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Title:

Acoustic Radiation Force Impulse accuracy and the impact of hepatic steatosis on liver fibrosis staging

Running title:

ARFI accuracy in hepatic steatosis

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Introduction:
The accuracy of Acoustic Radiation Force Impulse (ARFI) imaging has been validated in the setting of hepatitis C, however the accuracy in the setting of fatty liver disease (FLD) has been less well established. The aim of this study was to assess the accuracy of ARFI in the setting of hepatic steatosis.

Methodology:
Patients with biopsy proven or sonographically diagnosed liver steatosis were assessed for ARFI trends including: inter-operator concordance, interquartile range, ARFI failure rate, relationship between ARFI velocity and steatosis severity, and concordance between biopsy and ARFI fibrosis scores.

Results:
Three hundred and forty-nine patients were assessed (53 “biopsy” cohort and 296 “ultrasound” cohort), with 28 patients having biopsy on the same day as ARFI.
Low stages of fibrosis (F0/1) were over-estimated by ARFI in 62% of cases with biopsy correlation (n=16, $p<0.001$), with ARFI offering increased accuracy in regard to higher-stage fibrosis (14/15 cases, 93%). In both the biopsy and ultrasound cohorts the failure rate and median inter-quartile range increased with increasing steatosis, and the inter-operator concordance remained good across all liver steatosis severities.

Conclusion:
In the setting of steatosis, ARFI is very sensitive in detecting, and accurate in diagnosing, higher stages of fibrosis regardless of steatosis severity. It tends to overestimate the fibrosis category in lower stages of fibrosis. The present study does not show conclusively if the presence of steatosis or its severity independently alters ARFI measurements.

Keywords: Acoustic Radiation Force Impulse, ARFI, liver steatosis, accuracy, liver, fibrosis

Introduction:

Non-alcoholic fatty liver disease (NAFLD) has become a major health problem and is now the commonest cause of chronic liver disease in Australia, affecting approximately 5.5 million Australians[1]. It is estimated that NAFLD progresses to non-alcoholic steatohepatitis (NASH) in one third of cases, with a proportion of these progressing to cirrhosis and its complications[2,3]. Hepatic steatosis is also significant in alcohol-related liver disease, in chronic hepatitis C, and some drug induced liver injury, as well as a few rare metabolic disorders.

Liver fibrosis is the best prognostic indicator for all chronic liver diseases, and liver biopsy remains the gold standard for staging fibrosis. However, it is invasive, expensive, prone to sampling error and inter-observer variability, and carries a mortality risk[4,5]. Given these drawbacks and the prevalence of chronic liver disease and associated risk of fibrosis, non-invasive techniques to assess for fibrosis are becoming increasingly important. Ultrasonic based elastography is the most widely utilised non-invasive technique, with Transient Elastography (Fibroscan®, Echosens™, Paris, France) and Acoustic Radiation Force Impulse (ARFI) imaging (Siemens, Mountain View, CA, USA) the most commonly used[4,5].

ARFI imaging is the relative newer technique that measures tissue elasticity by measuring the velocity of ultrasound generated shear waves. Increasing stages of liver fibrosis decreases tissue elasticity, and increases shear wave velocity through the liver parenchyma[4]. Unlike Fibroscan®, it has the advantage of being integrated into a conventional ultrasound-imaging unit, and has a lower reported failure rate in obtaining adequate readings[6]. Research has established the accuracy of ARFI in hepatitis C infection in particular; however, there have been concerns that results are affected by concomitant steatosis and/or inflammation. Similarly, the accuracy of data regarding ARFI in assessing fibrosis in other liver diseases, especially NAFLD is very limited. Truncal obesity is a major impediment to all ultrasound elastography techniques, with reduced accuracy with increasing skin to liver capsule distance[7]. Furthermore, ARFI has been shown to be less reliable in obese patients, a significant risk factor for NAFLD[8,9], and may be less reliable in the presence of fatty liver disease of any cause. Meta-analyses assessing the non-invasive ultrasound methods of investigating fibrosis have identified a need to assess the accuracy and failure rate of ARFI in the setting of steatosis[5,10].

The aim of this study was to assess the accuracy of ARFI in the setting of steatosis by reviewing patients with biopsy proven and sonographically identified hepatic steatosis.

