Conclusions:
2D-SWE showed low overall measurement variability, with a minimum of five readings providing equivalent precision to the existing method using 10 samples. Obesity, increasing abdominal wall thickness, sub-capsular measurements and a ROI SD/Speed >0.15 were all associated with increased measurement variability. ROI SD/Speed warrants further evaluation as a quality assessment metric, to allow objective operator assessment of individual 2D-SWE measurement reliability in real-time.

Variability of liver shear wave measurements using a new ultrasound elastography technique.

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Running Title:
Measurement variability of a new 2D-SWE technique.

Abstract

<u>Objectives:</u> A new two-dimensional shear wave elastography (2D-SWE) device has been developed for the non-invasive assessment of liver fibrosis. Guidelines on measurement acquisition parameters are not yet well established for this technique. Our study aimed to assess 2D-SWE measurement variability and to determine the number of measurements required per patient to reliably assess liver stiffness.

<u>Methods:</u> 2D-SWE was assessed in fifty-five patients with mixed etiology chronic liver disease using the Toshiba Aplio 500 ultrasound system. Ten measurements were obtained per patient by an operator blinded to all preceding readings. Results were analyzed with clinical information obtained from medical records.

<u>Results:</u> The median IQR/Median ratio for 2D-SWE was 0.131 (q1-q3: 0.089–0.174). Five readings provided an approximation within 0.11m/s or 4.2% of the median velocity of ten measurements. Factors associated with increased measurement variability included BMI (rho=0.388, $p=0.003$), increased skin-to-liver capsule distance (rho=0.426, $p=0.002$) and measurements taken within 1.5cm of the liver capsule ($p<0.001$). Measurements with heterogeneous shear wave profiles (indicated by a ROI SD/Speed ratio >0.15) showed greater deviation from the set's median velocity than those with a ROI SD/Speed ≤0.15 (0.42 vs. 0.22m/s, $p=0.001$).

<u>Conclusions:</u>

2D-SWE showed low overall measurement variability, with a minimum of five readings providing equivalent precision to the existing method using 10 samples.

Obesity, increasing abdominal wall thickness, sub-capsular measurements and a ROI SD/Speed >0.15 were all associated with increased measurement variability. ROI SD/Speed warrants further evaluation as a quality assessment metric, to allow objective operator assessment of individual 2D-SWE measurement reliability in real-time.

Key words:

Ultrasound, 2D shear wave elastography (2D-SWE), liver, fibrosis, interquartile range (IQR), Obesity

Introduction

The management of chronic liver disease relies heavily on the accurate estimation of liver fibrosis, as this is an important prognostic indicator of future clinical outcome. Whilst liver biopsy remains the gold standard for fibrosis assessment, it has a number of inherent limitations which have seen its clinical use decline over recent years. The invasive test is painful, results in hospitalization in 3% of patients and has a procedural mortality rate of approximately 0.01%.[1,2] The test is also prone to sampling error, with only 1/50,000th of the total liver volume obtained at biopsy.[3-5]

Increasing numbers of non-invasive elastography tools have been developed, which allow clinicians to estimate the severity of a patient's liver fibrosis whilst avoiding the risks of biopsy. These include Transient Elastography (Fibroscan®; Echosens, France), Acoustic Radiation Force Impulse (ARFI) imaging (Siemens Medical Solutions, Germany), Magnetic Resonance Elastography (MRE), Aixplorer® (Supersonic Imagine, Aix-en-Provence, France) and more recently ElastPQ® (Philips Healthcare, USA). Reliability issues however exist for the most widely utilized of these tools, Fibroscan® and ARFI, whose performance is variably affected by necroinflammation,[6,7] obesity,[8,9] ascites, narrow intercostal spaces,[10] and operator inexperience.[8]

Shear Wave Elastography utilizing ARFI technology is now sub-classified as point SWE (pSWE) where a very small volume of tissue is stimulated, and two-dimensional (2D) SWE where the elasticity profile of a larger section of tissue is evaluated.[11] A new 2D-SWE technique has been developed by Toshiba Medical Systems

Corporation (Tochigi, Japan), which uses shear waves to interrogate the viscoelastic properties of tissues. Ultrasound ‘push pulses’ are used to generate shear waves in the liver, whose propagation is subsequently monitored with ‘tracking pulses’; similar to some other technologies in use. Shear wave arrival times (or ‘wavefronts’) at different locations are plotted, allowing a 2-Dimensional map of shear wave properties within a section of liver to be generated. These shear wave display maps can be viewed in ‘continuous’ mode, however a higher quality ‘single shot’ mode is chosen for quantitative analysis. This involves obtaining a static image of shear wave characteristics within an arc shaped section of liver measuring approximately 35mm in maximal width and 30mm in axial depth. A circular measurement ‘region of interest’ (ROI) of fixed 1cm diameter is subsequently positioned in an area suitable for quantitative assessment (Figure 1). The device automatically calculates the shear wave velocity (meters/second) and Young modulus (kPa) for the chosen ROI; the former recommended by the manufacturer for liver fibrosis quantification. The standard deviation of shear wave velocities within the measurement ROI (ROI SD) is also generated for each individual measurement (Figure 2). Like other ultrasound based elastography tools, 2D-SWE is only able to evaluate areas of liver accessible via a reliable acoustic window (i.e. predominantly the right hepatic lobe). Whilst this introduces a degree sampling error, these technologies are nonetheless able to assess a significantly larger area of liver parenchyma than biopsy.

