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Ultrasound based shear wave elastography of the liver:

a non-invasive method for evaluation of liver disease

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Dedication

To my family and friends, with love

“When the liver is stiff, the prognosis is bad”

—Hippocrates

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Abstract

Background: Detecting liver disease at an early stage is important, given that early intervention decreases the risk of developing cirrhosis and subsequently hepatocellular cancer (HCC). The non-invasive ultrasound-based shear wave elastography (SWE) has been used clinically for a decade to assess liver stiffness. This method is reliable, rapid and can be performed in an outpatient setting without known risks for the patient. However, increased variance in SWE results has been detected, without clear explanation. Factors that affect SWE results needs to be identified. Data are insufficient regarding the reliability of SWE with different body positions and probe pressures. Men have higher SWE results than women, also for unclear reasons. Increasing the reliability of SWE is crucial for understanding how factors such as overweight and obesity, cardiovascular and antiviral medication, age, sex, smoking habits, hepatic steatosis and cirrhosis affect SWE results.

Aims: The overall aim of the studies included in this thesis was to increase the reliability of SWE liver. The specific aims were to investigate patient-related factors associated with increased uncertainty in SWE results. Another aim was to investigate the influence of increased intercostal probe pressure on liver stiffness assessment with SWE liver. The final aims were to investigate the influence of postural changes, sagittal abdominal diameter (SAD) and skin-to-liver capsule distance (SCD) on SWE results, along with sex-based differences for SWE results and cardiovascular medication.

Methods: All enrolled participants in these studies were consecutive patients with various liver diseases presenting at the radiology department Östersunds Hospital. The patients were examined using SWE liver method at the ultrasound unit between April 2014 and May 2018. Inclusion criteria were that participants be adults (age ≥18 years) who had provided written consent for participating in the study. The exclusion criterion was an inability to communicate. Current guidelines for SWE of the liver were used in the thesis with the following exceptions: In study II, increased intercostal probe pressure was used, and in study III, postural change was used. Study I included 188 patients; study II included 112 patients, and studies III and IV involved 200 patients. The four studies were conducted as cross-sectional and clinical trial, using quantitative methods.

Results: Factors associated with low variance for SWE results were age, sex, and presence of cirrhosis, the use of antiviral and/or cardiovascular medication, smoking habits, and body mass index. Factors associated with increased uncertainty in SWE results were increased SCD and the presence of steatosis.

With increased probe pressure SCD decreased and the quality of shear wave increased. The results showed that the number of required measurements can be reduced. A postural change to left decubitus decreased SCD. For patients with increased SAD and increased SWE result in the supine position, SWE result decreased with a postural change to left decubitus. The SWE results, SCD and SAD significantly differed between women and men. SWE results was higher in the presence of increased SAD (≥23 cm) among men, but not among women.

Conclusions: SWE of the liver is a reliable, non-invasive method for diagnosing liver disease. Results in this thesis suggest that for patients with SCD ≥2.5 cm, shear wave measures could be of poor quality and the SWE exam less reliable. In these cases, increased probe pressure may facilitate a reliable SWE exam. With such adjustments in probe pressure, the ultrasound-based SWE method can be superior for examination in patients with overweight or obesity. An effect of SAD ≥23 cm was seen for men with liver fibrosis only, which may explain the higher SWE result for men compared to women. Depending on the severity of liver disease and SAD, a postural change to left decubitus can produce a different outcome. As SAD increased, liver stiffness did, as well. Increased SAD thus is linked to increased liver stiffness, indicating that SAD should be taken into account when performing SWE of the liver.

Keywords: adrenergic antagonist, anthropometric measurement, diagnostic imaging, elasticity imaging technique, blood supply, BMI, body position, fatty liver, liver disease, hepatic steatosis, liver fibrosis, liver stiffness, obesity, postural change, pressure, probe, sex-characteristic, shear wave elastography, skin-to-liver capsule distance, transducer and ultrasonography.

Abbreviations

2D two-dimensional imaging

AIH autoimmune hepatitis

ALD alcohol-related disease

ALT alanine aminotransferase

A-mode amplitude mode

ANOVA analysis of variance

APRI aminotransferase to platelet ratio index

ARFI acoustic radiation force impulse

AST aspartate aminotransferase

AUROC area under the receiver operating characteristic curve

B-mode brightness mode

BMI body mass index

CAP™ ultrasound-controlled attenuation parameter

CCC concordance correlation coefficient

CI confidence interval

CT computed tomography

DAA direct-acting antiviral agent

EASL European Association for the Study of the Liver

EFSUMB European Federation Society of Ultrasound in Medicine and Biology

HBV hepatitis B virus

HCC hepatocellular carcinoma

HCV hepatitis C virus

HIV human immunodeficiency virus

HSC hepatic stellate cells

ICC intraclass correlation coefficients

ICMJE International Committee of Medical Journal Editors

IQR inter-quartile range

kPa kilopascal

LLO 30° left lateral oblique

LLR left lateral recumbent

LOA limits of agreement

M-mode motion mode

MRI magnetic resonance imaging

MRE magnetic resonance elastography

NAFLD non-alcoholic fatty liver disease

NASH non-alcoholic steatohepatitis

OR odds ratio

PBC primary biliary cholangitis

PSC primary sclerosing cholangitis

p-SWE point shear wave elastography

ROI region of interest

SAD sagittal abdominal diameter

SCD skin-to-liver capsule distance

SD standard deviation

SNR signal-to-noise ratio

SPSS Statistical Package for Social Sciences

SVR sustained virological response

SWE shear wave elastography

TE transient elastography, 1D

Definitions

Aminotransferase to Platelet Ratio Index (APRI)

All blood tests were performed and analyzed at Östersunds Hospital. Blood samples were drawn from patients in study IV within 3 months after the SWE examination. The aminotransferase to platelet ratio index (APRI) calculation was based on publicly available formulas. APRI scores ≥0.7 identify significant liver fibrosis with 77.0 % sensitivity and 72.0 % specificity (1).

Ascites and cirrhosis

In the all four studies in the thesis, the evaluation of ascites was performed with brightness-mode (B-mode) ultrasound at the same time as the SWE examination. In all studies, the diagnosis of cirrhosis was clinically determined (Table 1 A-B).

BMI, overweight, and obesity

In this thesis, overweight and obesity were considered although in this population, the key factor was a diagnosis of HCV. When evaluating the study population, however, the prevalence of overweight and obesity was fairly well represented (Table 1 A-B). Obesity was defined as BMI ≥30 and overweight as BMI ≥25 according to the World Health Organization (2).

Body positions

The same custom-made pillow was used for all patients in study III to obtain a similar 30° oblique left lateral (LLO) body position for every SWE measurement. For the left lateral recumbent (LLR) position, the patient was placed with the right arm elevated over the head and legs straightened. For all body positions,

measurement was performed in same liver segment, at the same depth and zero angle of the beam with the probe held perpendicular to the liver surface.

Hepatic steatosis

In study I, the presence of hepatic steatosis was assessed by standard B-mode ultrasound examination in every patient comparing brightness to right kidney and liver and evaluation of deep attenuation, visualization of the diaphragm, and vessel blurring (3,4) (Table 1 A-B).

In studies II–IV, steatosis was evaluated using the ultrasound-controlled attenuation parameter (CAP) in Fibroscan® program integrated with a GE Logiq S8 (GE Healthcare, Wauwatosa, WI, USA). The CAP was used to rule out (values <248 dB/m) or confirm (values ≥248 dB/m) with an area under the receiver operating characteristic curve (AUROC) of 0.82 for the presence of steatosis (5).

Medication

In study I, antiviral and/or cardiovascular medication [ATC codes: J05A (direct-acting antiviral agents, or DAAs), C01, C07, C08, C09] were registered. Of the 188 participants, 136/188 (72.3%) used no medication, 7/188 (3.7%) used antivirals, 69/188 (36.7%) used cardiovascular medications, and 2/188 (1.0%) used both antivirals and cardiovascular medication.

In study II, medication was not used in analysis.

In studies III and IV, 40/200 (19.7%) patients used cardiovascular medication (ATC codes: C07 AB03, C07 AA05, C07 AB07, C07 AG02, C07 AB02, C07 AB02, C07 AA07).

Metavir

In clinical medicine grading is frequently used to indicate the severity of a diagnosis. In the studies in this thesis, the severity of liver disease, the fibrosis stage, was scored using the Metavir systems, which is commonly used in Europe. The Metavir system is based on histopathology, however used for SWE liver method. F0 indicating no liver fibrosis, F1 mild fibrosis, F2 moderate fibrosis i.e. significant fibrosis, F3 severe fibrosis and F4 cirrhosis (6). The ultrasound companies provide the shear wave elastography cut-offs in terms of shear wave speed (m/s) and Young's Modulus (kPa) for classifying fibrosis stage.

Percutaneous liver biopsy

The indication for liver biopsy differs by diagnosis and staging. Usually, the biopsy specimen represents 1/50000 of the total mass of the liver (7). Liver

biopsies for this work were performed by several radiologists in the ultrasound department. Ultrasound guidance is a common approach for non-targeted liver biopsies, although image guidance is not mandatory. Any coagulopathy should be ruled out, and any anticoagulative medications discontinued before the procedure. Premedication with a sedative agent can be considered if the patient experiences anxiety. The patient should be in the supine position with the right arm over or behind the head. Using ultrasound allows for planning an appropriate approach, followed by injection of local anesthetics subcutaneously and at the liver capsule. A skin incision is made before the spring load needle is inserted. The biopsy is obtained under apnea. Complication risk is low, but to minimize bleeding risk, the patient should stay in bed, preferably lying on the right side. Recommendations on the duration of bedrest vary. In the radiology department in Östersunds Hospital, the routine is 4 hours of bedrest.

Probe pressure

In study II, maximum probe pressure was defined as the maximum pressure allowed by the patient or at the most 1 cm between the outer border of the rib cage and liver capsule. Normal probe pressure was defined as sufficient pressure to obtain contact with the skin enabling propagation of ultrasound waves through tissue.

Sagittal abdominal diameter (SAD)

All 200 sagittal abdominal diameter (SAD) measurements were performed by the first author and at the same time as the SWE examination. With a spirit-level and a wooden ruler, SAD was measured as the height from the bed surface to the highest point of the abdomen, to the umbilical level, with the patient in the supine position and during relaxed breathing. Increased SAD was defined as SAD ≥23 cm (8). SAD measurements were used for analysis in studies II–IV (Table 1 A-B).

Skin-to-liver capsule distance (SCD)

Measurement of the skin to the liver capsule was performed on the ultrasound monitor display, using the same ultrasound image as for SWE, and saved as an image file. SCD measurements were used for analysis in studies I–IV (Table 1 A-B).

Svensk sammanfattning

Bakgrund

Röntgenavdelningen Östersunds Sjukhus implementerade mars 2014 en för röntgenavdelningen ny ultraljudsmetod som heter shear wave elastografi (SWE) av lever, populärt benämnd ultraljud leverelastografi. Metoden mäter leverns stelhet, som ökat ger uttryck för leversjukdom. Tidigare hade lever biopsi använts för att gradera lever fibros i kroniskt leversjuka patienter. Vid tidig utvärdering av metoden visade sig att i 84.0% av fallen stämde leverelastografi svaren överens med den kliniska bilden, och i de övriga fallen visades ökad osäkerhet i mätresultaten. Aktuella vetenskapliga artiklar studerades och internationella kontakter med andra SWE operatörer gjordes mellan åren 2014 och 2017, för att inhämta ytterligare kunskap i metoden. Trots följsamhet till guidelines saknades djupare kunskaper hur undersökningarna skulle utföras för att få tillförlitliga mätresultat. De frågor som kvarstod utmynnade i det här doktorandprojektet vid Umeå universitet. Ultraljudsprojektet har utförts i samarbete med en docent onkologisjuksköterska-handledare vid Institutionen för Omvårdnad Umeå universitet och en docent/senior ultraljudsradiolog/- handledare, knuten till Karolinska Institutet. Forskningsvistelse i Italien har genomförts på specialklinik för leversjukdomar för att inhämta state of the art kunskap om metoden. Fyra artiklar är skrivna (varav 2 publicerade) som belyser dessa frågeställningar. Doktorandprojektet har pågått mellan januari 2017 och april 2020.

Ultraljuds leverelastografi metoden

Vid kroniska leversjukdomar kan fettlever, leverfibros, skrumplever och i värsta fall levercancer uppstå. Leverbiopsi har tidigare använts för att diagnosticera dessa aspekter av leversjukdomar. Metoden är invasiv och innebär ett stick i levern med risk för blödning och infektion. SWE metoden, ultraljuds leverelastografi, har istället kommit att användas för att detektera leverstelhet och därmed leverfibros, utan stick i levern.

Studiernas syften

I den här avhandlingen har det övergripande syftet varit att undersöka hur ultraljuds leverelastografi metoden kan göras ännu mer tillförlitlig och användbar. Syftet var därför att utforska vilka faktorer som påverkar mätresultatet, hur ändrad kroppsposition, bukhöjden och ökat tryck med ultraljudsproben påverkar mätresultatet, samt hur skillnader mellan män och kvinnor ser ut.

Studiernas resultat och sammanfattning

Resultaten från studierna i avhandlingen visade att förekomst av fettlever och det ökade avståndet mellan proben och leverytan, som ses vid övervikt och fetma, påverkar leverelastografi mätresultatet. Med metoden ultraljuds leverelastografi kan avståndet till levern minskas genom att använda ökat tryck med ultraljudsproben, vilket också ger bättre tillförlitlighet i mätresultatet. Avståndet till leverytan minskade med patienten i sidoläge. Patienter med högre bukhöjd och högre uppmätt leverstelhet i ryggläge, fick lägre uppmätt leverstelhet i sidoläge. Hög bukhöjd påverkar mätresultatet framförallt hos män, jämfört med kvinnor.

List of papers

This thesis is based on the following papers, referenced in the text by Roman numerals (I–IV).

I. Byenfeldt M, Elvin A, Fransson P. On patient-related factors and their impact on ultrasound-based shear wave elastography of the liver. Ultrasound in Medicine and Biology, 2018, 44, 1606-1615. DOI: 10.1016/j.ultrasmedbio.2018.03.031

II. Byenfeldt M, Elvin A, Fransson P. Influence of probe pressure on ultrasound-based shear wave elastography of the liver using comb-push 2-d technology. Ultrasound in Medicine and Biology, 2019, 45, 411-428. DOI: 10.1016/j.ultrasmedbio.2018.09.023

III. Byenfeldt M, Elvin A, Fransson P. Influence of postural changes on shear wave elastography of the liver. Submitted for journal publication, 2019.

IV. Byenfeldt M, Elvin A, Fransson P. Sex-based differences in shear wave elastography of the liver. Submitted for journal publication, 2020.