Methodology:

This was a retrospective study of consecutive patients with chronic liver disease (regardless of aetiology) referred for ARFI examination at our tertiary level institution over a nine-month period. During this period, two operators obtained ARFI measurements independently of each other and blinded to the others' results ("ultrasound" cohort). Additionally the ultrasound imaging and liver biopsy histopathology results were reviewed of any patient who underwent a liver biopsy within 6 months of having ARFI imaging performed since the commencement of ARFI imaging at our institution in August 2012 ("biopsy" cohort).

Cases were identified using a prospectively collected database of all ARFI cases, which included patient demographics, clinical findings, biochemistry, histopathology, and all ARFI readings. The Institutional Human Ethics committee approved this study.

Sonographic liver steatosis analysis:

The 2D ultrasound images were assessed retrospectively by a senior radiology trainee (NH) and a senior radiologist with 30 years experience (RG). Discrepancies were resolved by consensus. The presence and degree of steatosis was categorised into two groups: (i) absent or mild steatosis, and (ii) moderate or severe steatosis. The absent or mild steatosis category included cases with normal appearances of liver echogenicity (absent steatosis), or a slight but definite increase in liver echogenicity (mild steatosis) without any of the changes described below in the moderate to severe category. Cases in the moderate to severe steatosis category had a moderate or marked diffuse increase in liver echogenicity with impaired or no visualisation of the portal tracts, plus or minus focal fatty sparing (moderate) or these changes plus beam attenuation (severe)[11].

ARFI analysis:

ARFI was performed using a Siemens Acuson S2000® ultrasound machine (Siemens, Mountain View, CA, USA) and the Virtual Touch Tissue Quantification (VTTQ) imaging application. Studies were performed after a minimum six-hour fast, with the patient supine. Measurements were acquired with the 4C1 convex transducer via a right intercostal approach. Measurements were obtained, during suspended respiration, from the right lobe of the liver in parenchyma away from biliary or vascular structures (as identified on B-mode imaging). Measurements were acquired until ten valid readings had been obtained. No attempt was made to target specific segments of the right lobe.

ARFI was performed by sonographers and one radiologist working within our radiology department, all of who had undertaken basic ARFI training. Patients were routinely examined by two independent operators who were blinded to each other's results.

The median velocity of the ARFI set was converted into corresponding F-scores using cut-offs based on the meta-analysis of Friedrich-Rust *et al*[12], with the threshold for diagnosis of significant fibrosis 1.35 m/s, severe fibrosis 1.55m/s and cirrhosis 1.80 m/s. In our analyses, inter-operator concordance was defined as the two operators having median ARFI velocities that were in the same or adjacent F score range. ARFI failure was defined as a study that failed to obtain, with either operator, the currently accepted quality parameters of an inter-quartile range (IQR):median velocity of >0.3 and/or a success rate (SR) of <60%, with the SR being the number of valid readings divided by the total number of readings[9].

Accuracy was assessed by ARFI median interquartile range, inter-operator concordance, and ARFI failure rate in both groups; and concordance between biopsy fibrosis scores and ARFI fibrosis scores in those with histological results.

Liver biopsy:

The liver biopsies were all percutaneous ultrasound guided core biopsies of the right lobe of the liver using an intercostal approach. The biopsies were performed using a 14 g needle biopsy apparatus (Bard® Magnum reusable core biopsy device, Bard technologies Tempe Arizona, USA; or Achieve programmable automatic biopsy system, CareFusion, San Diego, California, USA). Biopsies included in the study

fulfilled our quality parameters of >15 mm in length and >5 portal tracts visualised. A single pathologist with expertise in hepatology reviewed all of the biopsies. Liver fibrosis was scored using the METAVIR system: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis with few septa, F3 = portal fibrosis with numerous septa without cirrhosis, F4 = cirrhosis[13]. The steatosis component of the NAFLD activity score system was used for categorising steatosis, with the proportion of total hepatocytes affected by steatosis graded as: minimal <5%, mild 5-33%, moderate 33-66%, and severe >66%[14,15].

Statistical analysis:

Statistical analyses were performed using Stata 13 (StataCorp, College Station, TX, USA). Initial descriptive analysis was undertaken to describe biopsy and ultrasound cohorts.