The technique is novel in the provision of two display maps for the ‘single shot’ acquisition, which provide different visual representations of the liver’s shear wave profile. The first is the ‘Speed Smart Map’ (Figure 1) which provides a color representation of shear wave velocities within a section of liver; similar to that used by Supersonic Shear Imaging (Aix-en-Provence, France). The 'Propagation Map' is however unique to the Toshiba system, and uses contour lines to depict shear wave arrival times at different points in the tissue (Figure 1). These two display maps provide different but complementary information regarding regional shear wave propagation. The additional information provided by the Propagation Map is designed to allow operators to better assess the suitability of 'single shot' acquisitions for quantitative analysis and to optimize ROI positioning.

The impact of scan acquisition parameters on these technical innovations has not been established, with limited published data currently available for this 2D-SWE technique. Whilst best acquisition practices for related elastography technologies provide a good starting point, there are several unknowns which prevent their direct implementation for 2D-SWE. The aim of our study was therefore to evaluate these technical questions in subjects with diffuse liver disease to assist in the formation of acquisition guidelines. This included analyzing the variability of 2D-SWE measurements obtained within each patient, as well the number of 2D-SWE measurements required per subject to provide a precise assessment of liver elasticity.

Materials and Methods

Subjects:

Fifty-five patients with diffuse chronic liver disease of mixed etiology were prospectively enrolled in the study. Participants were recruited consecutively, having been clinically referred for the assessment of liver fibrosis. Patients were required to be over 18 years of age for study entry. There were no further exclusion criteria relating to patient demographics, clinical history or chronic liver disease severity. The study was approved by the Melbourne Health Research Ethics Committee and all participants provided informed consent prior to participation.

Patient clinical information:

Information relating to patient demographics and liver disease etiology was obtained from medical records. Height and weight measurements were recorded at the time of 2D-SWE for use in Body Mass Index calculations (BMI = weight (kg) / height $(\text{meters})^2$). The presence and severity of steatosis was graded according to the level of echogenicity and beam attenuation observed on B-mode ultrasound.[12] Liver biopsy results were not available.

Shear Wave Elastography:

2D-SWE measurements were acquired with the Aplio 500 Platinum Series ultrasound machine (Toshiba Medical Systems Corporation, Tochigi, Japan) using the PVT-375BT probe (6-1.9MHz). Measurements were acquired by a single operator, which included one of three experienced sonographers. All three operators received basic training in 2D-SWE prior to study commencement, however had significant pSWE experience having completed in excess of 100 ARFI scans previously. The absence of ascites was confirmed on B-mode imaging prior to quantitative assessment.

Patients were fasted for six hours prior to testing and measurements were acquired in the supine position with the right arm abducted. The scan was performed via an intercostal approach with breathing held in light suspension. Measurements were taken in a single imaging session of less than 10 minutes duration, with the probe firmly applied to achieve acoustic coupling, whilst avoiding compression. Measurements were taken from the right hepatic lobe through a single intercostal space with a good acoustic window. Measurements were obtained in liver parenchyma away from vascular or biliary structures. Samples were taken from non-identical positions within these above parameters. The measurement ROI was positioned in areas of greatest shear wave uniformity; based on the combined and equal review of the Propagation Map and Speed Smart Map. Areas ideal for quantitative assessment were indicated by parallel lines on the Propagation Map and relatively homogeneous color on the Speed Smart Map (Figure 1). Note was made to avoid areas of non-filling on the Speed Smart Map and irregular or fragmented lines on the Propagation Map; indicative of unreliable shear wave characteristics. On the occasion that a 'single shot' acquisition showed no areas suitable for quantitative assessment, the 'single shot' image was reacquired. Whilst fibrosis may not be uniform the assumption is made that the most accurate results are likely to be when there is visible homogeneity within the measurement ROI, as this removes artefactual or technical sources of variation. Ten readings were obtained per patient, one per 'single shot' acquisition. Operators were blinded to all individual measurement velocities during the acquisition process via a screen shield. The ratio of ROI SD to the mean shear wave velocity of the corresponding individual measurement (ROI SD/Speed) was calculated. This was assessed as a potential indicator of individual measurement reliability, akin to the use of IQR/Median in assessing the reliability of measurement sets overall. The skin-to-liver capsule distance (SLD) was measured to two decimal points using the picture archiving and communication service (PACS) ruler function (Figure 2). The distance from the liver capsule to ROI center was also manually measured for each individual measurement.

Statistical analyses:

The interquartile range to median ratio (IQR/Median) was assessed for 2D-SWE measurement sets. This indicates the amount of spread between acquired measurements and is a primary indicator of measurement variability in both ARFI and Fibroscan®.[13-15] Cronbach's alpha was used to determine the internal consistency of readings and Bland Altman plot for the analysis of optimal measurement number.[16,17] Measurement distribution was assessed using skewness and kurtosis.[18] The relationship between patient factors and IQR/Median ratios were assessed using Spearman's rank correlation for continuous data and Kruskal-Wallis test for categorical data. The impact of factors affecting the reproducibility of individual measurements (i.e. ROI depth or ROI SD/Speed) were assessed by calculating the deviation of individual 2D-SWE measurements from the set's median velocity of 10 readings. Intra-operator and inter-operator reliability were not assessed.