Introduction

Preface

Several methods are available for assessment of liver fibrosis in patients with chronic liver diseases (9). At the radiology department in Östersunds Hospital, liver biopsy has been used to stage and grade liver fibrosis. This invasive procedure carries risk for serious complications (7), small tissue sample (10), inter-observer variability (11), and patient discomfort (12). In March 2014, the implementation of non-invasive SWE of the liver was begun in the radiology department at Östersunds Hospital. With this non-invasive method (13), the patient arrives in a fasting state at the radiology department, where the scanning is performed without known risks for the patient.

During the implementation of SWE, the sonographer responsible for applying the SWE method at the radiology department, also the first author, conducted two early, unpublished comparison studies. These unpublished results showed a discrepancy between the clinical evaluation and SWE result in 84.0% of cases. No clear explanation for this discrepancy was apparent from known factors, raising questions. The SWE results also involved increased variance for median kPa, so that assessment of liver fibrosis was more difficult. There was uncertainty about which factors affected SWE measurements and how reliable measurements could be obtained.

A radiography study, performed as a literature review by the first author, within ultrasound liver elastography (14), made apparent the need for more knowledge about SWE, the factors influencing measurements, and how to improve reliability of SWE liver exams. The sonographer responsible for SWE liver in the radiology department, also the first author of all four studies presented here, conducted this research project within the radiography subject area.

Background

Imaging methods at radiology departments

In Sweden, the first x-ray examination was performed in 1896. As the new method was advanced technically, diagnostic progress was fundamental for developing and refining surgery and neurosurgery. Ultrasound as a diagnostic medical tool was introduced in Sweden during the 1950s and takes a central place in all basic diagnostic imaging today (15).

The history of ultrasound

The use of ultrasound for diagnostic purposes has a great advantage over other radiology methods because it uses non-ionizing methods. Ultrasound enables repeatable, rapid, non-invasive, portable, inexpensive, and dynamic examinations (16), which often makes it the first choice in radiology and emergency departments.

Sound

The history of ultrasound goes back to Lazzaro Spallanzani (1729–1799), an Italian physiologist and priest. In 1794, he showed that blinded bats managed to navigate perfectly. However, with one ear blocked, they could no longer fly safely. Spallanzani hypothesized that bats navigated by sound and not by vision (17).

Echo

In a later experiment in 1938, two Harvard students first coined the term “echolocation” when explaining that bats use high-frequency sound waves for sending and then receiving after the waves bounce off surfaces (18).

Speed of sound

In 1826, using a clock bell under water and gunpowder, Swiss physicist Daniel Colladon and his assistant showed that the speed of sound is faster in water than in air. The speed in water was calculated as 1435 m/s, quite close to modern calculations of 1482 m/s (19).

Piezoelectric effect

In 1880, brothers Jacques and Pierre Curie discovered piezoelectricity. They demonstrated that crystals of tourmaline, quartz, topaz, cane sugar, or Rochelle salt generate electricity under pressure and when a voltage is applied to these crystalline materials (20). The reverse also was demonstrated in 1881 when French physicist Gabriel Lippmann exposed the crystal to an electrical pulse and triggered a sound wave (21). This inverse piezoelectric effect has ever since been used to produce ultrasound waves.

First medical ultrasound machine
In 1954, cardiologist Inge Edler and physicist Carl Hellmuth Hertz build the first medical ultrasound device in Sweden, a supersonic reflectoscope used to diagnose diseases of the heart (22). Professor Dugald Cameron designed and built an ultrasound machine, the Diasonograph, for obstetrics scanning in the early 1960s in collaboration with physician Ian Donald. The Diasonograph machine was built after the clinicians witnessed ultrasound being used in Glasgow's shipyards to look for cracks in the metal. Donald's team used B-mode sonograms of the pregnant uterus in 100 patients, publishing findings in *The Lancet* in 1958 (23).

The ultrasound method

Longitudinal and shear waves
Acoustic waves come in a variety of forms. Three types are longitudinal, shear, and torsion waves (24).

Longitudinal wave
Probably the most common forms of acoustic wave are the longitudinal compressional waves, in which the particles are displaced parallel to the direction of motion of the wave (24).

$$\mathbf{c=\lambda \times f_o}$$

c=the speed of sound wave, m/s
λ=wavelength, m
f_o=frequency, number of wave in one second, Hz

The wave travels as a transference of pressure and density variations from one tissue element to another, in compressions and rarefactions. The difference in pressure, the amplitude of the curve, is expressed in decibels, dB (16). The particles themselves hardly do not move or oscillate, and it is the wave that travels from source to detector (24). The longitudinal wave is the type that builds up B-mode imaging (16).

Shear wave
In the tissues, shear displacement occurs when the wave moves as a transference of variations in shear force and deformation from one tissue element to another, across and 90 ° of the direction of wave propagation. Shear waves travel about 1000 times more slowly than longitudinal waves and attenuate more rapidly in soft tissues, and in fluids, shear waves do not propagate at all (25).

The image

The modes

The pulse-echo method is used in A-, B-, and M-mode ultrasound imaging. A-mode (amplitude-mode) is one-dimensional (1D) and detects depth and proportions of organs. B-mode (brightness-mode), or gray-scale, displays two-dimensional (2D) images, adding directionality to the A-mode (amplitude mode) data. Each sound wave that reflects back to the probe represents a point in the grey-scale (16). M-mode (motion-mode) displays a 1 D -image of echo over time. The brightness in the point is proportional to the strength of the returning sound wave. The location of the point depends on the probe position and the time it takes for the echo to return to the probe (16).

Echoes

The frequencies normally applied in clinical imaging lie between 1 and 24 MHz. The probes used in medicine are both transmitters and receivers of sound waves. The ultrasound image is built up by reflected echoes occurring when media in two different tissues have different acoustic impedance (Z). The vibrations from the probes can transfer into the body when a coupling medium, such as a gel, is applied between the probe and skin. The intensity of the reflected echo when passing boundaries of different media is determined by the equation $Z=\rho*c$, where the media density is ρ (kg/m^3) and sound speed is c (m/s). Not all sound waves are reflected; some transmit to deeper tissue structures (16).

Loss of energy

As the sound wave travels, there is loss in energy, and the deeper the sound waves travel into the body, the weaker the wave becomes when the amplitude decreases with increasing depth. The energy loss is because of attenuation and absorption. Attenuation results from reflection (echoes), refraction (the beam divergence and the wavelength change, giving an oblique echo to the probe), and scattering (sound waves hit a boundary with an uneven surface, giving echoes in every direction and also to the probe, or backscattering). Scattered waves will interfere, either by constructive interference, where waves add in intensity, or by destructive interference, where waves terminate each other. This interference gives rise to a random speckle pattern of bright and dark spots in ultrasound images. Absorption is the greatest reason for energy loss, the ultrasound energy is converted to heat (16).

Artefacts

Speckle
A detailed echo pattern can result from interference effects of the scattered sound from all scatters within the tissue. The small scatters, smaller than a wavelength, are created from echoes and can combine constructively or destructively. This combination produces the familiar pattern of bright and dark spots in a gray-scale image, a phenomenon called “acoustic speckle” (16).

Reverberation
Multiple reflections can occur between two strong reflectors or between a probe and a strong reflector. These echoes can be presented in the displayed image although they not represent real structures (16).

Mirror artefact
Mirror artefact is a form of reverberation, showing structures that exist on one side of a strong reflector as being present on the other side as well (16).

Acoustic enhancement and shadowing
Enhancement is strengthening of echoes from reflectors that lie behind a weakly attenuating border. Shadowing and enhancement result in reflectors being displayed on the image with amplitudes that are too low or too high, respectively. A strongly attenuating or reflecting structure weakens the sound distal to it, causing echoes from the distal region to be weak and thus appear as darker, like a shadow (16).

Resolution
Ultrasound has superior spatial resolution compared to magnetic resonance imaging (MRI). However, in deep-lying objects, MRI has an advantage, as in diagnosing diseases in the peripheral nervous system (26).

The spatial resolution of an ultrasound image is the ability to discriminate between two adjacent objects. It is commonly divided into three components. *Axial resolution* is the component discriminating along the ultrasound beam. The axial resolution depends on the length of the transmitted pulse, which in turn depends on the sound wavelength and the number of cycles in the pulse. For discrimination of two echoes from adjacent interfaces, the echoes need to be separated by at least half the pulse length or they will fuse into one. The *lateral resolution* depends on the width of the ultrasound beam, and the *elevational resolution* depends on resolution along the thickness of the ultrasound beam. Echoes from two objects separated by less than a beam width or thickness will be observed as one (16). In the axial plane with 15 MHz probe, the resolution is 0.1 mm and for a 33 MHz probe the resolution is 0.05 mm (27).

The axial resolution is higher than the lateral resolution because it is easier to generate short rather than narrow ultrasound pulses. However, tissue absorption of the sound wave increases with shorter wavelengths (higher frequencies), so there is an upper limit to the frequencies depending on the desired scan depth (16).

The *contrast resolution* is the ability to discriminate objects with small differences in acoustic characteristics, shown in different shades of grey, known as the signal-to noise ratio (SNR). *Time resolution* is the ability to detect moving targets and is set by the frame rate (16).

The use of ultrasound method

Medical ultrasound is based on the use of high-frequency sound to aid in the diagnosis and treatment of patients. Diagnostic medical ultrasound uses ranges from 2 MHz to 20 kHz. Sound is mechanical energy that requires a medium to propagate. Thus, in contrast to electromagnetic waves, which are used in MRI and computed tomography (CT), sound waves cannot travel in a vacuum (16). For medical purposes, in ultrasound, the sound wave speed in body tissue is set to 1540 m/s because the soft tissue in the body is considered to be similar to fluid (16).

Ultrasound use today is not confined to hospitals but can be applied in extreme and low-resource environments using portable machines and is effective for assessing patients during triage (28). Portable ultrasound scanners in hospitals are currently used, e.g., at bedside, which allows for clinical imaging without replacing a comprehensive ultrasound. This practice is called point-of-care ultrasound (PoC-US) (29).

The Liver

Anatomy

The liver is divided into eight functionally independent segments, numbered in a clockwise manner, according to Couinaud (Figure 1). Each segment has its own vascular inflow, outflow, and biliary drainage. In the center of each segment is a branch of the portal vein, hepatic artery, and bile duct. In the periphery of each segment is a vascular outflow through the hepatic veins. The right hepatic vein divides the right lobe into anterior and posterior segments. The middle hepatic vein divides the liver into right and left lobes. This plane runs from the inferior vena cava to the fossa of the gallbladder. The Falciform ligament divides the left lobe into a medial part (segment 5) and a lateral part (segments 2 and 3). The portal vein divides the liver into upper and lower segments. The left and right

portal veins branch superiorly and inferiorly to project into the center of each segment (30,31).

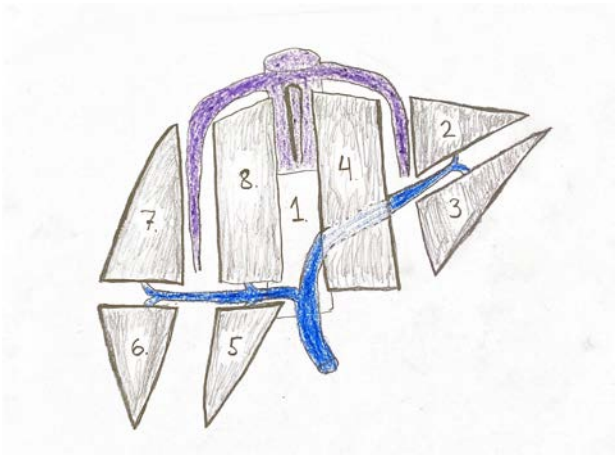

Figure 1. The anatomical architecture of the liver; the eight liver segments according to Couinaud. Blue color represents portal veins and purple color represents hepatic veins. *Illustration by Associate Professor, MD radiologist Anders Elvin.*

The liver is one of the most important metabolic organs and has multiple functions. It receives a dual blood supply from the portal vein and the hepatic artery, and from many additional vessels. The veins lead from digestive organs (splanchnic circulation) or the systemic circulation (veins of Sappey). The splanchnic circulation describes the non-portal venous supply to the liver, or the gastrointestinal circulation, and includes the celiac trunk, superior mesenteric artery, and inferior mesenteric artery. Organs served by this circulation are the stomach, liver, spleen, pancreas, small intestine, and large intestine. The blood flow exits the liver from hepatic veins (32).

Hepatocytes make up 70.0%–85.0% of the liver's cells. They have important roles in metabolic, secretory, and endocrine functions. Kupffer cells are specialized macrophages, and hepatic stellate cells (HSCs) are pericytes located in the space of Disse. HSCs can be activated in response to liver damage, leading to collagen formation, such as fibrosis or cirrhosis. Bile is secreted by hepatocytes and drained into biliary ducts, exiting the liver in the bile duct. The capsule of the liver, known as Glisson's capsule, covers the hepatic parenchyma and is a fibrous connective membrane mainly composed of collagen and elastin fibers (33).

Liver disease

Liver cancer

The most common type of primary liver cancer is hepatocellular carcinoma (HCC) (33). Acute inflammation of the liver can develop into chronic inflammation, which in turn can lead to fibrosis, cirrhosis, and in worst cases, HCC (34). A newly published meta-analysis has confirmed liver fibrosis as a strong predictor for all-cause mortality and morbidity in non-alcoholic fatty liver disease (NAFLD), with a 5- to 12-fold increased relative risk of liver-related events for each increased fibrosis stage and death (35). The global burden of chronic liver diseases has trended to an increase from 2012 to 2017. Hepatitis remains the most common cause of liver-related mortality. However, NAFLD is the most rapidly growing disease globally causing liver mortality and morbidity. Data from one 2017 global burden disease study identified 2.14 million liver-related deaths, an increase of more than 11.0% since 2012. Between 2012 and 2017, the age-standardized incidence rate for HCC increased from 11.1 to 11.8 per 100000. For cirrhosis, the incidence rate increased from 66.0 to 66.3 per 100000. Age-standardized death rates have increased annually for NAFLD, by 1.4%, but with no increase for hepatitis B virus (HBV) or hepatitis C virus (HCV) (36). In Sweden, the prevalence of NAFLD is estimated at 15.0% (37) and increasing. Moreover, the incidence of HCC in Sweden increased from 2009 to 2018, from 6.4/100000 to 9.3/100000 for men and 2.5/100000 to 2.8/100000 for women. For cirrhosis and other liver diseases in Sweden, surveillance is performed every 6 months with B-mode ultrasound, but too few cases of HCC (28.0%) are detected by surveillance. Thus, this gap represents an area for improvement (98). In 2015, the European Association for the Study of the Liver (EASL) recommended screening for NAFLD in individuals with obesity and overweight, and if NAFLD is present, further surveillance with elastography every 3 years (38).

