabecirovic, Odd Helge Gilja, Mette Vesterhus, Roald Flesland Havre.

**Validation:** Anesa Mulabecirovic, Mette Vesterhus, Roald Flesland Havre.

**Visualization:** Anesa Mulabecirovic.

**Writing – original draft:** Anesa Mulabecirovic.

**Writing – review & editing:** Anesa Mulabecirovic, Anders Batman Mjelle, Odd Helge Gilja, Mette Vesterhus, Roald Flesland Havre.

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# Paper III



● *Original Contribution*

## ULTRASOUND AND POINT SHEAR WAVE ELASTOGRAPHY IN LIVERS OF PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS

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**Abstract**—Point shear wave elastography (pSWE) is an ultrasound-based method for non-invasive quantification of liver fibrosis. The objective of this study was to explore liver pSWE in patients with primary sclerosing cholangitis (PSC) for assessment of fibrosis. Fifty-five non-transplant patients with PSC (38 males, 17 females; mean age: 46.4 y) were included and compared with 24 matched controls. Median (range) PSC duration was 8.1 (0–33) y. Ultrasonographic scanning followed by liver stiffness measurement by pSWE was performed using a conventional ultrasound system (Philips iU22). Signs of liver fibrosis on B-mode were identified in 21 patients (38%). Splenomegaly was found in 19 patients (35%) and ascites in two patients (4%). Successful pSWE measurements were achieved in the right liver lobe of all individuals and in the left liver lobe of 36 patients (65.5%). PSC patients had significantly higher median shear wave velocity (SWV) than controls in the right liver (median [range] SWV 1.26 [0.73–2.57] m/s vs. 1.09 [0.88–1.25] m/s,  $p < 0.001$ ). SWV measured in the left liver lobe and spleen did not differ between PSC patients and controls. Our findings indicate that PSC patients have increased median SWV, indicating more fibrosis compared with controls; however, a wide range of SWV values were obtained among PSC patients, possibly reflecting the various stages in disease development. (E-mail: [mette.vesterhus@helse-bergen.no](mailto:mette.vesterhus@helse-bergen.no)) © 2016 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

**Key Words:** Primary sclerosing cholangitis, Point shear wave elastography, Non-invasive, Liver fibrosis, Elastography, Ultrasound.

### INTRODUCTION

Primary sclerosing cholangitis (PSC), a chronic inflammatory disease affecting the biliary tree, leads to liver fibrosis and cirrhosis over time, with a reported median transplant-free survival time of 12–21 y (Boonstra et al. 2013; Broomé et al. 1996). Medical therapy with proven benefit is lacking, and PSC is a frequent indication for transplantation.

A major challenge in PSC is the lack of valid prognostic markers and biomarkers of disease activity (Hirschfield et al. 2013; Karlsen et al. 2014). Fibrogenesis is an important pathogenetic pathway in PSC and a target of treatment in several clinical trials.

A serum marker panel of fibrosis, the enhanced liver fibrosis (ELF) test, was reported to distinguish mild from advanced disease in PSC by an area under the curve of 0.81 and to predict prognosis independently of other biomarkers, underscoring the importance of accurate liver fibrosis estimation in PSC (Vesterhus et al. 2015). However, for other etiologies of liver fibrosis, some studies indicate an improved performance of ultrasound elastography compared with ELF or an incremental value of the combination of the ELF test and liver stiffness evaluation by ultrasound elastography (Cobbold et al. 2010; Wahl et al. 2012). Hence, better methods for the diagnosis, grading and monitoring of liver fibrosis are warranted.

Ultrasound elastography is a technique measuring liver stiffness as an expression of fibrosis and has emerged as an important tool in the diagnosis and follow-up of liver fibrosis and cirrhosis, largely replacing

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liver biopsy in hepatitis B and C (Cosgrove *et al.* 2013; Ferraioli *et al.* 2015). The status of liver biopsy as the gold standard for liver fibrosis assessment has long been challenged because of its invasiveness and risk of serious complications, as well as the substantial sampling error and inter-observer variation between pathologists (Castera and Pinzani 2010; Cholongitas *et al.* 2006; Thampanitchawong and Piratvisuth 1999). Liver biopsy is generally not indicated in PSC for either diagnosis or follow-up because of the patchy disease distribution and consequent sampling bias, except in cases of suspected small-duct disease or autoimmune hepatitis overlap (Chapman *et al.* 2010; European Association for the Study of the Liver 2009). Ultrasound elastography has the advantages of being non-invasive and repeatable and offers the possibility of investigating several regions of the liver, thus reducing sampling bias. Guidelines for the use of elastography in clinical practice have been published (Bamber *et al.* 2013; Cosgrove *et al.* 2013); however, reports on elastography in PSC are scarce (Corpechot *et al.* 2006; Hagstrom *et al.* 2012; Righi *et al.* 2012).