Associations between ARFI velocity and fibrosis or steatosis grade were assessed using Kruskall-Wallis test. Logistic regression and sensitivity analysis were used to determine optimum cut-off points for ARFI velocity and fibrosis stage. Chi-square or Fisher's exact tests were used to determine agreement between fibrosis stage defined by ARFI and biopsy.

Results:

Patient characteristics:

There were 55 patients in the biopsy cohort and 296 patients in the ultrasound cohort. On review of the biopsy cohort, the ARFI results for two patients were noted to be outliers from the remainder of the cohort and were excluded from the analysis leaving a biopsy study cohort of 53.

The characteristics of the patients are demonstrated in Table 1.

I: Biopsy Cohort

Timeline between liver biopsy and ARFI performance:

The number of days between biopsy and ultrasound ranged from 182 days before the biopsy to 159 days after the biopsy, with the majority (28 patients, 52.8%), having their biopsy performed on the same day as the ARFI examination.

Correlation between ARFI median velocity and histology:

Table 2 demonstrates the relationship between median ARFI velocity and histopathology for the entire biopsy cohort. A significant correlation was demonstrated between the median ARFI velocity and the biopsy fibrosis stage with increasing median velocity associated with an increasing fibrosis stage. No significant association was demonstrated between ARFI and degree of steatosis. The difference in median ARFI value was small and was slightly higher in the moderate and severe steatosis patients.

Table 3 demonstrates the relationship between median ARFI velocity and histopathology for those in whom liver biopsy was on the same day as ARFI. As with the total biopsy cohort a significant increase in median ARFI velocity was demonstrated with increasing fibrosis stage. The median ARFI velocity was slightly, but not significantly, lower in cases with moderate and severe steatosis.

Diagnostic performance of ARFI:

Receiver operating curves were used to determine the cut off median ARFI values for each stage of fibrosis (Table 4, Figure 1). ARFI demonstrated poor accuracy in regards to significant fibrosis (F ≥ 2, AUROC of 0.65, 95% CI 0.50-0.80), however improved at detecting more severe fibrosis compared to lower stages (F ≥ 3, AUROC of 0.76, 95% CI 0.61-0.90).

Within the biopsy cohort (n=53) ARFI overestimated fibrosis in the lower stages of fibrosis and was accurate in staging higher-stage fibrosis (p<0.001). ARFI overestimated the fibrosis stage in 62% (16/26) of cases that had a biopsy proven fibrosis score of F0 or F1. ARFI accurately assigned fibrosis scores of F3 or F4 in 93% (14/15) of cases that were biopsy proven F3 or F4. ARFI showed poor reliability in biopsy proven F2, overestimating the degree of fibrosis in 42% cases (5/12) and underestimating in 25% cases (3/12). The performance of ARFI on the same day biopsy cohort was essentially the same as those done within 182 days.

ARFI accuracy also did not improve when adjusted for degree of steatosis. Review of the biopsy cohort (n = 53) demonstrated that in the cases with minimal or mild steatosis (n = 43) ARFI overestimated fibrosis scores in 60% (12/20) with a biopsy proven fibrosis score of F0 or F1, and accurately assigned an F3 or F4 score in 92.3% (12/13). In the cases with moderate or severe steatosis (n = 10) ARFI overestimated fibrosis in 67% (4/6) cases with a biopsy F0 or F1, and was accurate in both patients with F3 or F4. Similar findings were demonstrated on analysis of the biopsy on the same day as ARFI cohort (n = 28). Therefore, steatosis made no difference with accurate readings in F3 and F4, and there was a tendency to over estimate fibrosis in F0-2, regardless of steatosis grade.

II Biopsy and Ultrasound Cohort (combined):

Interquartile range, failure rate and inter-operator concordance:

The interquartile range of the ARFI velocities obtained on an individual patient is used as a surrogate for reliability of readings. Increased steatosis was associated with increased IQR, suggesting lower reliability. The median IQR of 0.23 was seen in cases of no or mild steatosis compared with 0.37 for cases with moderate or severe steatosis (p= 0.001). This trend was the same in the biopsy cohort although the relationship was not statistically significant (p 0.143).