Hepatitis

HCV infection is one of the main causes of chronic liver disease worldwide. The long-term impact of HCV infection is highly variable, ranging from minimal histological changes to extensive fibrosis and cirrhosis with or without HCC. The number of chronically infected persons worldwide is estimated to be about 160 million (39). Regimens incorporating DAAs are the standard of care in HCV treatment (40). The epidemiology of HBV has changed because of vaccination programs and migration. All patients with chronic HBV infection are at increased risk for progression to cirrhosis and HCC, depending on host and viral factors. Approximately 240 million people are infected globally. Patients with compensated or decompensated cirrhosis need treatment (41).

Autoimmune hepatitis (AIH) was the first liver disease for which a controlled clinical trial demonstrated an effective treatment. AIH remains a diagnostic and

therapeutic challenge because it is relatively rare and has a highly heterogeneous course. AIH prevalence ranges from 16 to 18 cases per 100000 inhabitants in Europe. AIH affects mainly women, and if untreated leads to cirrhosis, liver failure, and death. HCC is also a known consequence of AIH-related cirrhosis (42).

Alcohol-related liver disease

The enlargement of and presence of fibrosis in alcoholic livers was described in 1896 (43). Liver disease caused by alcohol is currently called "alcohol-related disease" (ALD). Alcohol can lead to an increase in fat deposits in the liver. Hepatic inflammation due to ALD is referred to as alcoholic steatohepatitis (ASH), and the annual incidence of progress of cirrhotic livers to HCC is 2.6%. Any level of alcohol consumption represents a health risk (44,45). Liver-related death is a major consequence of excessive alcohol consumption, but risk also is present for moderate drinkers without an alcohol abuse syndrome (46). Of all cirrhotic livers globally with a mortality outcome, alcohol is the cause in 48.0% (47).

Hepatic steatosis

Hepatic steatosis is a histologic finding in many liver biopsies with elevated liver biomarkers. Steatosis is caused by an increased level of triglycerides inside the hepatocytes, with at least 5.0% of hepatocytes changed to fat drops. The histologic grading for steatosis is as follows: no significant evidence of fatty liver disease, steatosis (with inflammation/with nonspecific fibrosis), steatohepatitis (adult), steatohepatitis (young children), and cryptogenic fibrosis/cirrhosis (no steatosis where other explanation to fibrosis should be considered) (48).

Hepatic steatosis can arise due to ALD or NAFLD. NAFLD is the most common liver disease globally and is histologically divided into two major subgroups, non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). Approximately 10.0%–20.0% of patients with NAFLD have NASH, whereas the majority have NAFL. Some patients with NAFLD develop progressive fibrosis, which eventually may progress to cirrhosis. Fibrosis stage correlates well with clinical outcomes and is the strongest predictor for overall and liver-related mortality. NAFLD patients without steatohepatitis may develop progressive fibrosis, and those with progressive fibrosis appear to have a higher mortality risk irrespective of baseline NASH status (48).

Steatohepatitis

Steatohepatitis designates a unique diagnostic pattern observed in non-alcoholic adults with obesity. A ballooning injury is the distinctive pattern, and refers to the enlargement of cells where steatotic vacuoles may be seen. The term "steatohepatitis" implies steatos and inflammation, but these manifestations can vary greatly. Some findings suggest that SWE can distinguish between steatosis

and steatohepatitis (49), but this has not been adequately confirmed. Moreover, the progression of fibrosis in steatohepatitis can possibly reverse into regression (48). Fibrosis stage is the strongest predictor for mortality in patients with NAFLD (50), which makes the staging of liver fibrosis the most important factor overall.

Diagnostic methods for detecting liver disease

The invasive method liver biopsy is the gold standard for assessing liver diseases, but has limitations related to risks for bleeding and infections (7) and for pain (51).

Non-invasive diagnostic methods available to detect hepatic steatosis currently are B-mode ultrasound, Fibroscan® CAP™, MRI, and CT (5,52-55). However, a 20-year-old study found that neither MRI, CT, nor ultrasound can differentiate NASH from NAFLD (56).

Serum biomarkers can be used to evaluate the presence of significant liver fibrosis. Aspartate aminotransferase (AST) levels and platelet count are calculated in APRI, with a median AUROC of 0.77 for assessing significant fibrosis (≥F2). Age, AST, alanine aminotransferase (ALT), and platelet count are calculated in FIB-4, with a median AUROC of 0.74 for assessing significant fibrosis (≥F2) (57).

Imaging methods for detecting liver fibrosis are ultrasound devices or magnetic resonance elastography (MRE). The non-imaging devices can be used for 1D SWE transient elastography using a Fibroscan® device from Echosence, Paris (13). The imaging method of ultrasound-based SWE has been used for a decade to assess the degree of fibrosis in the liver and has been recommended since 2015 over invasive liver biopsy (38). With these methods, liver fibrosis can be staged for severity of liver disease. In Europe, the Metavir system is commonly used, which stages fibrosis in five grades, with F0 representing no liver fibrosis and F4 representing cirrhosis (6).

Two forces are used to create shear waves: mechanically induced force and acoustic radiation force impulse (ARFI), a strong push pulse used in ultrasound devices. Mechanically induced shear wave is seen with 1D SWE transient elastography (TE) with the device Fibroscan® from Echosence, Paris, and in MRE (13).

Ultrasound-based shear wave elastography

Before the technological age, palpation was used to determine viscera conditions such as liver stiffness and volume. Non-invasive ultrasound-based

SWE of the liver that is used in the studies described in this thesis could be viewed as a "virtual palpation" and is often considered an advanced ultrasound method. With this method, the ultrasound probe first generates an acoustic radiation force and multiple strong push pulses, inducing tissue displacement that creates shear waves (motion) in the tissue. This motion travels 1000 times more slowly than longitudinal ultrasound waves, enabling tracing of these shear waves with the same ultrasound probe, using tracking algorithms. The purpose is to measure liver stiffness via propagation of shear waves in the liver tissue. Increased shear wave speed reflects a higher severity of liver disease. A wide variety of technology solutions is used (13) to generate ultrasound-based shear waves in a target such as liver tissue.

As the shear waves propagate through the liver tissue and displace it, this displacement is detected by the same ultrasound probe that images the tissue structures (58). It is assumed that in homogeneous tissue, the speed of propagation (c) is determined by the density (ρ) and the shear elastic modulus (G). In soft tissue, G is much smaller than the bulk modulus of elasticity (K), which reflects that shear waves propagate more slowly than longitudinal waves and attenuate rapidly in soft tissues. In non-viscous pure fluid, shear waves do not propagate. The high speed of ultrasound enables observation of tissue displacements and their definition as shear deformation. Calculating the speed of the shear wave is complicated in biological tissues because these equations assume a linearly elastic, homogeneous, isotropic, infinite, and continuous medium (25).

Point shear wave and 2D shear wave

By using B-mode, the shear wave sample box within the region of interest (ROI) can be placed at certain locations in liver tissue, where shear waves are created with ARFI, at places and depths that the SWE operator determines (13). The speed of the shear wave propagation is measured within a ROI, using a system similar to Doppler imaging (13,59). For point shear wave, or p-SWE, no elastogram is displayed, but it can be provided in 2D SWE. Elastogram is a color overlay on the B-mode image, and the color scale shows Young's modulus in kPa. Its high frame rate allows tracking of shear waves in 2D, and RF-echo tracking over several points also enables displacement to be followed. 2D SWE provides the SWE operator a guideline for optimal placement of the ROI measurement because disturbances and areas of decreased SNR are shown as blackout pixels (25). The ultrasound scanner measures shear wave speed in m/s, which can be converted to kPa, the unit of elastic modulus. Then Young's modulus can be used with the equation $E=3\rho c_s^2$ (ρ=density and c_s=speed of shear wave propagation), with the assumptions of density always at 1000 m^3, linear liver tissue elastic response, purely elastic, mechanically isotropic, and with no boundary structures affecting the behavior of the shear wave. These

assumptions may not be correct, which makes elastic modulus an indirect measurement (13).

The performance of SWE liver

According to current guidelines (13), the performance of SWE in liver is conducted with patients fasting and placed in a supine position with the right arm elevated over the head. The ultrasound probe is placed intercostally and perpendicular to skin and liver surface, with an angle of the beam close to zero. SWE measurement is to be performed at least 1 cm below the Glisson capsule, preferably at a depth of 3-4 cm, during a relaxed breath hold and in liver segement 5, 7 or 8. For every breath hold, 3-4 frames are collected, and in each frame, one SWE measurement is saved. The data are not normally distributed (60), so SWE results should be reported as the median value of 10 SWE measurements with the quality of parameter interquartile range (IQR)/median <30.0%. The SWE result can be presented in m/s or with Young's modulus in kPa (13).

Factors affecting ultrasound image and SWE measurements

All methods for diagnostic imaging could result in inadequate examinations. BMI and male sex are the strongest predictive factors for imaging failures. In patients with obesity and ≥8 cm of subcutaneous fat, the sound wave attenuates by 94.0% before reaching the peritoneal cavity. Problems positioning the patient comfortably and in the same time in the best position for examination are also seen for patients with overweight or obesity (61-63). In abdominal ultrasound exams, the quality of the ultrasound image is affected by the acoustic characteristics of the specific body tissue through which the sound wave passes. The velocity of the sound wave depends on the density of body tissue (16). In screening for HCC with native B-mode ultrasound, 20% of all examinations are of inferior quality because of imaging difficulties (64).

Despite following current guidelines for the SWE liver method (13), an increased uncertainty in SWE results (65,66) has been identified. Reproducibility has been evaluated for intra- and inter-reliability, and the overall intra-operator agreement was better than the inter-operator agreement, with intraclass correlation coefficients (ICCs) respectively of 0.90 and 0.81 (67). With liver biopsy as the reference method, SWE performs with good diagnostic accuracy for the non-invasive staging of liver fibrosis (68). Although SWE has been used for a decade, further investigation is needed of factors that affect this method, given the remaining uncertainty. A better understanding of how to handle the effect of factors such as age, sex, SAD, smoking habits, cardiovascular medication, hepatic steatosis, cirrhosis, BMI, SCD, body position, and increased probe pressure on the SWE reliability is important.

SWE results tend to be higher for men compared to women, without a clear explanation (69,70). Older age has been associated with failed and unreliable SWE results (71). Variations in attenuation and different artefacts, such as absorption and reflection of the pushing ultrasound beam and shear wave scattering, reflection, or refraction, can affect both the push pulse and the estimation of shear wave speed (25,72,73). For patients with increased BMI, an increased distance to the liver is usually seen on the ultrasound image. Increased BMI and metabolic syndrome both increase liver stiffness values (74), and obesity is associated with unreliable and failed SWE exams (71). For patients with increased distance to the liver, it is difficult to place the ROI accurately, and the potential for a partial volume effect needs to be considered for ROI positioning. It is also important to place the ROI in the accurate liver segment (75), which can be difficult to achieve depending on body habitus. Shear wave propagation near boundaries and within thin layers can affect the shear wave speed calculation (73), as can the depth of ROI, with uncertainty in SWE results (76). A different body position might facilitate increased quality of the B-mode window for patients with higher BMI and decrease the distance to the liver. The left decubitus position earlier was found to yield better SWE results in healthy individuals (70,77), but no studies have been performed on patients with liver fibrosis.

The probe position has been assessed for effects from an intercostal or subcostal probe position (75). The intercostal approach is assumed to prevent shear wave speed artefacts because the ribs do not allow pre-stress and do not transmit to the liver. An increase in probe pressure would decrease distance to the liver capsule, but discussion has focused on whether pre-stress affects the shear wave calculation in terms of superficial tissues (25). However, at the time, no studies have reported SWE of the liver using increased intercostal probe pressure, a factor that needs to be investigated.

The shear modulus in tissues increases with vascular and interstitial pressure, which makes SWE more sensitive than other methods (72). This phenomenon is reported in studies of elevated AST and ALT, which can increase SWE result (78). When hepatocytes transform into fat droplets, the liver morphology and vascularity change, resulting in overestimations in SWE results (79,80).

The blood flow, use of cardiovascular medication and SAD impact on SWE results has not been clearly investigated. Reproducible differences in liver perfusion parameters during the development of fibrosis in the liver have been found (81). Previous studies also have yielded different SWE results depending on body position – standing or supine – and post-prandial state (77). In addition, venous pressure (82), extrahepatic cholestasis, and heart failure are known to increase SWE results (77,83). However, factors that have not been investigated for SWE of liver include SAD, which increases intraabdominal pressure (84).

An increase in the understanding of how factors such as age, sex, SAD, smoking habits, cardiovascular medication, hepatic steatosis, cirrhosis, BMI, SCD, body position, and increased probe pressure affect SWE could increase the reliability of ultrasound-based SWE of the liver method.

Rationale for the thesis

There is a globally growing burden of liver diseases (85) with increasing liver-related mortality (36). Liver fibrosis, cirrhosis, and HCC can develop from chronic liver diseases with different etiologies, including NAFLD (39,41,86). Moreover, symptoms of liver diseases are often discovered at a late stage. Therefore, detection, staging, and monitoring of liver disease at an early stage are important. Liver biopsy has historically been used to diagnose liver disease but is an invasive method associated with a risk for complications (7). A preferable method is a non-invasive diagnostic tool to detect liver disease at an early stage. Ultrasound-based SWE of the liver is a reliable method for staging liver fibrosis (87), but some factors that still interfere with performance of SWE despite following current guidelines (13) needs to be investigated. Increased variability in SWE result also means decreased reliability (65) in staging liver disease severity and treatment decisions, suggesting a great interest in finding factors that affect SWE outcomes and ways to increase test reliability.

In the presence of overweight and obesity, the need for a better acoustic window and increased quality of shear wave often becomes obvious. Therefore, the use of increased probe pressure and/or postural change can be introduced to increase reliability and technical success. However, increased probe pressure has been assumed to increased SWE result, and increased SWE result also have been obtained with a left decubitus position in healthy individuals (70,88). Data are insufficient regarding cardiovascular medication and anthropometric measurements impact on the reliability of SWE liver. Moreover, several studies have shown increased SWE result for men compared to women (75,89,90), for unknown reasons. To increase the reliability of ultrasound-based SWE of the liver method, relevant factors need to be explored and, if possible, managed.

Aims

The overall aim of the thesis was to investigate patient-related factors affecting SWE results, and to conduct a clinical trial to increase the reliability of the ultrasound-based SWE of the liver method.

The specific aims of each study were as follows:

I. To investigate patient-related factors associated with increased variance in median kPa SWE result. A secondary aim was to see how body constitution, expressed as either BMI or SCD, best associates with increased variance for median kPa SWE result.