Interestingly, a recent publication reported that baseline values of transient elastography (TE), as well as the change in liver stiffness measured by TE, are associated with clinical outcome in PSC (Corpechot *et al.* 2014). Point shear wave elastography (pSWE) is a more recent technology than TE, with the advantage of being incorporated into high-end ultrasound equipment, allowing B-mode ultrasound guidance of elastography measurements and an integration of liver stiffness measurement with a full evaluation of the liver. Some studies of pSWE in patient populations with chronic liver disease of heterogeneous etiologies have included PSC patients in small numbers insufficient for sub-analysis (Righi *et al.* 2012). To our knowledge, there are no studies exploring pSWE in PSC alone. In this study, we aimed to evaluate liver stiffness in PSC patients and compare them with healthy controls using ultrasound pSWE.

## METHODS

### *Patient population and data collection*

The protocol was in accordance with the Declaration of Helsinki and approved by the Regional Committee for Health and Research Ethics in Western Norway. Patients invited to participate in the study belonged to a known cohort of non-transplanted PSC patients in western Norway. Informed written consent was obtained from each patient enrolled. PSC patients with a histologically confirmed diagnosis of autoimmune hepatitis (AIH) were classified as PSC–AIH overlap. Patients were examined, and patient records were searched for information on clinical data, including ascites, encephalopathy,

esophageal varices, variceal bleeding and inflammatory bowel disease status at the time of serum extraction. On the day of ultrasound and elastography, blood was sampled and biochemical analyses were performed using the standard routine laboratory protocols, including C-reactive protein, hemoglobin, leukocytes, platelets, creatinine, total bilirubin, albumin, International Normalization Ratio, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and  $\gamma$ -glutamyl transferase. The Aspartate Aminotransferase-to-Platelet Ratio Index (APRI) and Fibrosis-4 (FIB-4) scores of fibrosis were calculated using published algorithms (Sterling *et al.* 2006; Wai *et al.* 2003). Mayo risk scores were calculated using the algorithm for the revised Mayo risk score (Kim *et al.* 2000). Blood samples were not taken from healthy controls.

### *B-Mode ultrasound examination*

Immediately before pSWE examination, all patients underwent B-mode ultrasound scanning of the liver and spleen. All examinations were performed by a single operator (M.V.) using a standardized scanning protocol on a Philips iU22 (Philips Healthcare, Andover, MA, USA) scanner. Scores were registered for liver capsule regularity, parenchyma heterogeneity, liver angle appearance, presence of ascites, gallbladder stones or polyps and the presence of bile duct variability or sludge. Measures were taken for liver size in a sagittal section in the medioclavicular line; gallbladder length, width and area; spleen length and width; and portal vein diameter. Splenomegaly was defined as spleen length > 12 cm.

### *Point shear wave elastography*

Liver and spleen stiffness was measured in the fasting condition by pSWE using a conventional ultrasound system (ElastPQ, iU22, Philips Healthcare) equipped with a convex probe (C5-1). For liver measurements, patients and controls were examined in the supine position with their right arm maximally abducted. A  $0.5 \times 1.5$ -mm region of interest was placed 2–6 cm deeper than the liver capsule in hepatic tissue, avoiding large vessels or bile ducts (Fig. 1). Right lobe measurements were made in an intercostal position, whereas left liver lobe measurements were performed in a subcostal epigastric position, with sampling from the central portion of the left liver lobe. Spleen stiffness was measured by pSWE from a left-side intercostal position. All pSWE measurements were acquired in relaxed mid-breath hold with minimal scanhead pressure being applied. A valid measurement was defined as the median value of 10 acquisitions, provided the requirement for a success rate  $\geq 60\%$  was also fulfilled. The acquisitions were performed during separate breath holds in the same general area within one segment, avoiding visible bile ducts and blood

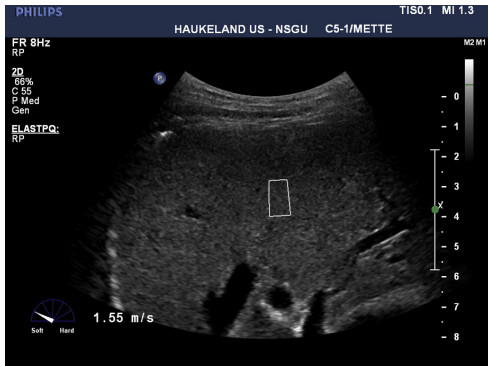


Fig. 1. Screen image of Philips iU22 in Elastography mode. B-Mode ultrasound image of a section of the right liver lobe in a patient with percutaneous sclerosing cholangitis. The *rectangular box* represents the region of interest where the elastography measurements are being performed. The region of interest has a fixed size but can be moved freely within the image down to a maximum depth of 8 cm. Measurements within 1 cm of the liver capsule or close to large vessels or bile ducts should be avoided. The measured SWV is given in the lower left corner; in this case it is 1.55 m/s, which indicates some degree of fibrosis. SWV = shear wave velocity.

vessels. Results were given as median shear wave velocity (SWV) in meters per second. All measurements were performed by a single investigator (M.V.). To evaluate the intra- and inter-observer variation, pSWE of the right liver lobe was performed twice in 16 healthy controls by the same investigator (M.V.) or two independent investigators (M.V. and A.M.), respectively, according to the protocol described previously.