Results

Patient characteristics:

The cohort consisted of 55 patients, of which 55% were male with an age range of 21 to 89 years. Patients with a normal BMI (<25kg/m^2, n=20), overweight BMI (25–30kg/m^2, n=19) and obese BMI (>30kg/m^2, n=16) were all represented. The most common liver disease etiologies were non-alcoholic fatty liver disease (NAFLD, n=17), hepatitis B (n=16) and hepatitis C (n=9). Patient characteristics are summarized in Table 1.

Measurement variability:

A large variation in shear wave velocities was observed between individuals (Figure 3), with the median overall 2D-SWE of patients being 2.10m/s (q1-q3: 1.81–2.89m/s).

The median interquartile range (IQR) of 2D-SWE measurement sets was 0.275 (q1-q3: 0.180–0.575) and the median IQR/Median ratio was 0.131 (q1-q3: 0.089–0.174). There was very high internal consistency between measurements obtained within each patient, with Cronbach's alpha being 0.937 for 5 readings.

The number of measurements required to adequately approximate the set's median of ten measurements was analyzed. When increasing numbers of measurements were obtained/analyzed, the calculated velocity became predictably closer to the set's overall median of 10 readings (Figure 4). The median of five measurements provided a velocity estimate within 0.11m/s or 4.2% of the set's overall median. The Bland Altman limits of agreement was -0.254 to 0.374m/s for five measurements.

2D-SWE velocities from each of the 55 measurement sets did not follow a normal distribution, with kurtosis ranging between 3.2 and 5.6 and skewness ranging between 1.0 and 1.6. Individual 2D-SWE sets followed a Gamma distribution, with measurements slightly skewed towards higher shear wave velocities.

Factors affecting measurement variability:

The association between patient factors and measurement reproducibility was assessed in univariate analyses (Table 1). BMI was the primary factor associated with increased IQR/Median ratios (rho=0.388, p=0.01), with overweight and obese patients (BMI>25g/m^2) demonstrating higher average IQR/Median ratios than those with normal BMI (0.149 vs. 0.112, p=0.013). Moderate to severe steatosis on ultrasound, increasing age and a clinical diagnosis of NAFLD were additional factors which showed non-significant trends towards higher IQR/Median ratios (Table 1).

Skin-to-liver capsule distance (SLD) also showed a moderately strong correlation with IQR/Median (rho=0.426, p=0.002, Table 1). Measurements with a SLD >2cm showed considerably greater deviation from the set's median of 10 readings (average deviation = 0.501 vs. 0.268m/s). SLD values were also closely related to BMI (rho=0.787, p=0.01).

The region of interest (ROI) may be positioned at different depths beneath the liver capsule. A greater spread of measurement velocities was observed when the ROI center was positioned within 1.5cm of the liver capsule (Table 2). These subcapsular measurements showed significantly greater deviation from the set's overall speed (average deviation = 0.39m/s) than measurements taken more deeply in the liver (average deviation = 0.20m/s, $p<0.001$).

The ROI SD/Speed ratio had a strong relationship with the amount individual measurements deviated from the set's overall median velocity (Table 3). Measurements with a ROI SD/Speed >0.15 showed low reliability, deviating an average of 0.42m/s from the set's overall median, compared to 0.22m/s for measurements with a ROI SD/Speed≤0.15 ($p=0.001$). Patient factors associated with increased ROI SD/Speed ratios included increasing BMI (rho=0.499, $p=0.003$) and SLD (rho=0.604, $p<0.001$). Weaker correlations were also seen with age (rho=0.307, $p=0.09$) and the clinical diagnosis of NAFLD ($p=0.04$). A positive correlation was also observed between ROI SD/Speed and median shear wave velocities (rho=0.496, $p<0.001$). The median ROI SD/Speed was also significantly higher amongst subcapsular measurements taken within 1cm from the liver capsule than those taken more deeply in the liver (ROI SD/Speed = 0.169 vs 0.117, $p=0.002$).

Discussion

The new 2D-SWE technique showed high measurement reproducibility, with a low spread in shear wave velocities (i.e. low IQR/Median) relative to the most widely utilized elastography tools in clinical use.[7,14,15] Measurement variability, as indicated by IQR/Median ratio, is a powerful predictor of accuracy for both ARFI[13,14] and Transient Elastography.[15] The low variability of 2D-SWE measurements is therefore encouraging for future accuracy studies.

The low measurement variability of 2D-SWE also translated into fewer measurements being required to adequately estimate liver stiffness. Acquiring five 2D-SWE measurements yielded a liver stiffness estimate within 5% of the overall median velocity of ten readings. This 5% deviation threshold has been previously used to recommend a minimum of eight measurements for ARFI.[19] Five measurements would therefore appear sufficient using this 2D-SWE technique. The level of deviation is unlikely to have significant impact on the assignment of fibrosis categories, in view of the broad range of median shear wave velocities observed between patients (Figure 3). Given the inherent technical differences between 2D-SWE tools, this measurement number recommendation is specific to the Aplio 500 device and may not be directly transferrable to related technologies.