II. To investigate the influence of increased intercostal probe pressure on liver stiffness assessment with ultrasound-based SWE and comb-push 2D technology. A secondary aim was to determine the number of measurements required to achieve technically successful and reliable SWE examinations.

III. To investigate the influence of postural changes, SAD and SCD on SWE results.

IV. To investigate sex-based differences in factors possibly affecting liver stiffness measurements; cardiovascular medication and anthropometric measurements

Methods

This thesis included a total of 388 patients (Table 1 A-B). The same group of patients were included in studies III and IV, and 112 of them also were included in study II. In this thesis, all four studies were prospective and quantitative investigations. Studies I and IV were cross-sectional. The current guidelines (13) was not completely followed in studies II and III, which were clinical trials (Table 2).

Study population

All enrolled participants in these studies were consecutive patients with various liver diseases presenting at the radiology department Östersunds Hospital. The patients were examined using the ultrasound-based SWE of the liver method at the ultrasound unit between April 2014 and May 2018. The patients were generally referred from the departments of infectious diseases and of internal medicine at Östersunds Hospital. Some patients were referred from regional general practitioners. The demographics of the populations of all four studies are presented in Table 1 A-B.

Inclusion and exclusion criteria

The inclusion criteria were age ≥18 years and consecutive patients referred to radiology department for ultrasound-based SWE liver who gave written consent to participate. The exclusion criteria were not having been examined according to currently described methods of SWE of the liver, an inability to communicate, and/or not giving written approval to participate in the study.

Ethical considerations

All studies in this thesis were conducted according to recommendations from the International Committee of Medical Journal Editors (ICMJE) and the World Medical Association Declaration of Helsinki, 2013. The studies were approved by the research ethics review board in Umeå, Sweden [Dnr 2015/355-31 (study I), 2017-417-32M (studies II and IV), 2017-78-31M (studies III and IV), 2017-302-32M (study III)]. All participating patients gave written informed consent. The studies were approved for establishment of a serum biomarkers collection by biobanking Region Jämtland Härjedalen, Sweden, RS/2731/2017.

Table 1 A. Overview of study population demographics

	Study I	Study II	Study III	Study IV
Number of patients	188	112	200	200
Age Years mean (SD; min-max)	47 (14.8;18-83)	46 (16.1;19-80)	47 (15.5;19-80)	47 (15.5;19-80)
BMI in kg/m², mean (SD; min-max)	27.5 (5.2;18.4-45.5)*	26.8 (5.6;16.3-45.3)	27.3 (5.3;16.0-45.0)	27.3 (5.3;16.0-45.0)
BMI overweight ≥ 25 kg/m²; n(%)	36 (19.0)**	66 (58.9)	128 (64.0)	128 (64.0)
BMI obesity ≥ 30 kg/m²; n(%)	16 (27.1)**	28 (25.0)	50 (25.0)	50 (25.0)
Metavir F0-F4 n (%)				
F0-F1	93 (49.5)	96 (85.7)	172 (86.0)	172 (86.0)
F2	74 (39.4)	9 (8.0)	13 (6.5)	13 (6.5)
F3	17 (9.0)	3 (2.7)	4 (2.0)	4 (2.0)
F4	4 (2.1)	4 (3.6)	11 (5.5)	11 (5.5)
SAD in supine (cm) median (range; min-max)	Not performed	21.0 (24.5;14-39)	21.5 (25;15-39)	21.5 (25;15-39)
SAD ≥23 cm n (%)	Not performed	45 (40.2)	88 (44.0)	88 (44.0)
SCD in supine (cm) Mean (SD; min-max)	2.05 (0.66;1.1-4.9)	1.98 (0.57;1.0-3.9)	1.97 (0.6;1.0-3.9)	1.97 (0.6;1.0-3.9)
SCD ≥ 2.5 cm n (%)	43 (22.9)	17 (15.2)	30 (15.0)	30 (15.0)
Sex Women n (%) Men n (%)	66 (35.1) 122 (64.8)	51 (45.5) 61 (54.5)	90 (45.0) 110 (55.0)	90 (45.0) 110 (55.0)
Smokers, daily n (%)	37 (19.7)**	32 (28.6)	53 (26.1)	53 (26.1)

BMI = body mass index; SAD = sagittal abdominal diameter; SCD = skin-to liver capsule distance; *only available in 59 patients; **only available in 97 patients.

Table 1 B. Overview of study population demographics				
	Study I	**Study II**	**Study III**	**Study IV**
Number of patients	188	112	200	200
Diagnosis n (%)				
AIH	0 (0)	1 (0.9)	2 (1.0)	2 (1.0)
ASH	7 (3.7)	4 (3.6)	6 (3.0)	6 (3.0)
Chronical cholestasis	0(0)	2 (1.8)	2 (1.0)	2 (1.0)
Cirrhosis	12 (6.4)	8 (7.1)	9 (4.5)	9 (4.5)
Cryptogen	11 (5.8)	0 (0)	8 (3.9)	8 (3.9)
Diabetes	Not performed	8 (7.1)	13 (6.5)	13 (6.5)
HBV	51 (27.1)	30 (26.8)	53 (26.1)	53 (26.1)
HCV	111 (59.0)	62 (55.4)	108 (53.2)	108 (53.2)
HIV	1 (0.7)	0 (0)	0 (0)	0 (0)
NAFLD	0 (0)	8 (7.1)	12 (5.9)	12 (5.9)
NASH	5 (2.6)	1 (0.9)	2 (1.0)	2 (1.0)
PBC	2 (1.5)	1 (0.9)	2 (1.0)	2 (1.0)
PSC	0 (0)	1 (0.9)	3 (1.5)	3 (1.5)
Psoriasis*	0 (0)	2 (1.8)	2 (1.0)	2 (1.0)

*Treated with methotrexate; PSC=primary sclerosing cholangitis; PBC=primary biliary cholangitis; HCV=hepatitis C virus; HBV=hepatitis B virus; NASH=non-alcoholic steatohepatitis; AIH= autoimmune hepatitis; NAFLD=non-alcoholic fatty liver disease; ASH=alcoholic steatohepatitis; HIV=Human Immunodefiency Virus

Table 2. Summary of studies included in this thesis

Study	Studies	Design	Participants
I	Patient-related factors	Cross-sectional	N=188
II	Probe pressure	Clinical trial	N=112
III	Body positions	Clinical trial	N=200
IV	Sex-based differences	Cross-sectional	N=200

Data collection

A total of 12120 SWE measurements were analyzed for these studies. ARFI was used to created shear wave in the liver tissue, with two different ultrasound systems and two different SWE technologies (Table 3).

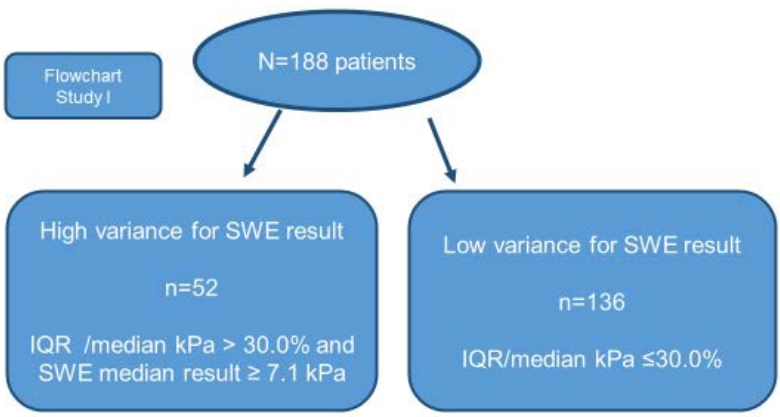

Figure 3. Flowchart for groups in study I. High variance for SWE result indicates increased uncertainty in SWE result. Reproduced with permission from the publisher.

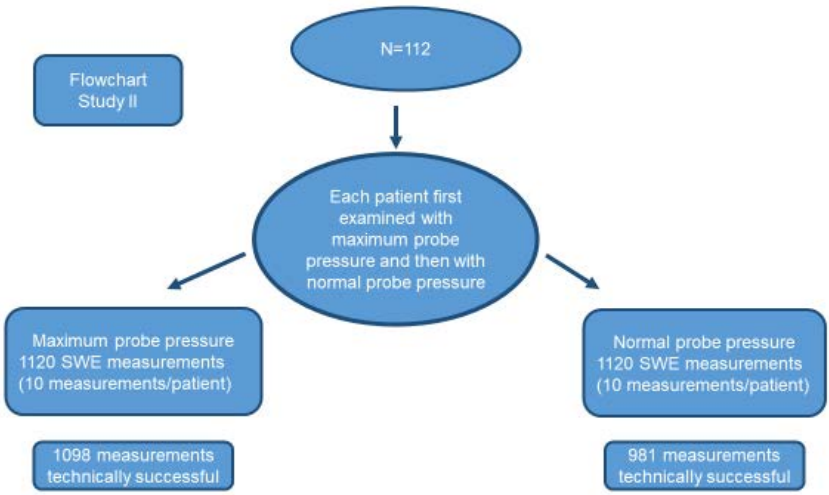

Figure 4. Flowchart for groups and measurements in study II. Reproduced with permission from the publisher.

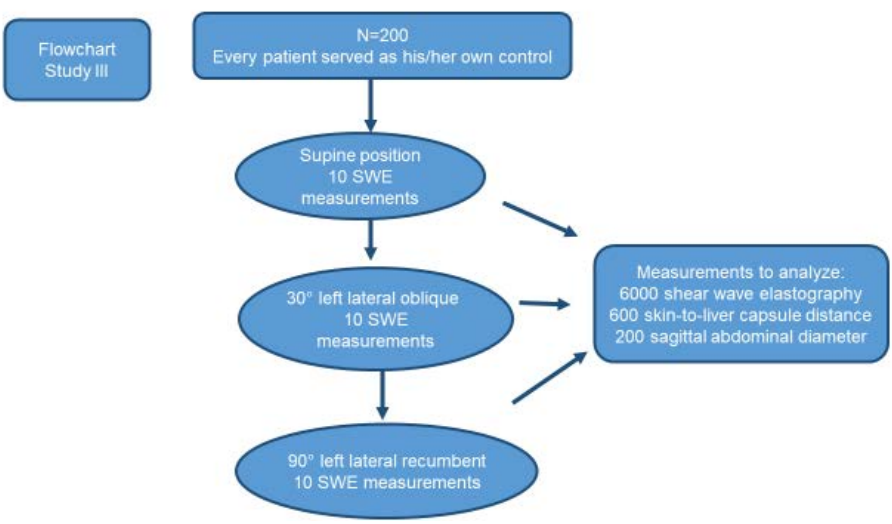

Figure 5. Flowchart for groups and measurements in study III.

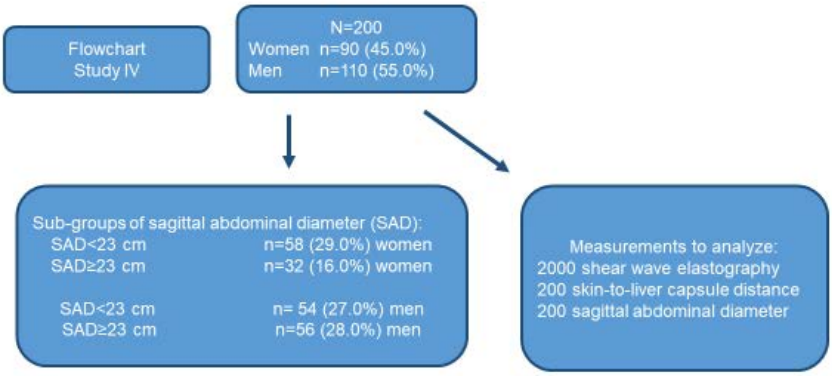

Figure 6. Flowchart for groups and measurements for study IV.

Ultrasound systems and SWE technology

When ARFI is activated, shear waves propagates in the tissue. Some vendors provide elastogram maps that indicate the reliability of shear wave quality and propagation. SWE technologies with elastograms were considered technically successful with at least 50% colorfill in the elastogram map.

Table 3. Summary of shear wave elastography technologies and measurements in each study

	Study I	Study II	Study III	Study IV
Ultrasound device	Philips iU22	GE LOGIQ E9	GE LOGIQ E9	GE LOGIQ E9
SWE technology	point-SWE Elast PQ	2D-comb push	2D-comb push	2D-comb push
Probe	Convex C5	Convex C1–6	Convex C1–6	Convex C1–6
Probe pressure	Normal	Maximum and Normal	Normal	Normal
Size of ROI (cm)	0.5×1.5	Ø 1.25	Ø 1.25	Ø 1.25
Elastogram map available	No	Yes	Yes	Yes
Body position	Supine	Supine	Supine, LLO, LLR	Supine
Patient fasting (h)	5	5	5	5
SWE exams (n)	188	112	200	200
SWE measurements in each patient (n)	10	10+10	10+10+10	10
Total SWE measurements in study (n)	1880	2240	6000	2000
SWE operator	First author	First author	First author	First author

ROI=region of interest; LLO=left lateral oblique; LLR=left lateral recumbent; SWE=shear wave elastography

Shear wave speed is expressed in m/s, but in this thesis, the speed was converted to the Young modulus (E), expressed in kPa, and given from $E=3\rho c_s^2$, where c is the shear wave speed and ρ is the tissue density. This equation assumes an elastic, linear, isotropic, and homogeneous material that is almost incompressible (13).

In study I, the point-SWE in Philips iU22 (Philips Healthcare, Bothell, WA, USA) was used, applying the ElastPQ technique with a C5 convex probe. In the ROI, multiple ARFIs were sent, generating tissue displacement. In the image, a fixed ROI of 0.5×1.5 cm was used, with a depth decided by the SWE operator. No

elastogram map was provided with this SWE technology (Table 3). However, intrahepatic vessels were avoided with the help of power Doppler to identify fluid-filled vessels. The ultrasound device ElastPQ program displayed 00.00 for invalid measurements, and these were not included in the examination. A reference list for cut-off values used in study I is presented in Table 4.

Table 4. Study I Philips iU22 Elast PQ cut-offs in terms of Young's modulus for classifying fibrosis stage

Metavir score	kPa
F0	2.0–4.5 kPa
F0-F1	4.5–5.7 kPa
F2-F3	5.7–12.0 kPa
F4	12.0–21.0 kPa

In studies II–IV, a GE Logic E9 (GE Healthcare, Wauwatosa, WI, USA) 2D comb-push was used with a convex probe (C1-6). In the elastogram, multiple ARFIs spatially created shear waves. Measurements were performed within a fixed ROI with a diameter of 1.25 cm and area ($A=\pi d^2/4$) of 1.23 cm^2, placed in an artefact -free region of the elastogram map. Measurements were performed non-overlapping with only one measurement at each elastogram (Table 3). The reference list for cut-off values used in studies II–IV is presented in Table 5.