#### Statistical analyses

Version 12.7.0.0 of SPSS 22 (IBM, Armonk, NY, USA) and MedCalc were used to perform all statistical analyses, and  $p$  values  $< 0.05$  were considered to indicate significance. Variables were tested for normal distribution, and Student's  $t$ -test or the Mann-Whitney  $U$ -test were applied as appropriate. Data are presented as mean (SD), or as median (range) when the data were not normally distributed. Correlations between SWV and clinical parameters, biochemical scores of fibrosis or Mayo risk scores were tested by Spearman's rank order correlation coefficient ( $\rho$ ). Intra-observer agreement was tested using the limits of agreement method (Bland and Altman 1999).

## RESULTS

Sixty-four non-transplant PSC patients in a region of western Norway were identified and invited to participate; 55 (86%, 38 males, 17 females; mean age: 46.4 y;

95% confidence interval [CI]: 42.0–50.8) were included and compared with 24 healthy controls matched for age and gender. Median (range) PSC duration was 8.1 (0–33) y. Baseline demographic characteristics and clinical and laboratory data are summarized in Table 1. One patient with small-duct PSC and 5 patients with PSC–AIH overlap syndrome were included. In total, 3 patients had biochemical signs of clinically significant cholestasis or hepatitis as determined by a bilirubin level  $>30$  (2 patients) or an alanine aminotransferase or aspartate aminotransferase level  $>5\times$  the upper limit of normal (1 patient). There were no significant differences in distribution of age, gender or body mass index (BMI) between patients and controls. On the basis of B-mode ultrasound evaluation, signs compatible with advanced liver fibrosis, including liver capsule irregularity, periductal fibrosis and coarse liver parenchyma, were identified in 16 (29%), 5 (9%) and 12 (22%) patients, respectively, whereas 34 (62%) patients displayed no signs of fibrosis on B-mode ultrasound. Splenomegaly was found in 19 patients (35%) and ascites in 2 (4%) patients. Bile duct

Table 1. Baseline characteristics of 55 patients with PSC and 20 healthy controls undergoing ultrasound elastography

Baseline characteristics	PSC patients	Controls	$p$
N	55	24	
Males	37 (67%)*	11 (46%)	0.07
Age, mean (95% CI)	46 (42, 51)	43 (37, 49)	0.35
Age at diagnosis	34 (12–73)	NA	
Body mass index, mean (95% CI)	25.8 (24.8–26.8)	24.3 (22.9–25.8) <sup>†</sup>	0.09
PSC duration, y	8.1 (–0.6 to 32.8)	NA	
Inflammatory bowel disease, ever	47 (85.5%)	0	
Ulcerative colitis	32 (58.2%)	0	
Crohn's disease	8 (14.5%)	0	
Indeterminate colitis	7 (12.7%)	0	
Cholecystectomy	4 (7.3%)	0	
Mayo risk score	–0.4 (–2.1 to 1.9)	NA	
Laboratory data			
ALP, U/L	138 (25–838)	NI	
AST, U/L	45 (20–129)	NI	
ALT, U/L	49 (19–390)	NI	
Total bilirubin, $\mu$ mol/L	11 (5–75)	NI	
$\gamma$ -Glutamyl transpeptidase, U/L	177 (17–1576)	NI	
Albumin, g/L	46 (36–53)	NI	
INR	1.0 (0.9–1.2)	NI	
Platelets, $10^9/L$	227 (60–765)	NI	

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CI, confidence interval; INR = International Normalization Ratio; NA = not applicable; NI = not investigated; PSC = primary sclerosing cholangitis.

\* Values are expressed as n (%) or median (range) unless otherwise noted.

<sup>†</sup> N = 17.

dilation was identified in 25 (46 %) patients. B-Mode ultrasound findings are summarized in Table 2.

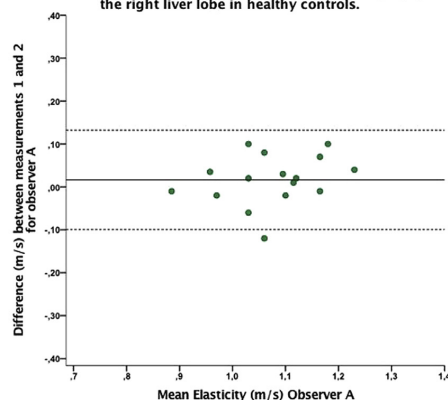
### pSWE of the right liver lobe

The intra- and inter-observer agreement for pSWE of the right liver lobe in the healthy controls was good, as evaluated by the limits of agreement method (Fig. 2). In PSC patients, valid pSWE measurements were achieved in all patients for the right liver lobe. The median success rate for the individual patients was 100% (range: 71.4%–100%). PSC patients had higher median SWV compared with the healthy controls (1.26 [0.73–2.57] and 1.09 [0.88–1.25] m/s, respectively,  $p < 0.001$ ) (Fig. 3a). Area under the receiver operating characteristic curve (AUROC) analysis revealed fairly good discrimination for median SWV of the right liver lobe between PSC patients and controls, with an AUROC of 0.775 (95% CI: 0.67–0.86) (Fig. 4). For the discrimination of PSC patients from controls, the statistically optimal cutoff for SWV as decided by Youden's index was 1.24 m/s, with a sensitivity and specificity of 56.4 and 95.8, respectively.

