LIVER

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Comparison of Transient Elastography and Acoustic Radiation Force Impulse for Non-Invasive Staging of Liver Fibrosis in Patients With Chronic Hepatitis C

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OBJECTIVES: **Transient elastography (TE) is adequate for a diagnosis of cirrhosis, but its accuracy for milder stages of fibrosis is much less satisfactory. The objective of this study was to compare the performance and the discordance rate of acoustic radiation force impulse (ARFI) and TE with liver biopsy in a cohort of chronic hepatitis C (CHC) patients.**

METHODS: **One hundred thirty-nine consecutive patients with CHC were enrolled in two tertiary centers, and evaluated for histological (Metavir score) and biochemical features. All patients underwent TE and ARFI.**

RESULTS: **TE was unreliable in nine patients (6.5%), while in no cases (0%) were ARFI invalid measurements recorded ($P=0.029$). By area under receiver operating characteristic curve (AUROC), the best cutoff values for TE and ARFI for significant fibrosis (≥F2) were ≥6.5 kPa (AUROC: 0.78) and ≥1.3 m/s (AUROC: 0.86), respectively. For severe fibrosis (F3–F4), these cutoff values were 8.8 kPa (AUROC: 0.83) for TE and 1.7 m/s (AUROC: 0.94) for ARFI. For cirrhosis, TE had its best cutoff at ≥11 kPa (AUROC: 0.80) and ARFI at ≥2.0 m/s (AUROC: 0.89). By pairwise comparison of AUROC, ARFI was significantly more accurate than TE for a diagnosis of significant and severe fibrosis ($P=0.024$ and $P=0.002$, respectively), while this difference was only marginal for cirrhosis ($P=0.09$). By partial AUROC analysis, ARFI performance results significantly higher for all three stages of fibrosis. The average concordance rates of TE and ARFI vs. liver biopsy were 45.4 and 54.7%, respectively. By multivariate analysis, ARFI was not associated with alanine aminotransferase (ALT), body mass index, Metavir grade, and liver steatosis, while TE was significantly correlated with the ALT value ($P=0.027$).**

CONCLUSIONS: **In a cohort of patients with CHC, ARFI imaging was more accurate than TE for the non-invasive staging of both significant and severe classes of liver fibrosis.**

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INTRODUCTION

In hepatitis C virus (HCV)-infected patients, the prognosis and the clinical management of chronic liver diseases are dependent on the extent of fibrosis (1). Biopsy, even if invasive, painful and with potentially life-threatening complications, remains the reference standard for staging liver disease (2). The diagnostic accuracy of a liver biopsy for assessing liver fibrosis is influenced by many factors: (i) sampling errors, (ii) technical processing of the specimens (length and width of biopsy sample), and (iii) inconsistency in defining pathological features (variety of scoring systems) (3,4). In addition, interobserver and/or intraobserver diagnostic discrepancies are estimated to affect 10–20% of assessments of hepatic fibrosis (5). Moreover, the high prevalence of chronic hepatitis C (CHC), in addition to the cost and constraints generated by this procedure, has triggered an intensive search for alternative, non-invasive methods for staging the disease. These methods include methodologies derived from elaboration of parameters obtainable with the current liver imaging techniques (ultrasound, CT (computed tomography) scan, and magnetic resonance), or from innovative uses of the principles of physics (Fibro-CT, MRI (magnetic resonance imaging)-elastography, MRI-spectroscopy, transient elastography (TE)), and acoustic radiation force impulse (ARFI) (6–8).

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Fibroscan is a rapid and user-friendly standalone device that can easily be used at the bedside or in the outpatient clinic with immediate results and good reproducibility (9,10). A recent meta-analysis confirmed that TE is adequate for the diagnosis of severe fibrosis or cirrhosis compared with mild fibrosis, but its accuracy is variable for the diagnosis of significant fibrosis (≥F2) according to underlying liver diseases (11). TE is burdened by a series of confounding factors, which might reduce its diagnostic accuracy. Obesity, insulin resistance, aminotransferases, and gamma glutamyltransferase (GGT) values have been recently been independently associated with liver stiffness measurement by Fibroscan (12–15).