Table 5. Studies II–IV GE LOGIQ E9 cut-offs in terms of Young's modulus for classifying fibrosis stage

Liver fibrosis staging	Metavir score	kPa
Normal	**F0**	<5.48 kPa
Normal-mild	**F1**	5.48–8.29 kPa
Mild-moderate	**F2**	8.29–9.40 kPa
Moderate-severe	**F3**	9.40–11.9 kPa
Cirrhosis	**F4**	>11.9 kPa

Shear wave elastography performance

The first author performed all elastography measurements. Current guidelines (13) were followed for the studies presented in this thesis, with the following exceptions: in study II, maximum probe pressure was used, and in study III, different body positions were used. In study II, the SWE operator was blinded during examination to the median kPa SWE results with maximum probe

pressure, which were calculated after the SWE examination. All patients rested for a minimum of 10 minutes and fasted for at least for 5 hours before the SWE examination. Patients unable to independently hold their breath were provided a nose clip and instructed to close their mouth to suspend breathing during measurements. Patients were measured on site for height and weight while wearing light clothes at the same time the SWE examination was performed.

Assessment of quality parameters

Probe maintenance
In the ultrasound probe, piezoelectric crystals convert electricity to mechanical waves in the ultrasound frequency range and convert the energy in the mechanical waves into electricity (16,21). The track pulses used to measure shear wave speed in the tissue depend on the piezoelectric crystals, and damage to these crystals can affect the reliability of speed measurements. In study I, the probe was tested within a machine test onboard program by the technician at the radiology department. In all the other studies included in this thesis, the probes were tested by the manufacturer before lending the ultrasound system.

Shear wave quality
The ARFI and the tracking pulses are affected by boundary conditions. Shear wave attenuates rapidly because of liver morphology and is very sensitive to rib shadow (73). In studies II–IV, an elastogram map in GE Logiq E9 was set as a guide for shear wave quality. According to the manufacture, at least 50% colorfill in the elastogram map defines when the volume of shear wave signal is accurate and amenable for successful technical measurement (Figure 2).

ROI for measurement was placed in the middle of the elastogram map where artefacts such as pixelated areas and sharp blue lines could be avoided. Artefacts within the elastogram map tend to increase kPa and therefore are important to avoid for reliable measurements. Sharp blue lines on an elastogram are a feature of motion, which can result in measurement bias.

When an elastogram map was not technically successful, the penetration program was activated. This activation increased the terminal index, which increased the pulse duration and decreased the frame rate.

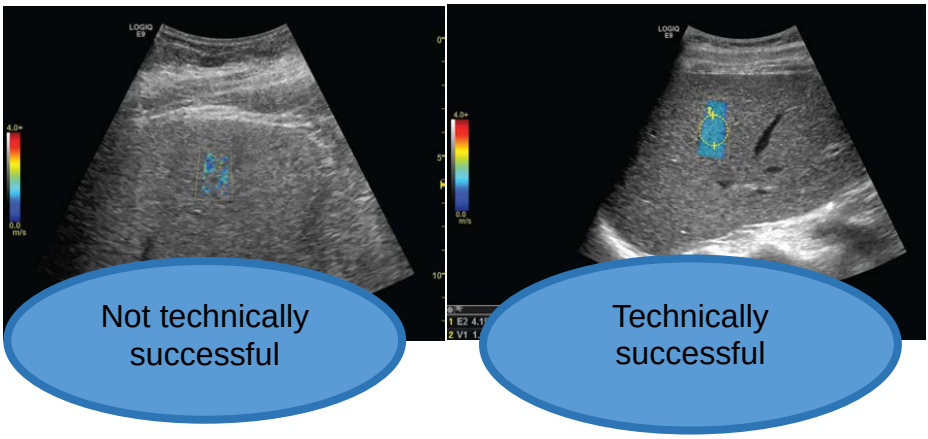

Figure 2. With at least 500% colorfill in the elastogram map, the criteria for a technically successful measurement are fulfilled (right). The image on the left illustrates a lack of technical success, with a corresponding low quality of shear wave signals in the elastogram map.

Statistical analysis

The statistical analyses were conducted using the IBM Statistical Package for Social Sciences (SPSS) Statistics (IBM, Armonk, NY, USA) Version 23 in studies I and II. SPSS version 25 was used in studies III and IV. In all studies, statistical models were chosen in collaboration with a statistician. SWE data were not normally distributed, so nonparametric tests were used, and 95% confidence intervals (CIs) were calculated. Tests were two-sided and the level of significance was set at $p<0.05$.

Study I

In study I, 10 SWE measurements were was used for a complete SWE examination. Each SWE examination was allocated to one of two groups using liver stiffness reliability criteria (66) as the cut-off. In the high variance group, high variance for median kPa SWE result was defined as IQR/median kPa >30.0% and a liver stiffness median of ≥7.1 kPa, and indicating increased uncertainty in SWE result. In the low variance group, low variance for median kPa SWE result was defined as IQR/median kPa ≤30.0%, and indicating reliable SWE results (Figure 3).

Binary and multivariate logistic stepwise regression analyses were used to investigate patient-related factors of age, sex, smoking habits, BMI, SCD, presence of steatosis and/or cirrhosis in the liver, and use of antiviral and/or cardiovascular medication in association with the low or high variance group.

Nagelkerke, Cox and Snell, and Hosmer–Lemeshow tests were used to evaluate the model.

The Fisher's exact test and Chi^2 test were used for dichotomous variables and the Mann–Whitney U test for continuous variables. Spearman's r was used to test the correlation between liver biopsy result and SWE result.

Study II

In study II, 10 SWE measurements were performed in each patient at the maximum intercostal probe pressure and then another 10 SWE measurements were performed with normal applied probe pressure (Figure 4). SWE measurement and SCD differences between maximum and normal probe pressures were tested with Friedman test, correlation test, and Spearman rho. Differences in technically success, i.e. shear wave quality, between the two groups were assessed using the Wilcoxon signed rank test.

The required amount of measurement needed for a reliable SWE exam was evaluated. Used as reference, SWE result from 10 SWE measurements were considered as a reliable SWE exam. These SWE result were then used in analyses for every test with an increasingly reduced number of measurements in a SWE exam. The SWE results (10 measurements) were compared to other SWE results (10 – x measurements) with Friedman's test. Scatterplot was used to define SCD values for less reliable SWE examinations, in which IQR/median kPa ≤30.0% was used as the cut-off for a reliable SWE examination.

Univariate logistic regression was applied to assess the association of SAD with reliable SWE examination. SWE reliability was defined by an IQR/median kPa of ≤30.0% as the cut-off.

Study III

In study III, the SWE result from supine, LLO, and LLR positioning was tested for differences using the Friedman test and Wilcoxon signed-rank test. SCD values from each body position were evaluated with repeated-measures analysis of variance (ANOVA) and Huynh–Feldt correction. Low variance for the SWE result was defined as IQR/median kPa ≤30.0% and used as an expression for a reliable SWE examination. SWE result corresponding to Metavir ≥F2 obtained with the supine position were used as the cut-off when dividing SWE examinations into two groups (Figure 5). McNemar's test was used to evaluate the amount of cases between groups.

To study the agreement between supine, LLO, and LLR median kPa, Lin's concordance correlation coefficient (CCC) was used. CCC can be expressed as the

product of Pearson's r (a measurement of precision) and the bias-correction factor (Cb; a measure of accuracy) (91). CCC was classified as poor (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), good (0.61–0.80), or excellent (0.81–1.00).

Bland–Altman 95% CI limits of agreement (LOA) was evaluated within and between body positions. At first, a one-way t-test was performed to check accuracy for the Bland–Altman analyses. LOA represents the interval within which the absolute difference between SWE result from two different body positions, even with high agreement or concordance, can be expected to lie with a 95% probability.

Study IV

In study IV, the Mann–Whitney U test was used to analyze differences in SCD, SAD, and SWE results between men and women. Differences in SWE results between all subgroups were assessed with the Kruskal–Wallis test. Fisher's exact test was applied to evaluate differences in the numbers of cases with results corresponding to Metavir ≥F2, between results from SWE vs APRI. Linear regression was used to analyze the correlation between SAD and SWE result for the subgroup with SAD ≥23 cm. Serum biomarkers for APRI were not available for 2 cases, so this analysis included 198 patients (Figure 6).

Results

Study I

The group with a high variance for SWE result, indicating increased uncertainty in SWE result, comprised 52 of 188 cases (28.0%), compared to 136 of 188 cases (72.0%) in the low variance group, indicating reliable SWE result.

In univariate analysis, factors associated with low variance in SWE result were sex, use of antiviral and/or cardiovascular medication, and the presence of cirrhosis. In multivariate analysis, medication, age, BMI, and smoking habits were associated with the low variance group (IQR/median kPa <30.0%).

In multivariate analyses, factors associated with high variance in SWE result were presence of hepatic steatosis (odds ratio [OR] 2.89; 95% CI 1.33–6.28) and increased SCD (OR 3.08; 95% CI 1.70–5.60).

Data for BMI and smoking habits were collected for 52 of 188 patients. BMI and SCD were associated with a high variance in SWE results group, which was seen as an expression of the effect of body constitution. In multivariate analyses of data for these 52 patients, smoking habits, BMI, and SCD were tested. Only SCD was associated with high variance in SWE result group (n=59; OR 6.12; 95% CI 1.50–25.10). These results indicate that SCD is better than BMI as a proxy for body constitution.

SWE result showed a significant positive correlation with available liver biopsy results (n=48; Spearman's ρ=0.476; $p<0.001$).

Study II

The median SWE results with normal probe pressure was 5.49 kPa (IQR 2.41) and with maximum probe pressure, it was 5.02 kPa (IQR 1.96); this difference was significant ($p<0.001$). A significant strong correlation (n=112; Spearman's ρ=0.866; $p<0.001$) was found between median kPa SWE result for normal and maximum probe pressures.

With normal applied probe pressure, the mean SCD was 1.98 cm (±0.57 cm; range 1.00–3.87 cm), and with maximum applied probe pressure, the mean SCD decreased to 1.74 cm (±0.45 cm; range 0.96–3.18 cm). The difference between groups was significant ($p<0.001$; 0.69 cm, 21.7%). For patients with the largest SCD of 3.87 cm with normal probe pressure, this value decreased to 3.03 cm with maximum probe pressure.

A low variance for SWE result was found with a SCD cut-off of 2.5 cm with normal probe pressure and 3.7 cm with maximum probe pressure.

The number of technically successful measurements, i.e. measurements with high shear wave quality, obtained by normal probe pressure was 981 (87.6%); with maximum probe pressure, this number was 1098 (98.0%). The increase was significant with an additional 117 measurements (11.9%; $p<0.001$).

With maximum intercostal probe pressure, a reliable SWE examination was possible in all 112 patients in the study when three measurements were required. For normal probe pressure, 107 patients could achieve a reliable SWE examination with three measurements. No significant difference was found between SWE result for examinations with three vs ten measurements ($p=0.625$).

When using maximum probe pressure to decrease SCD, increased SAD remained associated with a high proportion of variance for SWE result among men, but not among women.

The number of cases that were ≥F2 did not differ significantly ($P=0.405$) between normal probe pressure (n=15/112; 13.4%) and maximum probe pressure (n=12/112; 10.7%). Also, when grouping cases by Metavir score (F0–F4), no significant difference was found ($p=0.197$).

Correlations were found between liver biopsy Metavir results and SWE Metavir results using maximum probe pressure (n=9; Spearman's $\rho=0.646$; $p<0.001$) and normal probe pressure (n=9; Spearman's $\rho=0.731$; $p=0.025$).

Study III

The SWE results in the supine (5.50 kPa), LLO (5.80 kPa), and LLR (5.84 kPa) positions showed a difference <1 kPa. The difference was significant between supine vs LLO ($p=0.041$) and supine vs LLR ($p=0.026$), but not between LLO vs LLR ($p=0.290$).

For the patient with the largest SCD measurement of 3.93 cm in the supine position, SCD values decreased to 3.05 cm in LLO and to 2.93 cm in LLR. SCD decreased 1 cm with a postural change from supine to left recumbent position for this individual. Mean SCD significantly differed ($p<0.001$; df, 1.584) among the supine (1.97 cm), LLO (1.88 cm), and LLR (1.91 cm) positions.

CCC values were 0.780 (0.720–0.829) for supine vs LLO, 0.662 (0.578–0.733) for LLO vs LLR, and 0.717 (0.643–0.778) for LLO vs LLR, indicating good agreement (Table 6).

Table 6. Agreement among values in different body positions

Body position	Obs.	CCC (95% CI)	Pearson's r	Cb
Supine vs LLO	200	0.780 (0.720–0.829)	0.782	0.998
Supine vs LLR	200	0.662 (0.578–0.733)	0.669	0.990
LLO vs LLR	200	0.717 (0.643–0.778)	0.719	0.997

Obs=observations; LLO=left lateral oblique; LLR=left lateral recumbent.
CCC=concordance correlation coefficient Cb=bias correction factor, a measure of accuracy

The mean kPa SWE result differences and 95% LOA values were −0.125 (−3.524, 3.274) for supine vs LLO, −0.228 (−4.315, 3.859) for supine vs LLR, and −0.103 (−3.764, 3.558) for LLO vs LLR, as shown in the three Bland–Altman diagrams (Figure 7A–C).

The mean differences in kPa among body positions (x-axis) (see Bland–Altman diagrams) were small and assumed to have no clinical impact. However, the 95% LOAs were wide (e.g., for supine vs LLR: -4.1 kPa to 3.8 kPa) for all three Bland–Altman diagrams (y-axis; Table 7). Such a difference, e.g., as that seen for supine vs LLR of approximately 8 kPa, is more likely to have a clinical impact.

Table 7. Limits of agreement (LOA) and mean differences in kPa among body positions

Body position	Mean difference*	95% LOA**
Supine vs LLO	–0.125	–3.524 to 3.273
Supine vs LLR	–0.228	–4.070 to 3.859
LLO vs LLR	–0.103	–3.764 to 3.558

LLO=left lateral oblique; LLR=left lateral recumbent; LOA=limits of agreement.
*Mean kPa differences between two body positions, x-axis seen as red line in Figure 7 A-C. **Represent the interval within which the absolute difference between median kPa from two different body positions can be expected to lie, within a 95% probability, y-axis seen as green corridor in Figure 7 A-C.

A trend can be seen in all three Bland–Altman diagrams (Figure 7A–C). The 95% LOA tends to increase as the mean SWE result (x-axis) increases among all body positions. Differences outside the 95% LOA appear when the mean of two median

kPa results was ≥8 kPa, which can be seen for all Bland–Altman diagrams. A SWE median kPa ≥ 8 corresponds to Metavir ≥F2.