Table 5 and 6 show the IQR, inter-operator concordance and failure rate in the biopsy cohort (Table 5) and the ultrasound cohort (Table 6). Failure rate was defined as IQR:median velocity >0.3 or success rate <60%.. In both the biopsy and ultrasound cohorts, the operator failure rate increased with increasing steatosis. In the biopsy cohort when assessing the moderate to severe steatosis cases, the failure rate increased by 14% for the first operator and by 19% for the second operator. In the ultrasound cohort when assessing the moderate to severe steatosis cases, the failure rate increased by 16% for the first operator and by 17% for the second operator.

There was good concordance at ≥ 80% between the first and second operators regardless of the degree of steatosis in both the ultrasound only and biopsy cohorts.

Discussion:

This is the first study to assess the accuracy of ARFI in the setting of hepatic steatosis in an Australian population. Our study has shown a step-wise increase in ARFI median shear wave velocity with increasing fibrosis stage, consistent with results demonstrated by Yoneda et al.[16] and Fierbintenau-Braticevici et al.[17]. The receiver operating curve analyses did not demonstrate ARFI to have the same degree of accuracy in diagnosing/staging fibrosis as some other reports, which may be due to the small numbers[16, 17,18].

ARFI was shown to offer improved accuracy and sensitivity with higher degrees of fibrosis (F ≥3) consistent with other studies[16,17,18]. One of the important findings on liver histology or ultrasound elastography is to establish if there is high stage fibrosis or cirrhosis present as these patients are those with the poorest prognosis and are at risk for complications. Whilst a high degree of accuracy in the lower stages of fibrosis would be ideal, in general the lower degrees of fibrosis have less importance in terms of management decisions. ARFI was 93% sensitive in detecting advanced fibrosis (F>3).

The ARFI failure rate (i.e. IQR:median velocity >0.3 or success rate <60%.) and median IQR increased with increasing steatosis. These variables have not been analysed in prior studies dedicated to ARFI analysis in the setting of hepatic steatosis. They have

been investigated in studies analysing the impact of obesity on ARFI, but obesity does not necessarily equate with steatosis.

This is the first study to use inter-operator concordance as a measure of the accuracy of ARFI in the setting of hepatic steatosis. We showed good concordance between the two ARFI examiners regardless of the severity of steatosis.

The small number of patients with moderate to severe steatosis was a limitation of this study as has been the case with other studies. Consequently we may not have adequately represented the full range of variability in this population in our study. Additionally we did not assess the impact of active inflammation on the ARFI and as such we are unable to comment on the potential influence of inflammation in this study.

In conclusion our study has shown that in the setting of steatosis, ARFI is very sensitive in detecting, and most accurate in diagnosing severe fibrosis and cirrhosis in regardless of the presence or degree of steatosis. The tendency of ARFI to upstage the fibrosis category in lower stages of fibrosis (F0/F1) needs to be considered when interpreting results. The present study does not show conclusively if the presence or severity of steatosis independently alters ARFI measurements. Previous reports have suggested that steatosis decreases ARFI readings[16,17] but this study shows that the effect, if real, is small. As might have been expected given previous studies examining the effect of obesity on ARFI, the failure rate and median inter-quartile range increased with increasing steatosis. However the inter-operator concordance remained good across all steatosis severities.

This may help clarify the accuracy and reliability of ARFI in the presence of hepatic steatosis, with the potential to aid those performing and interpreting results to facilitate a more meaningful test result for patient management.

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Figure 1: Receiver operating characteristic curves of the ability of ARFI to accurately predict different stages of fibrosis. i: Fibrosis ≥ F2, ii: fibrosis F ≥ F3.