The low intrinsic measurement variability observed with this 2D-SWE technique is likely attributable to the provision of the 'Speed Smart Map' and 'Propagation Map'. The novel 'Propagation Map' is not shared by other conventional elastography methods and provides complementary information which allows operators to better visualize and evaluate regional shear wave propagation characteristics.[20] This additional information may enable operators to better assess 'single shot' acquisitions, allowing the rejection of acquisitions which are of insufficient quality for quantitative analysis. The display modes also provide guidance for the optimization of ROI positioning; enabling operators to avoid regions with heterogeneous propagation characteristics which may yield aberrant results.

Our results likely overestimate 2D-SWE's measurement variability in optimal conditions. Study operators were inexperienced with 2D-SWE at study commencement, which resulted in a number of subcapsular measurements being acquired. These measurements are associated with reduced measurement reproducibility (described below) and their inclusion in composite analyses may have increased the observed IQR/Median ratios. Furthermore, our study had a relatively high prevalence of obesity which may similarly elevate the overall measurement variability observed. The recommendation of a minimum of five measurements is therefore likely conservative and fewer readings may be acceptable under optimal conditions. This is illustrated in Figure 4, in which the exclusion of subcapsular measurements alone reduced the measurement requirement to four. Five readings are however an appropriate recommendation for 'real-world' conditions and amongst similar patient populations.

The observation that 2D-SWE measurement sets do not follow a binomial distribution also suggests that the set's median velocity may be a more robust representation of a patient's overall shear wave velocity than the set's mean.

Obesity:

We found BMI to be the predominant factor affecting measurement variability, with particularly high IQR/Median ratios observed amongst obese patients. A similar association has been previously reported for ARFI[9,21] and Transient Elastography[8,9] and our results provide additional evidence to a possible 'class effect' of obesity on ultrasound-based elastography tools in general. Whilst the impact of obesity on the accuracy of this 2D-SWE technique requires assessment, our findings provide early caution that similar reliability issues may be encountered amongst this population subset.

Obesity's negative impact on ultrasound elastography has been hypothesized to involve ultrasound beam attenuation and degradation from increasing depths of subcutaneous adipose tissue.[9,21] The skin-to-liver capsule distance (SLD) approximates the amount of subcutaneous adipose tissue traversed by the ultrasound beam and has been found to be a powerful predictor of measurement reliability in ARFI.[22] We found SLD to have a stronger correlation with IQR/Median than BMI, which lends weight to this hypothesis. This is further supported by the observation of higher ROI SD/Speed ratios amongst patients with increased SLDs (Table 1), indicating degradation in shear wave quality from central adiposity.

Whilst we did not formally control for probe pressure in this study, we feel this is unlikely to be a major determinant of SLD. Drawing from unpublished results from our own group looking at 943 patients tested with ARFI by multiple independent operators, we found low inter-operator variability in SLD values obtained within each patient. This suggests any potential difference in operator acquisition technique does not significantly impact on SLD values. The strength of the correlation between BMI and SLD further supports body habitus being the key determinant of SLD, rather than operator technique.

Region of Interest Depth in the Liver:

We found poorer measurement reproducibility when the center of the ROI was positioned within 1.5cm of the liver capsule. This observation is consistent with existing pSWE studies, which have associated subcapsular readings with artificially elevated fibrosis scores, increased measurement variability and reduced accuracy.[23,24] ARFI guidelines generally now recommend measurements be taken at least 1cm, and preferably 2cm, from the liver capsule. Our results suggest a similar recommendation should be instituted for the Toshiba system. The phenomenon has been previously attributed to a band of physiologic fibrosis underlying the liver capsule, resulting in disproportionately high shear wave velocities relative to the remaining liver parenchyma. We also speculate that a more important factor is likely to be subcapsular reverberation resulting in regional shear wave degradation. This is suggested by 2D-SWE's Speed Smart Maps, which show an area of heterogeneous shear wave velocity immediately underlying the liver capsule in some patients (Figure 1 - 2A). This regional degradation of shear wave profiles is further evidenced by the observation of higher ROI SD/Speed ratios amongst subcapsular measurements (Table 2).

ROI SD/Speed:

The ROI SD/Speed reflects the variation in shear wave velocities recorded within a measurement ROI on the Speed Smart Map. Measurements with a low ROI SD/Speed reflect regions of liver parenchyma with uniform shear wave profiles, whilst noisy or heterogeneous ROIs translate into higher values. We expected measurements with uniform shear wave profiles would yield the most reliable liver stiffness readings and our results support this hypothesis. We found a strong positive correlation between measurement variability and ROI SD/Speed, with poor measurement consistency observed once the ROI SD/Speed ratio exceeded 0.15.