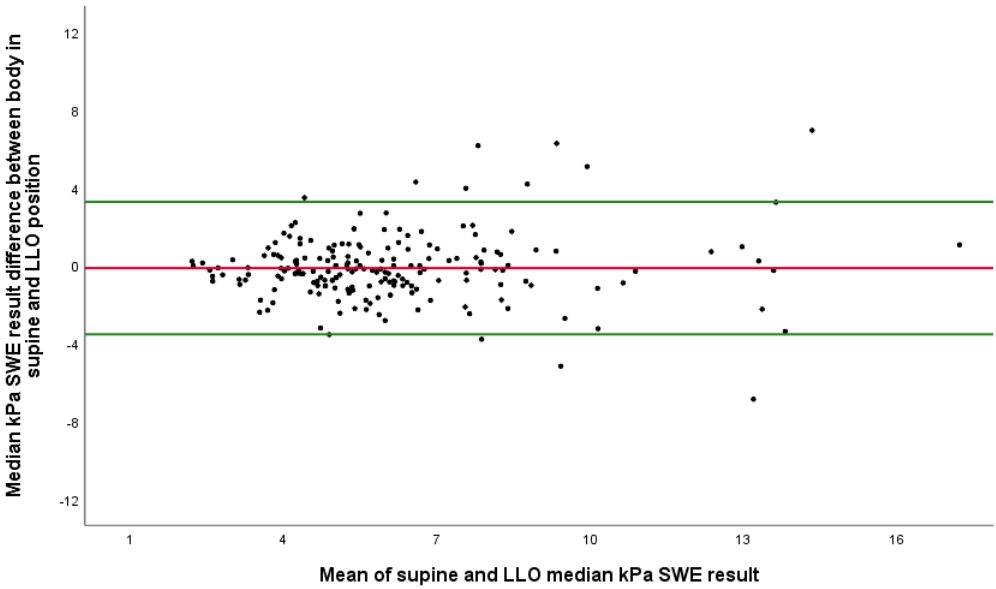

Figure 7A. Bland–Altman diagram for supine vs left lateral oblique (LLO) body position. The mean of supine and LLO median kPa SWE result is plotted on the x-axis, and the difference (median kPa in supine subtracted from median kPa in LLO) is shown on the y-axis. The central red line indicates a mean difference of −0.125. Green lines indicate the 95% limits of agreement (LOA), with an upper limit of 3.273 and lower limit of −3.524.

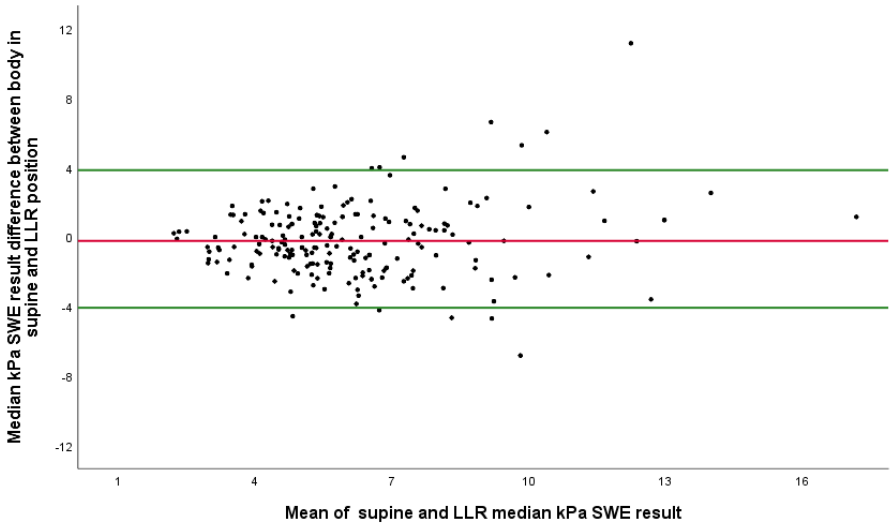

Figure 7B. Bland–Altman diagram for supine position vs left lateral recumbent (LLR). The mean for supine and LLR median kPa is plotted on the x-axis, whereas the difference (median kPa in supine subtracted from median kPa in LLR) is shown on the y-axis. The central red line indicates a mean difference of −0.228. Green lines indicate the 95% limits of agreement (LOA), with an upper limit of 3.859 and lower limit of −4.070.

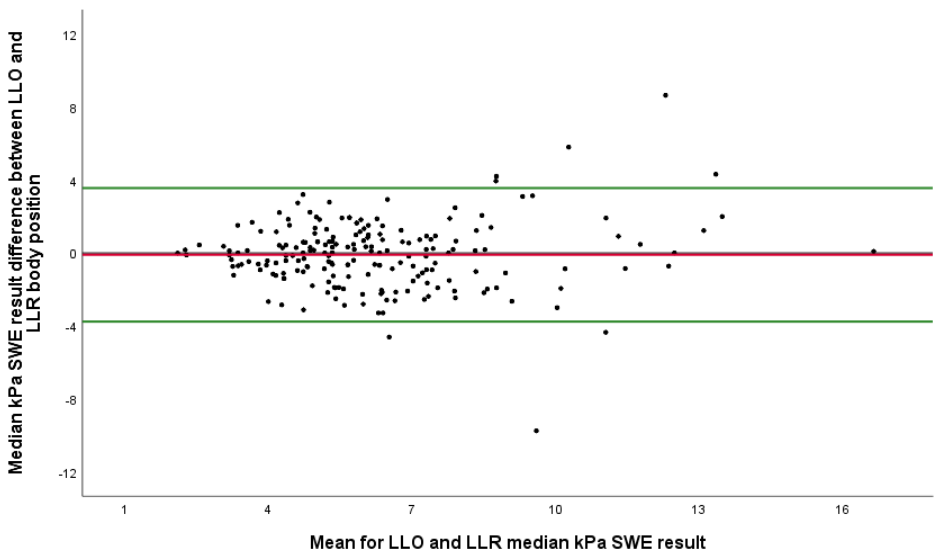

Figure 7C. Bland–Altman diagram for left lateral oblique (LLO) vs left lateral recumbent (LLR). The mean for LLO and LLR median kPa is plotted on the x-axis, whereas the difference (median kPa in LLO subtracted from median kPa in LLR) is shown on the y-axis. The central red line indicates a mean difference of −0.103. Green lines indicate the 95% limits of agreement (LOA), with an upper limit of 3.558 and lower limit of −3.764.

SWE reliability was expressed as the variance in median kPa SWE result, with a cut-off IQR/median kPa of >30.0%, i.e. high variance, indicating increased uncertainty in SWE result and therefore a less reliable SWE examination. For the positions, 28/200 patients (13.8%) in the supine position had a high variance, 25/200 (12.3%) in LLO did so, as did 12/200 patients (5.9%) in LLR. These numbers significantly differed between supine vs LLR (p=0.015) and LLO vs LLR (p=0.043), but not between supine vs LLO (p=0.742). Results indicate that the LLR position is the most reliable, based on variance for SWE results.

Hepatic steatosis was found in 87/200 patients (43.5%). Among these patients, there was a significant increase in SWE results in the supine (5.98 kPa) and LLO (5.98 kPa) positions compared to the LLR position (6.66 kPa; p=0.043).

In contrast, among patients without hepatic steatosis, SWE results did not differ significantly among the three different body positions. SAD did differ significantly between patients with and without hepatic steatosis (24.1 cm vs 20.6 cm; p<0.001; Table 8).

Table 8. The median kPa SWE result in different body positions for subgroups with and without hepatic steatosis

SWE median kPa result and body position	Hepatic steatosis (n=87)	P value*	No hepatic steatosis (n=113)	P value*
Body position		0.043		0.050
Supine (min–max; range)	5.98 (2.54–17.81; 15.27)		5.16 (2.21–15.25; 13.04)	
LLO (min–max; range)	5.98 (3.12–16.69; 13.57)		5.41 (2.09–14.48; 12.38)	
LLR (min–max; range)	6.66 (3.24–16.60; 13.36)		5.33 (2.11–12.72; 10.62)	

*Friedman's test. P<0.05 is regarded as statistically significant. LLO=left lateral oblique; LLR=left lateral recumbent

The influence of postural changes on SWE results clearly depends on high or low median kPa results in the supine position. Of note, among patients (n=28) with high SWE values in the supine position, the SWE results significantly decreased (p=0.002) in the LLO and LLR positions. With SWE result expressed in Metavir, there was a decrease from Metavir F3 (10.46 kPa) in the supine position to Metavir F1 (9.24 kPa) in LLR (Table 9).

Among patients (n=172) with low median kPa SWE results in the supine position, the SWE results significantly increased (p<0.001). With SWE results expressed in Metavir, there was an increase from Metavir F0 (5.18 kPa) in the supine position to Metavir F1 in LLO (5.33 kPa) and LLR (5.58 kPa; Table 9).

Moreover, SAD significantly differed (p=0.001) between subgroups. Patients with high median kPa in the supine position had a SAD value of 27.2 cm (27.5 cm for women and 27.0 cm for men), while those with low median kPa in the supine position had a SAD value of 21.0 cm (20.0 cm for women and 21.5 cm for men; Table 9).

Table 9. Subgroups with high or low median kPa SWE result obtained in the supine position, and their median kPa in different body positions

SWE median kPa result and body position	Metavir ≥2 (n=28)	P value*	Metavir <2 (n=172)	P value*
Body position		0.002		<0.001
Supine (min–max; range)	10.46 (8.34–17.81; 3.93)		5.18 (2.21–9.56; 1.92)	
LLO (min–max; range)	9.24 (4.44–16.69; 5.62)		5.33 (2.09–12.00; 2.23)	
LLR (min–max; range)	7.96 (4.57–16.60; 5.00)		5.58 (2.11–13.22; 2.52)	

Metavir ≥F2=SWE result corresponding to Metavir ≥F2. Metavir <F2=SWE result corresponding to Metavir <F2. *Friedman's test. P<0.05 is regarded as statistically significant. LLO=left lateral oblique; LLR=left lateral recumbent

As a test for validity of the study, the numbers of cases with a high median kPa SWE results corresponding to Metavir ≥F2 were compared among body positions. A total of 28/200 (13.8%) were identified using the supine position compared to 27/200 in LLO (13.3%) and 29/200 in LLR (14.3%). There were no significant differences between supine vs LLO (p=1.00), supine vs LLR (p=1.00), or LLO vs LLR (p=0.839), which supports the clinical validity of the study.

Study IV

This study was focused on sex-based differences in impact of cardiovascular medication, SAD and SCD on median kPa SWE results.

The median SWE results was 4.72 kPa for women and 5.90 kPa for men, which was significantly different ($p<0.001$).

No significant difference was found between men and women regarding intake of cardiovascular medication.

SCD significantly differed ($p=0.034$) between women and men, as well. The mean SCD for women was 1.9 cm (SD, 0.6 cm; min, 1.2 cm; max, 3.9 cm) compared to 2.0 cm for men (SD, 0.5 cm; min, 1.0 cm; max, 3.8 cm).

For SAD, men and women also differed significantly ($p<0.001$). The mean SAD for women was 21.4 cm (SD, 4.4 cm; min, 15 cm; max, 36 cm), compared to a mean of 22.8 cm for men (SD, 3.9 cm; min, 15 cm; max, 39 cm).

SWE result and SAD correlated significantly for men ($n=110$; Spearman's $\rho=0.461$; $p<0.001$) and for women ($n=90$; Spearman's $\rho=0.489$; $p<0.001$).

With a SAD measurement of ≥23 cm as a cut-off, men and women were divided into subgroups. A significant difference for median kPa SWE result was found between all four subgroups ($p<0.001$; Figure 8).

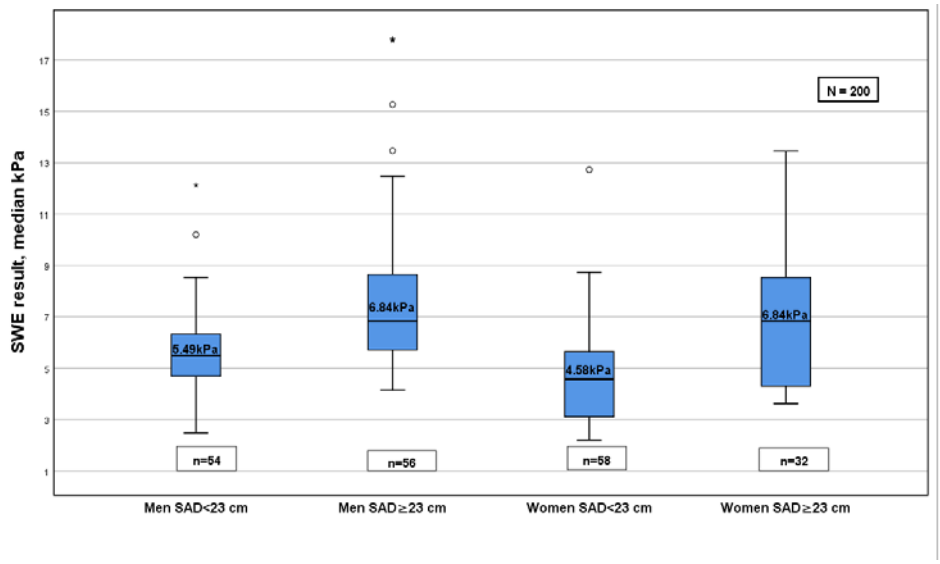

Figure 8. Median kPa SWE results for men and women, in subgroups with a cut-off SAD of ≥23 cm.

In subgroups with increased SAD (SAD ≥23 cm), the results showed a significant positive correlation between SAD and median kPa SWE result for both women (n=32; Spearman's ρ=0.375; P<0.045) and men (n=56; Spearman's ρ=0.527; P<0.001). Moreover, for every centimeter of SAD increase, the median kPa SWE result increased by a factor of 0.42 for men and 0.37 for women, with other factors held constant (Figure 9).

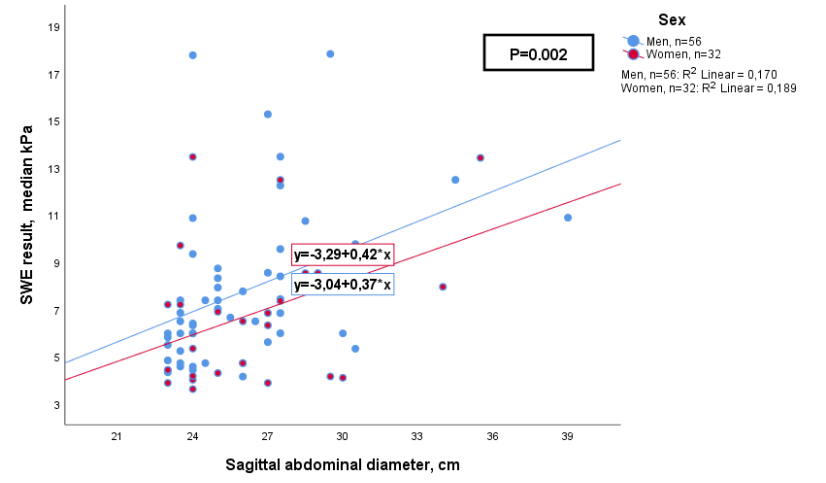

Figure 9. Correlation between SAD and median kPa for the subgroups of men and women with SAD ≥23 cm.