Table 1: Patients characteristics

Characteristic	Biopsy cohort	Ultrasound cohort
Mean Age (years)	48.2 (SD 15.1)	50.5 (SD 14.3)
Gender	Females: 26 (49.1%) Males: 27 (50.9%)	Females: 153 (51.7%) Males: 143 (48.3%)
Steatosis grade	Biopsy	Ultrasound analysis

	Minimal: 24 (45.3%) Mild: 19 (35.9%) Moderate: 4 (7.5%) Severe: 6 (11.3%)	Absent/mild: 188 (63.9%) Moderate/severe: 108 (36.5%)
Biopsy F stage	F0: 14 (26.4%) F1: 12 (22.6%) F2: 12 (22.6%) F3: 8 (15.1%) F4: 7 (13.2%)	
ARFI fibrosis category	F0/1: 14 (26.4%) F2: 9 (17.0%) F3: 12 (22.6%) F4: 18 (34.0%)	F0/1: 164 (55.4%) F2: 35 (11.8%) F3: 19 (6.4%) F4: 78 (26.4%)

Table 2: Relationship between histopathologic findings and ARFI velocity in patients with hepatic steatosis.

Histopathologic Findings		**ARFI velocity**	
		Overall median value (IQR)	**P value**
Fibrosis stage: n =53	**Steatosis grade**		0.038
F0 & F1 (n = 26):	Minimal and mild: 10 Moderate and severe: 16	1.41 (1.27 – 1.84)	
F2 (n = 12):	Minimal and mild: 6 Moderate and severe: 6	1.44 (1.31 – 1.71)	
F3 (n = 8):	Minimal and mild: 5 Moderate and severe: 3	2.26 (1.70 – 2.44)	
F4 (n = 7):	Minimal and mild: 3 Moderate and severe: 4	2.28 (1.59 – 2.53)	

Note – the p values describe the significance of the relationship between the median ARFI velocity and the fibrosis stage on biopsy, Kruskall-Wallis test.

Table 3: Relationship between histopathologic findings and ARFI velocity in patients with hepatic steatosis who had liver biopsy on the same day as ARFI

Histopathologic Findings		ARFI velocity	
		Overall median value (IQR)	**P value**
Fibrosis stage: n = 28	**Steatosis grade**		0.041
F0 & F1 (n = 12):	Minimal and mild: 5 Moderate and severe: 7	1.35 (1.25 – 2.00)	
F2 (n = 6):	Minimal and mild: 3 Moderate and severe: 3	1.39 (1.15 – 1.69)	
F3 (n = 4):	Minimal and mild: 3 Moderate and severe: 1	2.44 (2.28 – 2.76)	
F4 (n = 6):	Minimal and mild: 2 Moderate and severe: 4	2.39 (1.75 – 2.53)	

Note – the p values describe the significance of the relationship between the median ARFI velocity and the fibrosis stage on biopsy, Kruskall-Wallis test.

Table 4: Diagnostic performance of Acoustic Radiation Force Impulse imaging in determining biopsy stage of fibrosis using receiver operating curve analysis

	ARFI Median Velocity	
	Stage ≥ 2 vs. stage 0-1	**Stage ≥ 3 vs. stage 0-2**
Optimum cut-off (m/s)	1.48	1.58
Sensitivity (%)	74	93
Specificity (%)	58	58
Positive predictive value (%)	65	47
Negative predictive value (%)	68	96
Area under curve (CI 95%)	0.65 (0.50-0.80)	0.76 (0.61-0.90)

Table 5: Relationship between degree of steatosis, the Acoustic Radiation Force Impulse (ARFI) Imaging parameters and concordance with biopsy stage, in the biopsy cohort.

Steatosis on biopsy	**Median IQR**	**Inter-operator concordance**	**Failure rate**	**ARFI fibrosis score correlation with fibrosis stage on biopsy**
Minimal and	0.41	80%	First operator:	Agreement[†]: 23/43 (54%)

mild (n = 43)			50% Second operator: 45%	Underestimated: 3/43 (7%) Overestimated: 17/43 (40%)
Moderate and severe (n =10)	0.68	80%	First operator: 64% Second operator: 64%	Agreement†: 5/10 (50%) Underestimated: 1/10 (10%) Overestimated: 4/10 (40%)

† Agreement indicates within the same or adjacent histological F stage.

Table 6: Relationship between degree of steatosis and the Acoustic Radiation Force Impulse (ARFI) imaging parameters in the ultrasound cohort.

Steatosis on ultrasound	Median IQR	Inter-operator concordance	Failure rate
Absent and mild (n = 188)	0.23	88%	First operator: 34% Second operator: 33%
Moderate and severe (n = 108)	0.37	81%	First operator: 50% Second operator: 50%

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