The ROI SD/Speed ratio may represent an objective and quantifiable indicator of individual measurement reliability. It has the advantage of being readily assessable during scan acquisition and so has real potential for clinical application. ROI SD/Speed could potentially assist in the optimization of ROI positioning; providing operators with an additional quantifiable indicator of shear wave uniformity in addition to the Speed Smart Map and Propagation Map. The value could potentially alert operators to issues in acquisition technique and may present a means of providing dynamic feedback during operator training or technique modification. ROI SD/Speed ratio may also have utility in stratifying the relative reliability of individual measurements, potentially allowing the rejection of unreliable readings from the final measurement set.

Future studies are however required to further explore these potential clinical applications. The relationship between ROI SD/Speed and 2D-SWE accuracy also requires assessment, as this will help determine the true utility of ROI SD/Speed as a clinical tool. ROI SD/Speed may also have relevance for Supersonic Shear Imaging (SSI), which also generates a standard deviation of shear wave velocities obtained within each measurement ROI.

Limitations:

Our study did have limitations. Firstly, the study was a technical paper which aimed to address very specific questions regarding 2D-SWE measurement variability and required measurement number. Liver biopsy is performed in a minority of CLD patients as part of local clinical practice, and a histopathologic correlate was therefore only available for nine patients in the cohort (biopsy performed a median of 5.4 years from 2D-SWE) . Due to the very specific question being addressed, performing a liver biopsy for research purposes was felt unjustified in this study. As a consequence, the accuracy of this new 2D-SWE technology cannot be derived from our results. This will need to be assessed, together with intra-operator / inter-operator reliability in future studies. Nonetheless, our results assist in the formation of acquisition protocols which will facilitate the rigorous and standardized assessment of 2D-SWE accuracy and performance in future clinical trials.

The second limitation was the study's small size; the cohort comprising only 55 patients. The study's primary findings were nonetheless all drawn from results of high statistical significance, which underscores the potential clinical relevance of the study's findings.

Conclusion

The new 2D-SWE technique showed low variability between measurements, with a minimum of five readings required to provide a reliable estimation of liver fibrosis.

Factors associated with increased measurement variability include increased BMI, a SLD over 2cm and subcapsular measurements. ROI SD/Speed showed a strong relationship with measurement variability and may allow operators to objectively assess the reliability of individual measurements in real-time during the acquisition process.

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- There are no commercial interests which need to be disclosed for the above authors.

References

1. Janes CH, Lindor KD. Outcome of patients hospitalized for complications after outpatient liver biopsy. *Ann Intern Med* 1993; 118(2):96-98. 3.

2. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. *Hepatology* 2009; 49(3):1017-1044.

3. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; 38:1449-1457.

4. Lee RG. *Diagnostic Liver Pathology. St. Louis: Mosby-Year Book.*; 1994.

5. Maharaj B, Maharaj RJ, Leary WP, et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. *Lancet* 1986; 1:523-525.

6. Arena U, Vizzutti F, Corti G, et al. Acute viral hepatitis increases liver stiffness values measured by transient elastography. *Hepatology* 2008; 47:380-384.

7. Yoon KT, Lim SM, Park JY, et al. Liver stiffness measurement using acoustic radiation force impulse (ARFI) elastography and effect of necroinflammation. *Dig Dis Sci* 2012; 57(6):1682-1691.

8. Castéra L, Foucher J, Bernard PH, et al. Pitfalls of liver stiffness measurement: A 5-year prospective study of 13,369 examinations. *Hepatology* 2010; 51:828-835.

9. Cassinotto C, Lapuyade B, Aït-Ali A, et al. Liver fibrosis: noninvasive assessment with acoustic radiation force impulse elastography--comparison with FibroScan M and XL probes and FibroTest in patients with chronic liver

disease. *Radiology* 2013; 269:283-292.

10. Sandrin L, Fourquet B, Hasquenoph J-M, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; 29(12):1705-1713.

11. Barr RG, Ferraioli G, Palmeri ML, et al. Elastography Assessment of Liver Fibrosis: Society of Radiologists in Ultrasound Consensus Conference Statement. *Radiology* 2015; 0(0):150619.

12. Gerstenmaier JF, Gibson RN. Ultrasound in chronic liver disease. *Insights Imaging* 2014; 5(4):441-455.

13. Bota S, Sporea I, Sirli R, Popescu A, Jurchis A. Factors Which Influence the Accuracy of Acoustic Radiation Force Impulse (ARFI) Elastography for the Diagnosis of Liver Fibrosis in Patients with Chronic Hepatitis C. *Ultrasound Med Biol* 2013; 39:407-412.

14. Goertz RS, Sturm J, Pfeifer L, et al. ARFI cut-off values and significance of standard deviation for liver fibrosis staging in patients with chronic liver disease. *Ann Hepatol* 2013; 12:935-941.

15. Lucidarme D, Foucher J, Le Bail B, et al. Factors of accuracy of transient elastography (fibroscan) for the diagnosis of liver fibrosis in chronic hepatitis C. *Hepatology* 2009; 49(4):1083-1089.

16. Cronbach LJ. Coefficient alpha and the internal structure of tests. *Psychometrika* 1951; 16(3):297-334.

17. Bland JM, Altman DG. Statistical Methods for Assessing Agreement Between Two Methods of Clinical Measurement. *Lancet* 1986; 327:307-310.