ARFI is a novel method, with original technological solutions based on the use of shear acoustic waves remotely induced by the radiation force of a focused ultrasonic beam (16). Recently, ARFI has been investigated as a non-invasive method for the assessment of liver fibrosis (16–19).

In a recent pilot study by Friedrich-Rust *et al.* (18), ARFI imaging was able to assess liver fibrosis in chronic viral hepatitis with a diagnostic accuracy comparable to that of Fibroscan.

The aims of this prospective study in a cohort of patients affected with biopsy-proven CHC were

- to evaluate the reproducibility of ARFI technology in determining liver fibrosis,
- to determine the diagnostic accuracy of ARFI (index test) vs. TE (comparator test), and to evaluate the rate of discordances of these two tools compared with liver biopsy (reference standard).

METHODS

Patients

All consecutive patients with a virologic and histologic diagnosis of CHC admitted to the Infectious Diseases Units of the Garibaldi Nesima and Ferrarotto Hospitals in Catania and to the Hepatology Unit of the University Hospital, Palermo, from November 2008 to October 2009 were enrolled in the study.

The study was approved by the hospitals' Ethics Committees, and the patients gave their informed consent. None of the patients declined to give consent.

Diagnosis of CHC was based on the presence of active HCV replication, and on a liver histology consistent with chronic hepatitis. Patients with HBV/HIV coinfection, alcohol abuse (>20 g/day in the last year or more, evaluated by questionnaire), with Child B or C cirrhosis, and those under antiviral treatment were excluded from the study. Biochemical tests, TE and ARFI were always performed on the same day, and within 6 months from liver biopsy.

ARFI imaging in healthy subjects

Thirty-one healthy, adult blood donors (18 males and 13 females) who were matched for gender and age distribution with the patient group, and who did not have a history of relevant concomitant illness (autoimmune, heart, lung, liver, oncologic, or hematologic disease) were examined with ARFI imaging, and served as a healthy group.

Clinical and laboratory assessment

Clinical and anthropometric data were collected at the time of liver biopsy. Body mass index (BMI) was calculated on the basis of weight in kilograms and height (in meters). A 12-h overnight fasting blood sample was drawn at the time of biopsy to determine serum levels of alanine aminotransferase (ALT), GGT, total cholesterol, high-density lipoprotein and low-density lipoprotein-cholesterol, triglycerides, ferritin, plasma glucose concentration, and platelet count.

All patients were tested at the time of biopsy for HCV-RNA by qualitative polymerase chain reaction (Cobas Amplicor HCV Test version 2.0, Roche Molecular Diagnostics, Pleasanton, CA; limit of detection: 50 IU/ml). HCV-RNA-positive samples were quantified by Versant HCV-RNA 3.0 bDNA (Bayer, Tarrytown, NY) expressed in IU/ml. Genotyping was done with INNO-LiPA, HCV II, Bayer.

Liver stiffness measurement

Fibroscan measurements were done by two expert physicians, one (V.C.) in Palermo and one (A.M.) in Catania, both using FibroScan, Echosens (Paris, France) as previously reported (20), according to the manufacturer's instructions. Both examiners were blinded to clinical and pathological data.