The number of cases with results for either high or low indication for liver fibrosis, obtained with SWE and APRI methods, also was compared. Men and women were divided using SAD ≥23 cm as the cut-off. Results significantly differed between men with increased SAD vs men without increased SAD (p=0.039), but did not significantly differ between women with and without increased SAD (Table 10).

Table 10. Men and women compared for SAD and liver fibrosis using SWE and APRI methods

	SWE-method		APRI-method		
SAD	**≥F2**	**<F2**	**≥F2**	**<F2**	**P value***
Men <23 cm (n=59)	4 (2.0%)	55 (28.0%)	8 (4.0%)	51 (26.0%)	0.085
Men ≥23 cm (n=51)	15 (8.0%)	36 (18.0%)	8 (4.0%)	43 (22.0%)	0.039
Women <23 cm (n=60)	2 (1.0%)	58 (29.0%)	13 (6.0%)	47 (24.0%)	0.389
Women ≥23 cm (n=28)	6 (3.0%)	22 (11.0%)	1 (1.0%)	27 (14.0%)	0.214

Metavir ≥F2=SWE result corresponding to Metavir ≥F2. Metavir <F2=SWE result corresponding to Metavir <F2. SAD=sagittal abdominal diameter (cm). APRI=aspartate aminotransferase-to-platelet ratio index. *Fishers exact test. p<0.05 is regarded as statistically significant.

Discussion

Summary of results of the studies in this thesis

Results of the studies in this thesis show that ultrasound-based SWE of the liver is a reliable non-invasive method for diagnosing liver diseases. For patients with SCD ≥2.5 cm, the SWE operator may experience a bad quality of shear wave and less reliable SWE examinations. Results suggests that in these cases, with increased probe pressure, a reliable SWE exam can be achieved. Therefore, non-invasive ultrasound-based SWE liver method is superior for examination in patients with overweight and obesity because of the ability to increase probe pressure. In men but not women with liver fibrosis and SAD ≥23 cm, there was an impact of this SAD value on SWE results. This finding may explain the higher SWE values for men compared to women. Depending on the severity of liver disease and SAD, a postural change to left decubitus was associated with a different outcome. As SAD increased, liver stiffness did, as well. Therefore, SAD should be considered when performing SWE of the liver.

Methodological considerations

Strengths and limitations

In the studies included in this thesis, SWE of the liver was applied following the current guidelines (13) for a standardized method, which is a strength. This thesis is also strengthened by the consecutive enrollment of all patients referred for evaluation of liver fibrosis with ultrasound-based SWE to the hospital during the study periods, with written approval from the participant, minimizing risk for selection bias. The county has only one hospital and a large recording area, and no patient assessed for liver fibrosis was sent to another department or city. Also, the sample size in each study was large. Another strength is the access to a new ultrasound system with the latest updated 2D SWE program versions for all studies in this thesis, except study I.

This thesis is a single-site and single operator project, which may be a limitation on differences in the study population and thus a risk for bias. In addition, different results may arise if the method described in the studies is used for a different study population with other disease etiologies or ethnic differences. Moreover, results could vary with other ultrasound devices or SWE technologies or if inexperienced SWE operators perform the evaluations. Moreover, the patient perspective has not been given here.

Cut-off values for Metavir scores for liver fibrosis stages were provided by the manufacturer in all studies. The cut-off values differ depending on the etiology of

liver disease. According to EASL, the distribution of fibrosis varies with diagnosis. Cirrhosis in HBV is mostly macronodular in contrast to the mostly micronodular pattern seen in HCV. As a consequence, there is more fibrotic tissue in chronic hepatitis C, and at the same stage of Metavir, the liver is stiffer in HCV. For the ultrasound-based SWE liver method, the same cut-offs for grade of liver fibrosis is used for all liver diseases.

Another limitation is the difference in SWE technologies used, with the point-SWE in study I and 2D SWE with comb-push for the three others studies. However, supplementary unpublished studies with other technology have been performed and those results presented; as mentioned, these results confirmed the findings reported here. Thus, the results from this thesis could feasibly be generalized to patients with chronic liver diseases, at least in the western world.

Measurement bias

The distribution of fibrosis in the liver differs among etiologies (92), which might yield lower SWE result if measured in areas with a lower degree of fibrosis. Although fibrosis may not be uniform, the assumption is made that the most accurate results are likely with visible homogeneity within the measurement ROI. It is debated whether measurements from more sites in the liver should be performed because of heterogeneous fibrosis distribution (93), but this is not expressed in the guidelines (13). In the studies in this thesis, measurements were obtained from liver segments 5, 7, or 8, and from sites free from rib shadow, reverberation artefacts, larger vessels, and lesions. Variation in ROI placement regarding depth and liver segment, as well as angle of the beam, could be a source of bias. In these studies, all measurements were performed by the first author to minimize performance bias, but that factor could also imply a risk of systematic bias. In study I, the ICC was 0.900. In study II, the ICCs were 0.985 in supine position, 0.963 in LLO, and 0.988 in LLR. Intra-rater reliability is considered excellent with ICC values >0.9 (94). No inter-rater reliability test of interventions was possible in these studies, but the method has earlier been described as involving of high inter-rater reliability (13).

Motions from either the SWE operator or the patient might have a greater impact than known (95). In this work, the use of a nose clip turned out to be quite handy for patients unable to understand instructions on breath holding to avoid uncontrolled motions. Earlier results showed the importance of a good ergonomic position for the operator (96) to be able to relax the forearm and the importance of cooperation from patients being able to suspend their breathing during measurements. The first author performed all SAD measurements with a spirit-level and a wooden ruler to obtain the most accurate value. There is always a risk of bias, but the SAD measurement is not considered to have a great impact in

millimeter changes. For GE US SWE values, there is an ongoing discussion about whether the list of scores is accurate for cirrhosis (97).

Statistical methods
All statistical methods were chosen in collaboration with statisticians and applied by the first author. At least five statisticians have been involved in the project, none of them with a deep knowledge of the clinical aspects of the SWE method. This can be seen as a limitation because knowledge of clinical aspects could be crucial. However, it could improve the work because many questions had to be addressed, and several aspects were considered.

Reference method
A major limit in the research project is the validity. Liver biopsy remains the gold standard and is an invasive method associated with patient discomfort and a risk for serious complications and even death (7). Therefore, liver biopsy results were used in the study only when available. The lack of a reference method was obvious in study II when the median kPa SWE results from different body positions changed without any indication of which result represented the greatest accuracy. The use of MRE was not possible because of high costs, low grade of access to the appropriate department at the clinic, and no valid cut-off reference list for the stage of liver fibrosis.

There was an option to use TE as a reference method. TE is a well-established method introduced in 2003 and performed with Fibroscan® from Echosence, Paris. However, the Fibroscan® was not used as a reference method because measurements with the XL probe were known to yield unreliable results (98) at the time this PhD research project began. Furthermore, the TE method does not allow a push with the probe and or use in the left lateral recumbent position (13). We evaluated the presence of steatosis with Fibroscan® ultrasound-controlled attenuation parameter (CAP™) (5) in all our studies, except for study I. Serum biomarkers was used as a "true" comparison method in study IV with APRI, with the advantage of updated analysis reports. Moreover, the ultrasound-based SWE liver method is more accurate than TE performed with Fibroscan® (99-101).

The reliability criteria for liver stiffness evaluation (66) were used in study I for creating less reliable and reliable groups, respectively. Increased variance for SWE result accounted for increased uncertainty in SWE result, and therefore also less reliable SWE result. Moreover, the technically successful measurements with colorfill in the elastogram map, i.e. shear wave quality, were also used as criteria for reliability in study II. No significant differences were found between these two quality criteria parameters (Table 11).

Table 11. Number of cases compared between reliable and less reliable groups, technically successful measurements and not technically successful measurements

Amount of SWE exams	Technically successful	Not technically successful	Total N (%)	P value*
Reliable group n(%)	85 (91.4)	8 (8.6)	**93 (83.0)**	0.097
Less reliable group n(%)	13 (68.4)	6 (31.6)	**19 (17.0)**	
Total n(%)	**98 (87.5)**	**14 (12.5)**	**112 (100)**	

*McNemar's test. Increased variance for SWE result. Less reliable group=IQR/median kPa SWE result>30.0%. Technically successful=at least 50% colorfill in the elastogram map, i.e. high quality of shear wave.

Because data for SWE result are not normally distributed (102), non-parametric tests were used, which could be viewed as a limit because of weaker capacity for detecting significant differences. However, the SCD values were assumed to be normally distributed, and ANOVA was used instead of Friedman's in study III because ANOVA is a stronger test for detecting significant differences. Patients were used as their own individual controls on repeated measurements in the same day and at the same time as for the first SWE exam, which also is a strength.

Discussion of the main results

Study I

The results in study I show that patients with increased SCD, as seen for patients with obesity and overweight, have three times the risk for median kPa SWE result with IQR/median kPa >30%, i.e., increased varianc and increased uncertainty in SWE result. Similar results have been found for increased SCD in other studies, where with BMI compared to SCD, SCD was found to be the best measure for increased variance (103,104). Moreover, new results in study II identified SCD as the only factor associated with increased variance in comparison with BMI. The SWE measurements described in this thesis were produced by ARFI, which

creates the shear waves at a certain depth in the liver tissue. The ARFI pulse focus at 3–5 cm depth, called the sweet spot of ARFI (87), can be difficult to reach in the liver tissue because of increased distance to the liver capsule or increased area below the liver capsule from reverberation artefacts, as seen in B-mode imaging. As is known, ultrasound wave attenuation increases with depth (16), and attenuation affects SWE (105). With an increase in the push pulse energy output, shear wave measurement success increases (106). Distortion and shadow caused by rib structures also affect SWE (73,107). Upcoming solutions with an artificial intelligence program to find an accurate area of liver tissue to perform reliable SWE measurements could possibly be a coming aid for SWE operators, as seen with computer-aided systems have been tested to separate healthy cases from chronic liver diseases (108). Forthcoming developments with 3D and 4D SWE also can possibly increase reliability of the SWE liver method (109).

Steatohepatitis (NASH) is not associated with increased risk for progression of liver fibrosis. Indeed, patients diagnosed with NAFLD with no steatohepatitis are seen to develop progressive liver fibrosis with a higher mortality risk (110). The effects of steatosis and inflammation in the liver on SWE estimation remain uncertain (87), as shown in study I. If shear wave estimations vary depending on steatosis and inflammation parameters, the detection and treatment of an early stage of liver disease could be delayed. As liver stiffness seems to decrease with steatosis and increase with inflammation, scattering of shear waves can also influence liver stiffness estimates (111). Results in study I are in agreement with another study in which higher liver stiffness values were obtained in the presence of steatosis (79). In an earlier study, 26% of cases using liver biopsy as the reference method of the steatosis showed both over- and underestimation of liver stiffness measurements (112). In addition, patients with fatty livers commonly have increased distance to the liver, as seen in study I, resulting in low quality for shear wave because of severe attenuation. Measurement bias from fatty liver is a constant subject of research (60,80,113-115). Increased viscosity in liver tissue means an increase in the stiffness of the tissue, which increases SWE results. Shear wave speed depends also on the excitation frequency, which is a phenomenon called dispersion. Dispersion differs among ultrasound systems, creating different SWE results which can explain different results (72). Any unanimous agreement regarding how hepatic steatosis affects shear wave speed has not yet been established.

Study II

There have been concerns about whether increased probe pressure will increase median kPa SWE result. An earlier study with increased probe pressure in the left lobe of the liver also revealed increased liver stiffness (116). In contrast, results in study II with increased intercostal probe pressure in the right lobe of the liver showed a decrease in median kPa SWE result. In earlier guidelines, an increased intercostal probe pressure was recommended (98). The first author found very little on this question in the literature; however, one study revealed no differences in estimates in SWE with increased intercostal probe pressure (117).

With the increased intercostal probe push during SWE measurement in study II, a decrease in SCD was obtained. All patients accepted the increased intercostal pressure without exceptions. The mean difference was small, yet an improvement with reported low variance. The number of technically successful measurements (colorfill in elastogram map. i.e. shear wave quality) also improved. For patients with a small distance to the liver capsule, there is often no need for increased probe pressure. However, for patients with a larger distance, increased probe pressure often is needed to achieve a technically successful measurement. Of interest, with increased intercostal probe pressure, the SCD cut-off for reliable SWE result increased to 3.4 cm in study II. The increase in technically successful measurements when decreased SCD are possible because of increased shear wave quality when SNR improves and attenuation decreases from squeezed subcutaneous fat, which retains the push pulses' higher output energy. The presence of a thick fascial or fibrous layer at the distal boundary of the abdominal wall magnifies the amount of reverberation clutter (118).

In study II, the SCD cut-off for increased variance was 2.5 cm. From a clinical point of view, this means that with SCD >2.5 cm, the SWE operator may possibly experience a bad B-mode acoustic window and will not reach 50% colorfill in the elastogram map, i.e. low sheare wave quality, i.e. while also increasing the variance. However, the SCD cut-off value could alter depending on SWE technology, although this was not the case here. The SCD cut-off value stayed similar with another SWE technology, as shown in an unpublished study performed by the first author (performed in 2018 by M. Byenfeldt, in Östersund), comparing the technology in the studies reported here with a 2DSWE Canon in 34 cases with SCD >2.3 cm for increased variance. Moreover, these unpublished results are in agreement with ultrasound system from Canon with SCD >2.0 cm (104) and SCD>2.7 cm for GE 2D com-push (119).

Similarly, SCD cut-offs from the TE technology performed with Fibroscan® are SCD >2.5 cm for M-probe and SCD >3.5 cm for XL probe. These cut-offs correlate

with our results. It seems that the forces used to create shear wave, mechanically or with ARFI, give a similar SCD cut-off for median kPa SWE result with IQR/median kPa >30.0%.