18. Groeneveld RA, Meeden G. Measuring skewness and kurtosis. *J R Stat Soc Ser D (The Stat*) 1984; 33(4):391-399.

19. Karlas T, Pfrepper C, Wiegand J, et al. Acoustic radiation force impulse imaging (ARFI) for non-invasive detection of liver fibrosis: examination standards and evaluation of interlobe differences in healthy subjects and chronic liver disease. *Scand J Gastroenterol* 2011; 46:1458-1467.

20. Iijima H. Medical Review. Approaches to the Diagnosis of Liver Fibrosis. *Toshiba Medical Systems Corporation. White Paper.* 2014

21. Cui J, Heba E, Hernandez C, et al. Magnetic resonance elastography is superior to acoustic radiation force impulse for the Diagnosis of fibrosis in patients with biopsy-proven nonalcoholic fatty liver disease: A prospective study. *Hepatology* 2016; 63(2):453-461.

22. Nadebaum D, Gibson R, Howell J, et al. Central obesity negatively impacts the performance of Acoustic Radiation Force Impulse Imaging (ARFI) in the assessment of liver fibrosis. Hepatology 2014; 60(6): 1288A-1288A.

23. Sporea I, Sirli RL, Deleanu A, et al. Acoustic radiation force impulse elastography as compared to transient elastography and liver biopsy in patients with chronic hepatopathies. *Ultraschall Med* 2011; 32 Suppl 1:S46-S52.

24. Chang S, Kim M-J, Kim J, Lee M-J. Variability of shear wave velocity using different frequencies in acoustic radiation force impulse (ARFI) elastography: a phantom and normal liver study. *Ultraschall Med* 2013; 34(3):260-265.

Tables:

Table 1: Demographics and disease characteristics of the patient cohort, with the median IQR/Median and ROI SD/Speed listed for each patient subset. BMI and skin-to-liver capsule distance (SLD) showed the strongest associations with both IQR/Median and ROI SD/Speed.

Patient Characteristics	**No. of patients**	**IQR/Median, median (q1-q3)**	**Significance of IQR/Median differences (p value)**	**ROI SD/Speed, median (q1-q3)**	**Significance of ROI SD/Speed differences (p value)**
Gender					
Male	30	0.137 (0.088-0.269)		0.155 (0.101-0.208)	0.171
Female	25	0.129 (0.101-0.161)	0.636	0.123 (0.081-0.174)	
Age (years)					
<40	13	0.110 (0.102-0.149)	0.624	0.104 (0.077-0.174)	0.09
40-60	28	0.130 (0.104-0.136)		0.132 (0.101-0.174)	
>60	14	0.145 (0.089-0.282)		0.183 (0.157-0.212)	
Body Mass Index (BMI, kg/m^2)					
<25	20	0.112 (0.085-0.128)	0.013	0.101 (0.079-0.119)	0.003
25 – 30	19	0.134 (0.074-0.296)		0.153 (0.088-0.178)	
>30	16	0.165 (0.138-0.259)		0.180 (0.154-0.211)	
Liver disease etiology					
NAFLD	17	0.141 (0.129-0.250)	0.145	0.177 (0.151-0.196)	0.043
Hepatitis B	16	0.115 (0.072-0.154)	0.129	0.113 (0.101-0.201)	0.882
Hepatitis C	9	0.118 (0.110-0.269)	0.570	0.122 (0.113-0.222)	0.946
Alcohol	7	0.108 (0.088-0.152)	0.579	0.147 (0.099-0.177)	0.84
Other	12	0.168 (0.119-0.174)	0.757	0.174 (0.125-0.187)	0.105
Hepatosteatosis					
No	26	0.123 (0.089-0.168)	0.543	0.123 (0.099-0.187)	0.351
Mild	15	0.121 (0.088-0.161)		0.153 (0.076-0.184)	

Moderate/Severe	14	0.141 (0.108-0.272)		0.158 (0.141-0.195)	
SLD (Set average)					
<1.5cm	12	0.105 (0.070-0.119)	0.002	0.081 (0.073-0.107)	<0.001
1.5 to 2cm	21	0.118 (0.890-0.142)		0.147 (0.099-0.177)	
>2cm	22	0.164 (0.141-0.272)		0.177 (0.152-0.208)	

Table 2: Relationship between measurement depth in the liver (capsule to ROI distance) and measurement reliability. Sub-capsular measurements showed greater deviation from the set's overall median velocity and higher ROI SD/Speed ratios.

Capsule to ROI Distance	**Median deviation from the set's overall velocity (m/s)**	**Median ROI SD/Speed ratio**
<1cm	0.578	0.169
1 to 1.5cm	0.284	0.122
1.5 to 2cm	0.191	0.109
>2cm	0.218	0.119

Table 3: Relationship between ROI SD/Speed and measurement reliability. Individual measurements with a ROI SD/Speed ratio >0.15 showed increased deviation from the set's overall median velocity.