Figure 5 illustrates right liver SWVs correlated with APRI and FIB-4 scores ( $\rho = 0.494$ ,  $p = 0.001$ , and  $\rho = 0.368$ ,  $p = 0.017$ , respectively) (Fig. 5a and b), whereas there was no significant correlation with the Mayo risk score ( $\rho = 0.296$ ,  $p = 0.06$ ) (Fig. 5c). No correlation was found between right liver SWVs and BMI, age or PSC duration (Table 3).

SWV in the right liver lobe was significantly higher in PSC patients with coarse liver parenchyma (median [range]: 1.88 [0.99–2.57] m/s vs. 1.22 [0.73–2.34] m/s,  $p = 0.002$ ), irregular liver capsule (1.81 [1.11–2.57] m/

**a** Intraobserver agreement of point shear wave elastography of the right liver lobe in healthy controls.



**b** Interobserver agreement of point shear wave elastography of the right liver lobe in healthy controls.

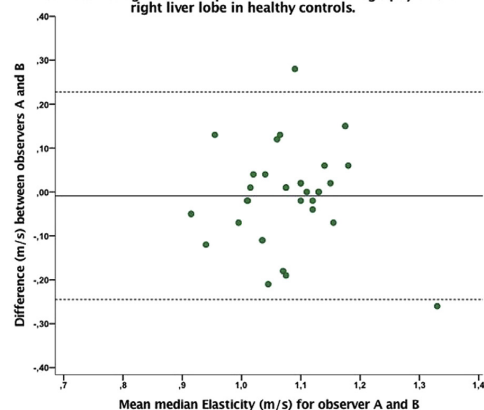


Fig. 2. Intra- and inter-observer agreement of point shear wave elastography of the right liver lobe in healthy controls. The Bland–Altman plots illustrate the (a) intra-observer and (b) inter-observer differences in liver stiffness evaluation measured by point shear wave elastography using ElastPQ (iU22, Philips) and expressed as shear wave velocity in meters per second. The horizontal solid lines represent the intra- or inter-observer mean  $\pm$  2 SD (limits of agreement, dashed horizontal lines), respectively. A valid measurement was defined as the median of 10 valid acquisitions with a success rate  $>60\%$ . The measurements were performed twice by one (a) or two (b) observer(s) on the same day in the right liver lobe of healthy controls for the intra-observer agreement assessment.

s vs. 1.17 [0.73–2.43] m/s,  $p = 0.001$ ) and periductal fibrosis (1.76 [1.27–2.09] m/s vs. 1.24 [0.73–2.57] m/s,  $p = 0.049$ ), compared with patients with normal findings (Fig. 6a–c). Patients with none of these three visual signs of liver fibrosis had a median right liver SWV of 1.17 (0.73–2.34) m/s, compared with 1.76 (0.99–2.57) m/s among patients with minimum one B-mode sign of fibrosis ( $p = 0.001$ ) (Fig. 6c). Right liver stiffness

Table 2. B-Mode ultrasound findings in PSC patients

Liver	
Liver size in MCL, cm	14.2 (8.0–28.5)*
Hepatomegaly (>16 cm)	10 (18.2%)
Liver capsule irregularity	16 (29.1%)
Coarse liver parenchyma	12 (21.8%)
Blunted liver angle	16 (29.1%)
Ascites	2 (3.6%)
Gallbladder and bile ducts	
Gallbladder length, cm	6.6 (2.1–10.0)
Gallbladder width, cm	3.0 (1.5–5.4)
Gallbladder wall thickness, mm	2.3 (0.3–10.4)
Gallbladder stone(s)	6 (10.9%)
Gallbladder polyp	1 (1.8%)
Cholecystectomy	4 (7.3%)
Bile duct variability	25 (45.5%)
Periductal fibrosis	5 (9.1%)
Spleen	
Spleen length, cm	12.6 (8.2–22.7)
Spleen area, cm <sup>2</sup>	58.3 (25.4–165.7)
Splenomegaly (>13 cm)	19 (34.5%)

MCL = medioclavicular line; PSC = percutaneous sclerosing cholangitis.

\* Values are expressed as n (%) or median (range).