Required number of measurements

The quality parameter IQR/median kPa is based on 10 measurements (13). A reduction in measurements in a SWE exam is of interest because a reduced examination time is valuable for the department, the patient (both adults and children), and especially the SWE operator, possibly with reduced musculoskeletal injuries (96). Results in study II showed that only three measurements were required to obtain a reliable SWE exam with increased probe pressure. A reduction in the required measurements for a reliable SWE exam has been confirmed in other studies (120-122). To reduce the number of measurements in a SWE exam, operator experience has to be considered as well as the quality of acoustic window because a challenging examination requires more measurements. Moreover, it is important to reflect that the studies with high reliability after a reduced number of measurements involved experienced SWE operators. With a reduced number of measurements in a SWE exam, the quality parameter IQR/median kPa loses its value, of course, and new quality parameters must be defined. It has been described that a rejection of all measurements should be necessary with SD more than 30% of the absolute value in each measurement (99). This quality check ensures a reliability of SWE exams independently of amount of measurements. In earlier studies, for patients with normal weight, no steatosis, and no increased distance to the liver, the number of measurements for a SWE exam can be reduced. However, in the presence of increased SCD, steatosis and/or increased liver stiffness, 10 measurements are preferable (123).

Study III

The ultrasound-based SWE method characterizes liver stiffness, which can be due to either liver fibrosis, impact from intra/extra-hepatic pressure, or a combination of both. The SWE results in study III were divided into two groups, one group with SWE results corresponding to Metavir <F2 and one group with results corresponding to Metavir ≥F2. The group with lower median kPa SWE result in the supine position showed an increase in SWE result obtained in LLO and LLR, which corresponds to earlier studies in healthy individuals (70,88). What was striking in study III was that in patients with higher SWE results in the supine position, with a postural change to LLO and LLR, the SWE result decreased. Because there was a significant difference in SAD for these two groups,

increased intra-abdominal pressure must be considered as a possible explanation.

The SWE method measures liver stiffness, which as noted can be due to either liver fibrosis or impact from increased intra- or extrahepatic pressure of different causes. Liver stiffness decreases after transjugular intrahepatic portosystemic shunt implantation (124). Supine SAD correlates with bladder pressure, which can be used as an expression for intraabdominal pressure (125). Liver stiffness is directly influenced by venous pressure (82), and SWE result from the upright position are higher than in the supine position (77). The impact of the upright position has been compared with postprandial splanchnic hyperemia in patients with cirrhosis and portal hypertension, with results showing a greater impact from prandial effects than the upright position (126).

In a comparison between patients with visceral adipose tissue (VAT) and subcutaneous abdominal adipose tissue (SAT), VAT is stronger correlated to metabolic risk factors (127). In study II-IV, the anthropometric measure SAD was used as a marker for VAT. In a study for cardiometabolic risk (n=4032), the optimal SAD cut-off for increased risk was in men approximaltely 22 cm and in women 20 cm (128). A Finnish population-based Health 2000 Study (n=6626), the association with liver-related mortality and death, showed SAD, waist-hip ratio, waist circumference can predict incident liver disease, whereas BMI was non-significant (129). Another study with biopsy proven NAFLD and controls (n=664), confirms that SAD is the predictor for liver fibrosis and NASH (130). In study II-IV, an increased SAD impact on median kPa SWE result was found. This finding confirms the relevance of SAD as a tool for identifying individuals at risk for liver disease, to be screened for increased liver stiffness with SWE method.

Study III results also showed that with increasing liver stiffness, mean differences among body differences also increased. This result accords as well with earlier observations from studies of sample box size impact (131) and comparing intra-rater-observer reproducibility (132). These findings emphasize morphology as a possible partial explanation for the increased mean difference in study III. Morphology in cirrhotic livers causes higher attenuation for shear wave, which results in more technical failures; however, for the SWE method, no overlap between healthy liver and liver with cirrhosis has been detected (97).

Cirrhosis is not an end-stage disease, and for some etiologies, there is the possibility for regression (133). Median survival for patients with decompensated cirrhosis is 2 years and for compensated status, 9 years (134). Because the SWE method is sensitive and can detect cirrhosis already at the compensated stage (135), in contrast to CT, MRI, or B-mode ultrasound (136), there is a role for SWE

in surveillance programs to identify patients at an early stage. B-mode ultrasound alone fails to identify cirrhosis in 64.0% of patients diagnosed with HCC (137).

Reduced shear wave propagation has been detected after direct-acting antiviral agent (DAA) treatment (138) with high sustained virological response (SVR). Cirrhotic liver due to HCV significantly reduces the response to DAA, whereas livers with no cirrhosis have 2-2.5 higher odds for response to treatment. However, there is a significant risk for development of HCC after treatment in the fibrotic stage (139). The population in the studies in this thesis included 29 cases with SVR after DAA treatment for HCV patients. For each patient, one SWE exam was performed after treatment. Because different methods were used to stage liver fibrosis, the Metavir score was applied for comparison. In 13 of 29 cases (45.0%), liver fibrosis decreased by 2 Metavir stages. In 7 of 29 cases (24.0%), liver fibrosis decreased by 1 Metavir stage, and in 7 of 29 cases (24.0%), there was no difference after treatment. In 2 of 29 cases (7.0%), liver fibrosis increased by 1 Metavir stage. The progression of fibrosis in SVR has been reported with similar percentage (6.0%) in a larger study population (140). This agreement underlines the need for evaluation of liver fibrosis after SVR. There are somewhat inconsistent results for the influence of treatment with DAA on the liver in terms of HCC development (141). Subgroups with progressive fibrosis correlating with development of HCC after successful treatment have been described (140,142). In summary, the importance of continued monitoring for liver fibrosis before, during treatment, and after sustained virological response must be considered.

It has been reported that DAA non-responders have significantly higher baseline BMI and a significantly lower albumin and platelet count (143). However, it is also known for individuals becoming drug free that a craving for sugar develops, which can increase weight. An increase in BMI has been found for post-treatment individuals (144). Therefore, a postural change to left decubitus could be used as a test for these patients, and a decrease in SWE results could indicate that a patient will have a positive outcome from lifestyle changes involving weight loss and a decreased SAD value. On the other hand, if SWE results do not decrease with postural change, the liver fibrosis may persist after HCV treatment. Liver stiffness value is a significant predictive factor for HCC occurrence (145), and a postural change to left decubitus could provide information regarding the severity of liver disease.

Comparison of body position SWE result with another SWE technology

The SWE results differences shown in study III could be altered if another SWE technology were used. In an unpublished study (performed in 2018 by M. Byenfeldt, in Östersund), 34 patients were examined with a 2D SWE technology Canon i700 system (Table 12). Analysis from this study showed significant differences in median kPa SWE result between supine vs LLR ($p=0.04$) and LLO

vs LLR ($p<0.01$), but not between supine and LLO ($p=0.60$). The results were similar to findings in study III, with a higher median kPa SWE results in left positions, but with no significant difference between the supine and LLO positions.

Table 12. SWE results with different body positions performed using Canon i700 2D SWE technology

SWE median kPa result and body position (n=34)	SWE result
Supine median kPa (min–max; range)	5.22 (2.5–16.7; 14)
Left lateral oblique median kPa (min–max; range)	5.55 (3.4–41.4; 38)
Left lateral recumbent median kPa (min–max; range)	6.20 (3.8–15.0; 11)

In these studies, the expectation was that the measurements would have better ICC values over time. With more practice, better results would be obtained because it usually takes some training to find an accurate place for measurements in the liver. However, when analyzing the ICCs for the body position study measurements 1–10 over time, this was not the case. Actually, the best results from the supine position were obtained in the 6th measurement, with an ICC of o.84. The 2nd to 6th measurements in LLO showed the best results, with an ICC of 0.91. The 6th measurement in LLR showed an ICC of 0.94. This analysis indicated that six measurements in a patient in LLO position would give the most accurate SWE results.

Study IV

In the last study, the median kPa SWE result, SCD, and SAD were higher for men compared to women. Several previous studies have reported similar higher median kPa SWE results for men (70,75,79,88,89,120,146). In contrast, other studies have found small or no differences in median kPa between men and women (120,147). There is no clear explanation for these sex differences. Only one group has offered a possible explanation, using rats under a fibrosuppressive effect of estrogen (148). However, no study has been performed on humans using the SWE method, and no published results for SCD and SAD in SWE studies by sex are available. The increased median kPa SWE result for men could result in a false-positive for liver disease in these patients because there are no sex-adjusted reference lists for any of the elastography methods for assessing liver fibrosis.

With results from study IV showing increased SAD and SCD for men, another result in study I was assumed then no association was found for sex and increased variance. This unexpected finding was interesting, but no clear explanation was found.

To the best of our knowledge, the difference in SAD between men and women has never been investigated in SWE for the liver. A correlation between SAD and median kPa SWE result was generally found for both men and women. Sex-based differences in pathophysiology have been previously reported (149). SAD is a predictor only of cardiovascular disease for men only (150). According to results in study IV, SAD seems to affect liver stiffness measurements for both men and women, but with a higher impact for men. When maximum probe pressure was used in study II to decrease SCD, increased SAD remained associated with increased IQR/median kPa SWE result among men but not women.

Men tend to accumulate fat around the abdomen, whereas women tend to store fat around the hips and thighs (151). In weight loss, sex-based differences also are manifested, for example with fat deposits in which men have more visceral fat than women (152). These sex-based differences in fat deposits indicate a possible explanation for our findings of increased median kPa SWE result for men with increased SAD. However, APRI used for comparison can be seen as a limitation because the best reference method is liver biopsy. Therefore, another outcome could be found with another reference method. Unfortunately, liver biopsy was not possible in study IV. However, the main population in the study was infected with HCV, and APRI is seen as the best biochemical parameter for liver fibrosis in HCV (153). It would be of great interest to evaluate the results in study IV against those from other studies with a larger study population and valid reference method to determine whether these differences have a clinical impact.

Ethical considerations

Research may be approved only if it can be conducted with respect for human dignity. Because this research was carried out with Umeå University as the principal, solid experience in research was assured. Moreover, if the expected result can be achieved in another way, which means fewer risks to the health, safety, and personal integrity of research participants, another way should be chosen. A non-invasive method was used in the studies, and the research implied no physical risks for the patients. All security was supplied, and all patients were included in the Östersunds Hospital patient security insurance program. Results in this thesis will contribute to the understanding of how to increase the reliability of the ultrasound-based SWE of the liver method and its feasibility in the presence of increased SCD, as seen in overweight and obesity, a potential future benefit for patients undergoing SWE liver examinations. Moreover, the findings

of increased SAD affecting SWE result can contribute to understanding men's higher SWE results compared to women. Another ethical benefit might be that individuals who earlier refused liver biopsy might now accept the non-invasive SWE method because the results of the research will be communicated to the public. A benefit for patients who chose to participate in the study was the opportunity for a more accurate diagnosis, because more chances to obtain a reliable SWE results were made available within these studies.

Oral and written information was given by first author at the time of the SWE examination, with allowance for questions. As a patient is undergoing approval for participation in a study, there can be an increased interest in findings and also anxiety. The first author was responsive to questions from all patients during SWE exams. All patients participating in studies gave their written approval. The first author also was the investigator for all patients, which could be an ethical disadvantage or advantage. The disadvantage may be that patients felt forced to participate in the study. The advantage may be that the patient felt safer attending appointments when they had the same SWE operator for all examinations.

All data were collected from consecutive patients examined within the clinical routine for SWE liver examination at the Östersunds Hospital radiology department. The code key was stored in a secure way and was not be passed to anybody except the first author. In a small city like Östersund, there is the risk that a single person would be recognized; however, the quantitative data were analyzed in the aggregate, leaving the risk negligible. General data protection and regulation was not in effect at the time of data recording. The studies were approved by the research ethics review board in Umeå, Sweden. The research was performed following the ICMJE Recommendations for the Protection of Research Participants, and in accordance with the World Medical Association Declaration of Helsinki 2013.

Conclusions and clinical implications

Clinicians have multiple options for noninvasively staging liver fibrosis, which also reduces the need for liver biopsy to stage disease and diagnose cirrhosis. The ultrasound-based SWE of the liver method is non-invasive, cost-effective, and without known risk for the patient. SWE liver can be used as a screening tool for evaluation of the severity of liver disease and is a reliable method even for individuals with overweight and obesity. As known from earlier research, SWE liver is sensitive and detects cirrhosis at an early stage, before CT, MRI, or B-mode ultrasound can do so. The findings summarized in this thesis show that body constitution is best measured with SCD rather than BMI. These results show that for patients with SCD ≥2.5 cm, there is an increased risk for low quality of shear wave and uncertainty in SWE results. This finding implies that in these cases, with an increase in probe pressure, a reliable SWE exam can be achieved. Therefore, the non-invasive ultrasound-based SWE liver method is superior for examination in patients with overweight and obesity because of the ability to increase probe pressure and to achieve a postural change to left decubitus.

Increased risk for uncertainty in SWE results was also seen for patients with fatty livers. However, for fatty livers, the impact on SWE remains unclear, and no consensus is yet available. Differences were seen for men and women for SAD, SCD, and SWE result, with higher results for men compared to women. In patients with liver fibrosis and SAD ≥23 cm, an increased impact on liver stiffness was seen for men only. This result may explain earlier studies finding higher SWE result for men compared to women. Increased SAD seems to increase liver stiffness for men. For patients with no increased SAD and no increased SWE result, a postural change to left decubitus increased liver stiffness values, as seen in earlier studies. In contrast, for patients with increased SAD and increased SWE result, a postural change decreased liver stiffness values. It seems that increased SAD also increases liver stiffness, implying that SAD needs to be considered when performing SWE of the liver for an accurate diagnosis.

It is hoped that the findings described in this thesis will contribute to an increased reliability of the method when performing SWE exams. Improve the feasibility for assessing liver diseases with SWE, especially for patients with higher SCD and SAD. With increased SAD, and subsequently, greater intraabdominal pressure, liver stiffness increases, leading to greater risk for liver diseases. Therefore, measuring liver stiffness, for individuals with increased SAD, with the ultrasound-based SWE liver method can be used with prognostic value for liver diseases. The findings described in this thesis hint at a promising future for the use of SWE in clinical work for liver diagnostics.

Future perspectives

In current guidelines and recommendations for SWE, no consensus describes how to handle SWE exams that involve increased variance for SWE result, defined as IQR/median kPa >30.0%, and/or presense of low quality of shear wave in the elastogram map.

It seems that SAD increases SWE result, and a postural change to left decubitus can be used to distinguish this impact from increased intraabdominal pressure from liver fibrosis. To confirm the clinical implications of the results described in this thesis, there is a need for further studies in larger study populations and for example, in multi-center studies.

The presence of hepatic steatosis was associated with discordance for SWE liver results, i.e increased uncertainty in measurements. The possibility of distingishing the impact of hepatic steatosis on liver stiffness measurements would increase the accuracy of liver diagnostics. Further studies are needed when this technology is established.

The patient experience of the ultrasound-based SWE of the liver method has not been addressed in this thesis and should be the subject of additional studies.

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Expert Commentary by:Steven L. Flamm, MDChief, Liver Transplantation ProgramProfessor of Medicine and SurgeryNorthwestern University Feinberg School of MedicineChicago, Illinois. Gastroenterol Hepatol (N Y) 2016;12:1-22.
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