Individual measurement ROI SD/Speed	Number of readings (%)	Average deviation of individual measurements from the set's overall median velocity (m/s)
<0.05	32 (6%)	0.090
0.05 – 0.099	163 (30%)	0.199
0.10 – 0.149	135 (24%)	0.272
0.15 – 0.199	92 (17%)	0.428
≥0.20	128 (23%)	0.413

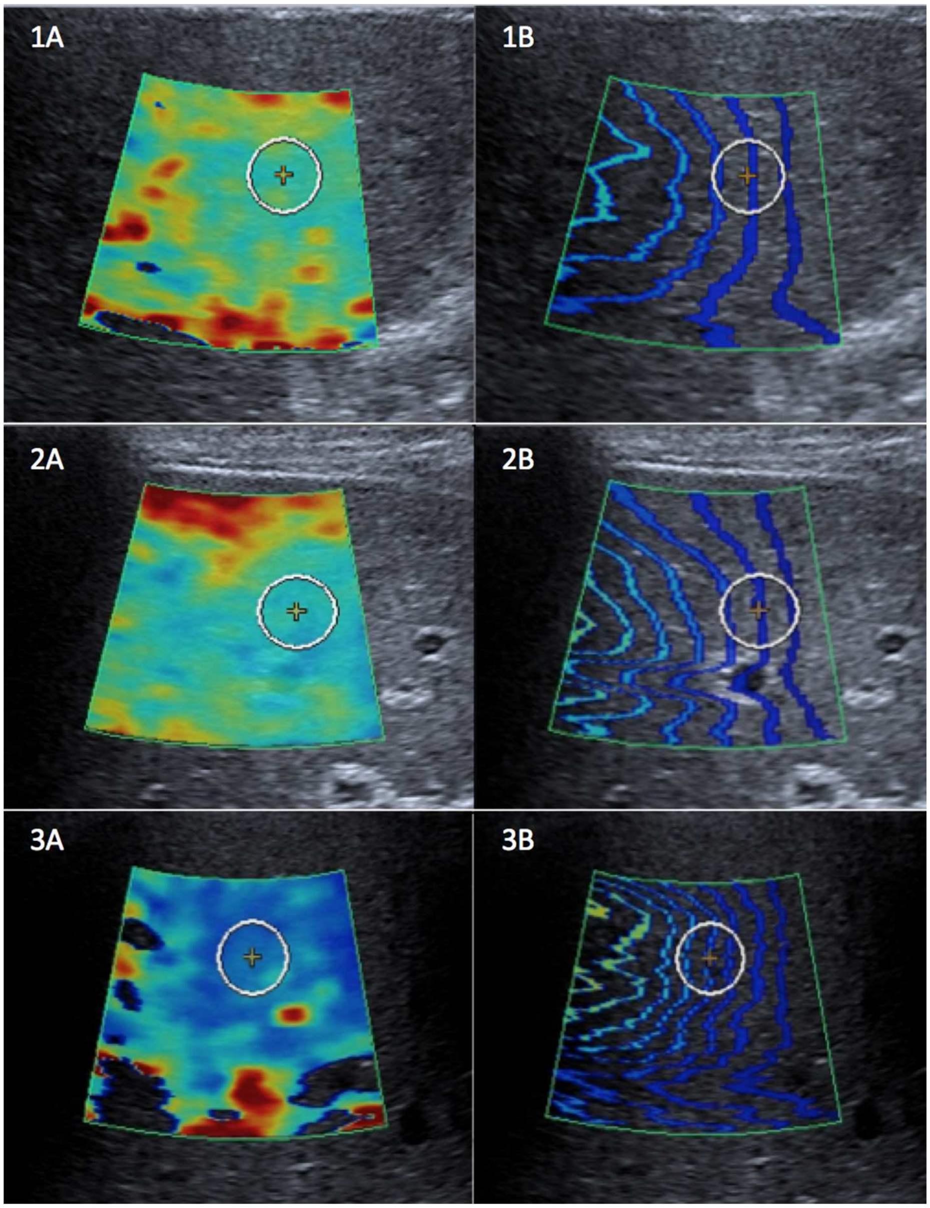
Figures:

Figure 1: Speed Smart Maps (1A, 2A, 3A) show the distribution of shear wave velocities through a section of liver; red areas representing high velocities and blue/green areas low velocities. Propagation Maps (1B, 2B, 3B) show the arrival time contours of shear waves. The region of interest (ROI) is positioned in an area with uniform shear wave characteristics; as indicated by homogeneous color on the Speed Smart Map and parallel contour lines on the Propagation Map.

Figure 2: Screenshot of a 2D-SWE measurement. The shear wave velocity and standard deviation of shear wave velocities within the measurement region of interest (ROI SD) are automatically generated and displayed. Skin-to-liver capsule distance and liver capsule to ROI depth were measured as indicated.

Figure 3: Spread in median shear wave velocities of the cohort's 55 patients. Overall 2D-SWE speeds ranged between 1.49 to 5.30m/s, with a median of 2.10m/s.

Figure 4: Closeness in approximation to set's overall median of 10 measurements, according to the number of measurements obtained/analyzed. The graph shows the average deviation +/- SEM. Five measurements yielded a liver stiffness approximation below the 5% deviation threshold (blue). Four measurements were however required if only readings taken >1.5cm deep to the liver capsule were analyzed (red).