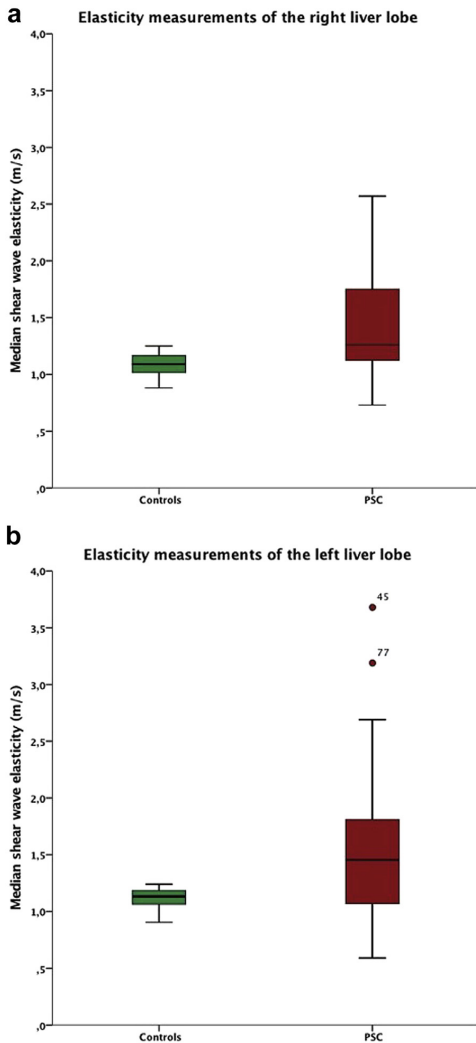


Fig. 3. Liver stiffness in PSC patients compared with controls. Liver stiffness evaluation by point shear wave elastography using iU22 (Philips) in 55 PSC patients and 24 healthy controls matched for age and gender in (a) the right liver lobe, and (b) the left liver lobe revealed increased liver stiffness in the right liver lobe of PSC patients compared with controls ( $p < 0.001$ ). No significant difference could be found in the left liver lobe ( $p = 0.11$ ). Liver stiffness is expressed as shear wave velocity in meters per second. PSC = primary sclerosing cholangitis.

assessed by median SWV did not differ significantly between patients with splenomegaly and patients without splenomegaly (1.42 [0.73–2.57] m/s vs. 1.24 [0.93–2.34] m/s,  $p = 0.11$ ). Bile duct dilation was identified

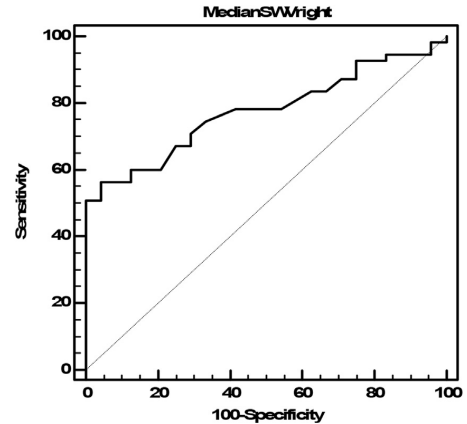


Fig. 4. Point shear wave elastography discriminates between primary sclerosing cholangitis patients and controls. Area under the receiver operating characteristic curve analysis revealed fairly good discrimination for median SWV measured by point shear wave elastography between 55 primary sclerosing cholangitis patients and 24 age- and gender-matched controls with an area under the curve of 0.775 (95% confidence interval: 0.67–0.86). The optimal cutoff for SWV as decided by Youden's index was 1.24 m/s, with a sensitivity and specificity of 56.4 and 95.8, respectively. SWV = shear wave velocity.

in 26 (47.3%) patients, but median right liver SWV did not differ between these patients and patients without bile duct dilation (1.32 [0.93–2.57] m/s vs. 1.24 [0.73–2.43] m/s,  $p = 0.61$ ).

#### *pSWE of the left liver lobe*

In the left liver lobe, valid SWV measurements were acquired in 36 patients (66%), whereas in 19 patients (35%) the measurements were considered invalid because of too many failed acquisitions (success rate: <60%). The median success rate was 83% (46%–100%). Left liver lobe SWV did not significantly differ between PSC patients and controls (median [range] SWV: 1.46 [0.59–3.68] m/s vs. 1.13 [0.91–1.24] m/s,  $p = 0.11$ ) (Fig. 3 b). There was no significant difference between median SWV of the right and left liver lobes in PSC patients ( $p = 0.41$ ). Paired SWV values of the right and left liver lobes in the individual patient did not significantly correlate ( $\rho = 0.233$ ,  $p = 0.17$ ). Similarly, no significant correlation was found between left liver SWVs and BMI, age or PSC duration (Table 3).

#### *pSWE of the spleen*

Valid measurements were obtained in 37 PSC patients (67.3%), whereas in the remainder of the patients, measurements either were not performed ( $n = 16$ ) or



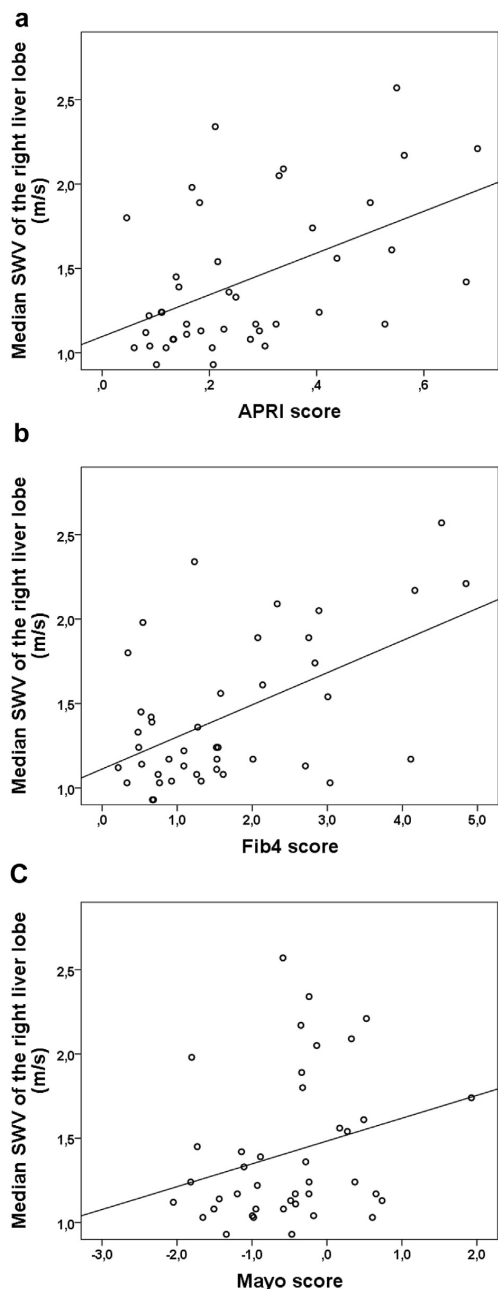


Fig. 5. Associations of liver stiffness with fibrosis scores and prognosis in percutaneous sclerosing cholangitis patients. The scatterplots with regression lines illustrate that SWV (m/s) of the right liver lobe as measured by point shear wave elastography using the iU22 (Philips) was correlated with the (a) APRI

failed to fulfill the quality criteria ( $n = 2$ ). The median success rate for the patients with valid measurements was 100% (76.9%–100%). There was no significant difference between PSC patients and controls (median SWV: 1.47 [0.79–3.13] m/s and 1.48 [1.17–1.80] m/s, respectively,  $p = 0.83$ ). A tendency toward higher spleen SWV in patients with splenomegaly compared with patients without splenomegaly did not reach statistical significance (1.71 m/s [0.89–2.71] vs 1.39 m/s [0.79–3.13], respectively,  $p = 0.05$ ). There was no correlation between spleen SWV and right or left liver SWV (1.47 m/s vs. 1.24 m/s,  $N = 37$ ,  $\rho = 0.104$ ,  $p = 0.54$ , and 1.39 m/s vs. 1.50 m/s,  $N = 22$ ,  $\rho = 0.331$ ,  $p = 0.13$ , respectively). One patient had variceal bleeding but did not have a high spleen SWV (median SWV: 1.55 m/s). No correlation was found between spleen SWV and BMI, age or PSC duration.

## DISCUSSION

Our study indicates how liver fibrosis can be evaluated by both SWE and traditional B-mode findings during the same procedure. We found excellent intra- and inter-observer variation for pSWE using the iU22 system for the right liver lobe, in line with previous reports (Ling *et al.* 2013). The non-invasive evaluation of the degree of and change in liver fibrosis in PSC may be of major importance in evaluating the stage and prognosis of the disease, as indicated by recent reports (Corpechot *et al.* 2014, Vesterhus *et al.* 2015).

Liver biopsy is generally not indicated in PSC, based on the inherent sampling error resulting from the patchy distribution of fibrosis (European Association for the Study of the Liver 2009). The flaws of biopsies were illustrated in a study of whole-section scanning of 50 liver explants from patients with primary biliary cirrhosis, another disease of the biliary tree, in which only 20% of the primary biliary cirrhosis livers had a consistent histologic stage of fibrosis throughout the liver at clinically defined end-stage disease (Garrido and Hubscher 1996). Likewise, the distribution of liver fibrosis in PSC is uneven and follows the bile ducts to a large extent. In our opinion, it may therefore be preferable to use non-invasive methods assessing liver fibrosis covering larger areas of the liver in PSC. Ultrasound shear wave elastography is non-invasive and repeatable, can be integrated into a full liver examination and has been documented in viral hepatitis as a means of measuring liver fibrosis, but has not been previously explored in PSC

and (b) Fibrosis-4 (Fib4) scores of fibrosis, but not with (c) the Mayo risk score, a commonly used prognostic score in primary sclerosing cholangitis. SWV = shear wave velocity; APRI = Aspartate Aminotransferase-to-Platelet Ratio Index.

Table 3. Correlations of median SWV with other continuous variables in PSC patients

Clinical and laboratory variables	N	Median SWV right liver		Median SWV left liver		
		Spearman's $\rho$	<i>p</i>	Spearman's $\rho$	<i>p</i>	
Age	55	-0.004	0.974	36	-0.064	0.709
Age at diagnosis	54	-0.06	0.669	36	0.115	0.504
PSC duration	54	0.01	0.945	36	-0.179	0.295
Bilirubin	49	0.175	0.23	32	0.207	0.255
Albumin	50	-0.358	<i>0.011*</i>	33	-0.222	0.215
ALP	50	0.246	0.085	33	0.335	0.057
AST	42	0.208	0.187	26	0.191	0.35
ALT	51	-0.072	0.616	34	0.222	0.207
Platelet count	51	-0.319	<i>0.022</i>	34	-0.301	0.084
Body mass index	55	-0.101	<i>0.463</i>	36	0.281	0.097
INR	47	0.325	<i>0.026</i>	31	-0.115	0.539
Mayo score	41	0.296	0.06	25	0.495	<i>0.012</i>
APRI score (AST/TRC) <sup>†</sup>	42	0.494	<i>0.001</i>	26	0.468	<i>0.016</i>

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; APRI = AST-to-Platelet Ratio Index; INR = International Normalization Ratio; NA = not applicable; PSC = primary sclerosing cholangitis; SWV = shear wave velocity.