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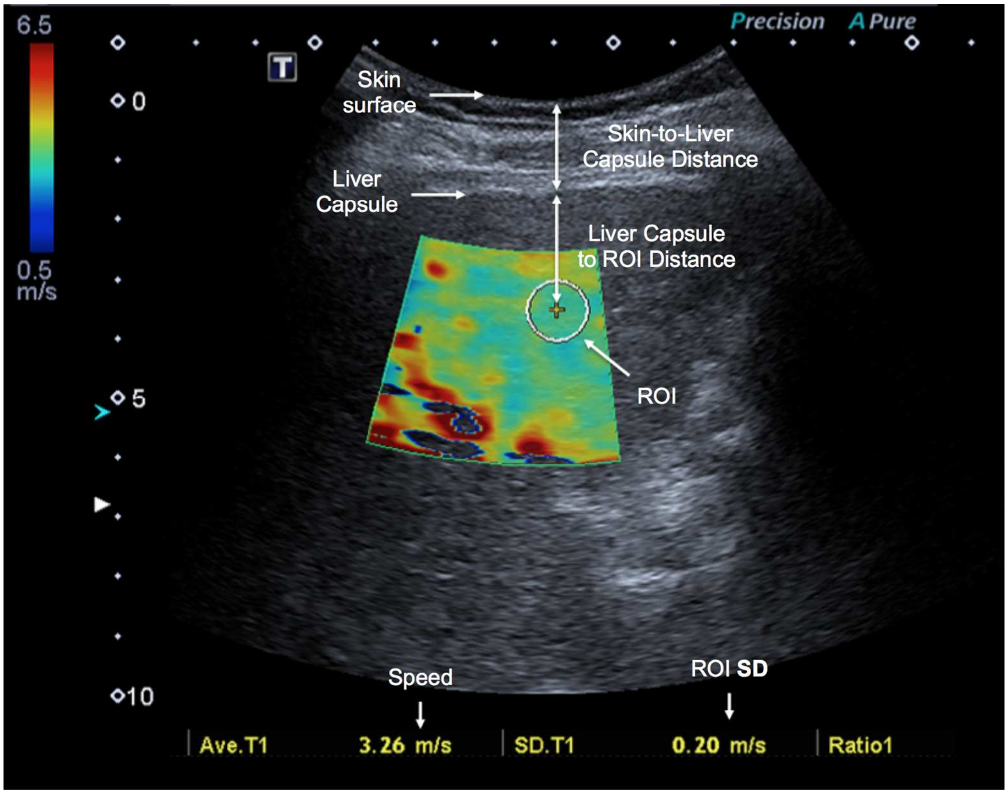

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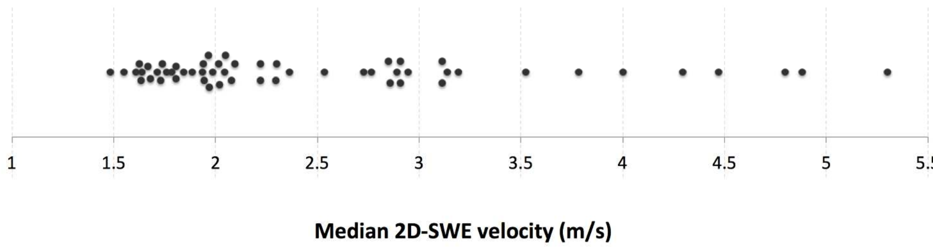

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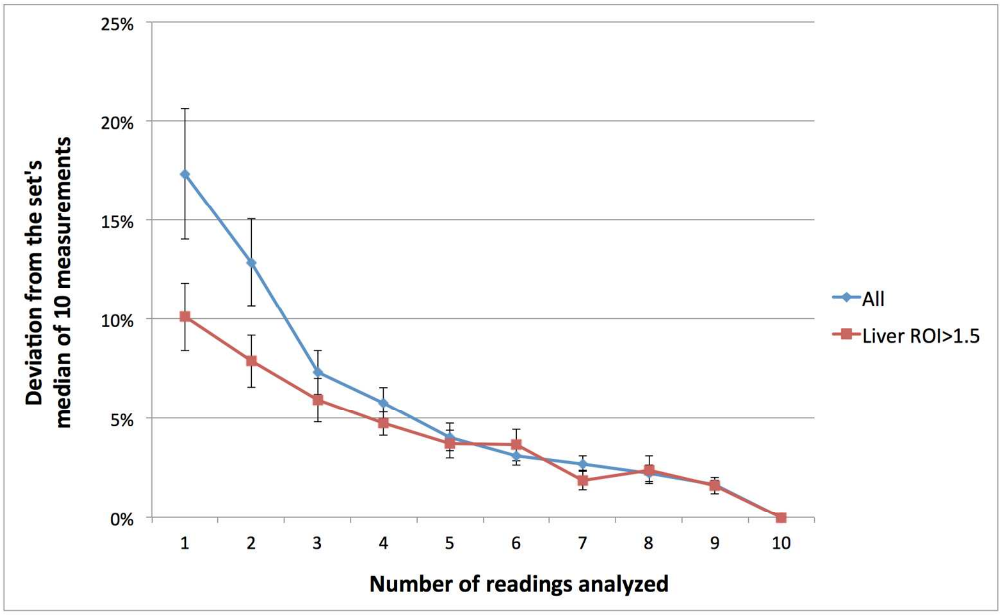

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