\* *p*-Values in italic denote significance.

<sup>†</sup> See Figure 5a.

(Bota et al. 2013a, 2013b; Friedrich-Rust et al. 2012; Sporea et al. 2012a, 2012b).

Our findings suggest that PSC patients have increased median liver stiffness as expressed by SWV compared with healthy controls. The literature reveals scarce information about pSWE in PSC, but our data are in line with pSWE findings in a pilot study of patients with autoimmune liver diseases causing fibrosis, including PSC (Righi et al. 2012), and support previous findings describing TE in PSC (Corpechot et al. 2006, 2014; Hagstrom et al. 2012). In the present PSC cohort, 21 (38%) of the patients expressed B-mode signs of liver fibrosis. SWV was significantly higher in patients with B-mode signs of liver fibrosis; however, 12 patients without visual signs of liver fibrosis also had increased SWV (>1.24 m/s). Previous studies have reported good to excellent AUROCs between pSWE and histologic evaluation of fibrosis, even in autoimmune liver diseases (Friedrich-Rust et al. 2012; Righi et al. 2012). Thus, our findings could suggest an increased sensitivity in identifying fibrosis in PSC patients by adding elastography to an ultrasound liver evaluation. Furthermore, pSWE was associated with currently acknowledged signs of fibrosis, including TE in cystic fibrosis liver disease, which displays a patchy disease distribution similar to that of PSC (Behrens et al. 2013; Karlas et al. 2012; Manco et al. 2012; Monti et al. 2012). Because liver biopsy is generally not indicated in PSC, histologic correlates are lacking

in the present study, but SWV correlated with serum-based scores of fibrosis, including APRI and FIB-4 scores.

Previous studies have reported that the performance of SWE and the cutoff values for significant fibrosis and cirrhosis may vary with the etiology of liver disease (Friedrich-Rust et al. 2012; Guzman-Aroca et al. 2012; Karlas et al. 2012; Sporea et al. 2012b). In the present study, the cutoff SWV value with the best statistical power to discriminate between PSC patients and healthy controls was 1.24 m/s with an area under the curve of 0.775, in line with previous findings suggesting 1.23 m/s as the statistically best cutoff between patients with chronic liver disease and controls (Sporea et al. 2014). It is an interesting characteristic of patients with PSC that this cutoff is similar to that of other liver disease populations, although it should be kept in mind that the clinically ideal cutoff value may differ depending on the aim of stratification (e.g., early diagnosis of liver fibrosis or identification of a high-risk group). Longitudinal studies are needed to resolve whether pSWE can be used to follow disease progression in the individual patient for prognostic purposes.

The wider variability and lower success rate of ultrasound elastography of the left liver lobe has been debated (Karlas et al. 2011; Ling et al. 2013; Toshima et al. 2011). We were able to obtain valid liver stiffness measurements of the left liver lobe in 66% of the patients. SWVs of the left liver lobe correlated with the APRI score of fibrosis. There was a wider range of SWV measurements in the left compared with the right liver, probably caused by respiratory and cardiac movements affecting elastography measurements and suggesting reduced reliability of measurements in the left liver lobe. The lack of correlation between the two liver lobes may be due to the higher variability in SWV of the left liver lobe, and the definition of stricter quality criteria for pSWE measurements of the left liver lobe might yield better correlation for the valid measurements.

Previously published studies have indicated that cholestasis influences the accuracy of pSWE for the non-invasive evaluation of liver fibrosis (Pfeifer et al. 2014). PSC is a cholestatic disease, and this might be expected to complicate the evaluation of liver stiffness by pSWE in this patient group. However, only two patients had significantly elevated bilirubin >30  $\mu\text{mol/L}$ ; and although some degree of cholestasis was indicated in 26 (47%) patients by bile duct dilation on B-mode ultrasound, there was no difference in liver stiffness by pSWE in these patients compared with patients without bile duct dilation.

Several articles have reported that increased spleen stiffness alone or the spleen/liver stiffness ratio may predict high-risk esophageal varices and, thus, aid the

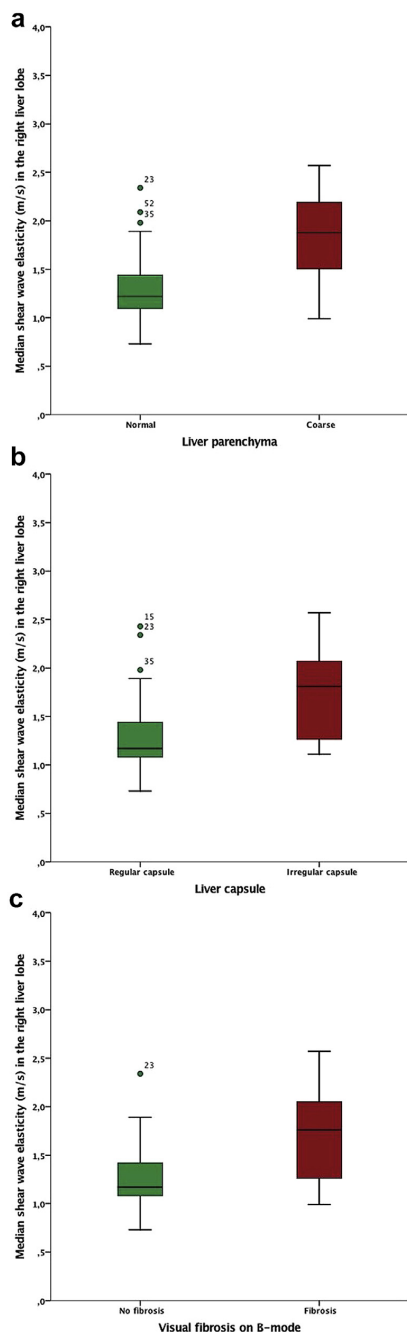


Fig. 6. Point shear wave elastography and B-mode signs of liver fibrosis. Boxplots illustrate SWV (m/s) reflecting liver stiffness as evaluated by point shear wave elastography using the iU22

identification of patients who should be selected to undergo gastroscopy (Berzigotti *et al.* 2013, 2014; Takuma *et al.* 2013; Sirlin *et al.* 2015). In view of this, the spleen stiffness of PSC patients is of interest. In the present study, spleen stiffness did not significantly differ between PSC patients and controls. However, the number of patients was small, and only one variceal bleeding was observed in this cohort, precluding definitive conclusions. The association of liver and spleen stiffness with portal hypertension and esophageal varices in PSC should be further investigated in a larger cohort and, preferably, in a prospective setting.

#### Limitations of the study

The lack of liver biopsies in our cohort represents a limitation to the study. However, liver biopsies are not indicated in PSC, and biopsy was considered unethical for study purposes. Previous studies of chronic liver disease with a range of etiologies have repeatedly indicated excellent correlation between pSWE and histology findings. The question of prognostic value cannot be answered by the cross-sectional design of the present study, and further studies, including prospective follow-up, are warranted.

Although no standards of quality control have been agreed on for pSWE, we applied a standardized protocol and strict quality criteria as previously proposed, demanding 10 valid acquisitions with a success rate >60% to have reliable results (Bota *et al.* 2013a, 2013b). Measurements were made in two selected sites in the right and left liver lobes, respectively. Considering the patchy disease distribution in PSC, it is conceivable that SWV measurements throughout the entire liver would have revealed variable results within each lobe of the individual patient, and further studies should attempt to explore this.

#### CONCLUSIONS

We found that PSC patients have increased SWV in their liver parenchyma compared with healthy controls, indicating increased liver fibrosis. However, a wide range of SWV values were obtained for PSC patients, possibly reflecting various stages in PSC disease development. This novel method exhibited low intra- and inter-observer variation, making it suitable for further studies analyzing prospective follow-up data evaluating pSWE as a prognostic tool.

(Philips) in patients with and without B-mode ultrasound signs of fibrosis, including (a) coarse liver parenchyma, (b) irregular liver capsule and (c) any of three signs of liver fibrosis (liver capsule irregularity, parenchyma coarseness, periductal fibrosis), compared with none of these.

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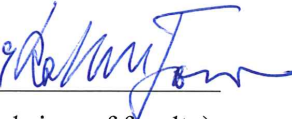
**Errata for**  
**In-vitro and in-vivo validation of ultrasound**  
**shear wave elastography for liver application**

**Anesa Mulabecirovic**



Thesis for the degree philosophiae doctor (PhD)  
at the Universitetet i Bergen

7.11.2015   
(date and sign. of candidate)

14.11.19   
(date and sign. of faculty)

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## Errata

- Page 10      The list of abbreviations has been arranged in an alphabetical order. Further abbreviations have been added, as they were not listed. These are: kilo Pascals (kPa), autoimmune hepatitis (AIH), brightness mode (B-mode), Hepatitis C Virus (HCV), Coefficient of variation (CV), meters per second (m/s), Region of interest (ROI), Supersonic Shear Imaging (SSI), Virtual touch quantification (VTq), interquartile range (IQR).
- Page 14      A section of five lines of text describing the requirements of the thesis abstract was erroneously included. This has been removed.
- Page 15      The publications terms and licenses of the papers included have been utterly specified to the following: *Paper I and II are published under the terms of the CC BY 4.0 license: <https://creativecommons.org/licenses/by/4.0>. Paper III is published under the terms of the CC BY-NC-ND 4.0 license: <http://creativecommons.org/licenses/by-nc-nd/4.0/>.*
- Page 21      Typing errors, misspellings and incomprehensive sentences have been corrected. “Initially the SE displayed solely a qualitative image, where the relative tissue elasticity was shown as a colour overlay on the conventional B-mode. Later, the method has been featured with an integrated quantitative approach to compare strain in two, or more, user selected areas such as the are one wants to measure and the reference are.” - corrected to “Initially the SE displayed solely a qualitative image, where the relative tissue elasticity was shown as a colour overlay on the conventional B-mode. Later, the method has been featured with an integrated quantitative approach to compare strain in two, or more, user selected areas such as the area one wants to measure, and the reference area.”
- Page 35      In the figure text describing figure 5, the text has been changed to include that the person on the image has consented to appear in the photo. “*The person on the image above has consented to appear on the photos in this thesis.*”



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