Acoustic radiation force impulse

B-mode standard ultrasonography scanning and ARFI elastography were performed using a Siemens Acuson S2000 (Siemens AG, Erlangen, Germany) with a 4Cl transducer. During the course of an ultrasound examination and by using the same probes used for abdominal exploration, the operator command (Push) generates mechanical energy into the examined tissue. This is achieved through a high-frequency ultrasound beam that produces a mechanical deformation at the focal point of the beam (Pulse). The micro-area of parenchyma affected by the compression, called ROE (region of excitation) undergoes a 10–20 μm deformation. This size of deformation is critical to trigger a shear wave with an adequate signal noise ratio. The propagation of three-dimensional shear wave strictly depends on the medium viscoelasticity. To simplify the calculation of speed, ARFI includes as representative only the wave perpendicular to the ROE axis of symmetry. To calculate the progression of the shear wave, ARFI measures the time-to-peak of the wave within a target area close to the ROE, denominated ROI (region of interest). The ROI area is 0.6×1 cm^2. As the shear wave velocities are 2,500–3,000 times slower than ultrasounds, the ultrasound-based sequence "Pulse-Tracking" can theoretically be cyclically repeated several times for each Push. The device is set to perform nine consecutive measurements for each Push without producing any significant increase in local temperature. If all nine sequences obtained for a single position-pulse fail in the confidence interval (CI) established at 95%, the data are considered reliable and the mean of the nine sequences is calculated and represented in the screen in m/s. On the contrary, in case of physical conditions affecting signal noise ratio, the device will reject the measure and report it on the screen as "XXX" (16). The box of the ROI can be freely positioned by the operator up to a depth of 5.5 cm during a traditional

ultrasound examination, as automatic corrections apply as automatic corrections to the ultrasound signal from different depths.

Liver stiffness was measured with ARFI elastography by two independent investigators: one in Catania (L.R.) and one in Palermo (N.A). Both investigators were blinded to all patients' clinical, serological, and histological data. ARFI elastography was performed on fasting patients, choosing as the target the right lobe of the liver, which was accessed through the intercostal spaces. The examined patients were lying in the dorsal decubitus position, with the right arm extended in abduction. The measurement was taken avoiding liver large vessels and scissures at a distance ranging between 30 and 55 mm from the liver surface. The velocity of the shear wave (in m/s) in the liver tissue was collected and recorded from 20 different sites, 5 sites for each segments (V, VI, VII, and VIII) within the right lobe. The overall volume of liver parenchyma examined by ARFI was 3.916 cm^3, which was slightly wider than that measured by a single Fibroscan scan. A median of the 20 results has been calculated. The duration of the whole ARFI examination lasted within a range of 8–14 min.

Since the procedure for collecting and evaluating data from the echographic outcomes could rely on some degree of subjective interpretation between the two investigators, we conducted a prior, parallel double blind experiment on 21 randomly selected patients with biopsy-proven CHC. The Metavir fibrosis stage of these groups of patients was 1 (mild fibrosis) in three patients, 2 (moderate fibrosis) in six patients, 3 (severe fibrosis) in six patients, and 4 (cirrhosis) in six patients.

These patients were examined with ARFI elastography by both investigators, both of whom reported 20 measurements for each patient, as described above.

Histology

All patients had undergone liver biopsy up to 6 months before TE and ARFI evaluation: the range interval was 1–6 months (median 3 months) to biopsy.

Liver biopsy specimens were obtained using Menghini 16G disposable needles, fixed in 4% buffered formalin and embedded in paraffin. Liver specimens were stained with hematoxylin and eosin and Masson trichrome. All biopsy specimens contained at least 10 portal tracts and were minimum 1.5 cm in length. Liver fibrosis stages were evaluated semiquantitatively according to the Metavir scoring system. The liver fibrosis stage was determined using a five-point (F0–F4) scale: stage F0 indicated the absence of fibrosis; F1 expressed portal fibrosis without septa; F2 was equal to portal fibrosis with a few septa; F3 indicated numerous septa without cirrhosis; and F4 was equivalent to liver cirrhosis.

In addition, the degree of steatosis in liver biopsies was assessed by a morphological semiquantitative approach (21). Steatosis was assessed as the percentage of hepatocytes containing fat droplets (minimum 5%) and evaluated as a continuous variable. Steatosis was classified as: mild 5–30% or moderate–severe ≥30%.

All biopsy specimens were coded and evaluated by a single experienced pathologist, who was blinded to the patients' clinical and imaging results.

Statistical analysis

Statistical analyses were conducted according to the following steps:

1. *Interobserver agreement.* Analysis of agreement between ARFI data obtained by two different observers on the same patients. Bland–Altman method was used to assess the agreement between the two esaminators (22).
2. *Analysis of ARFI and TE data on patients with biopsy-proven CHC.* Analysis of heterogeneity of the individual measurements in order to gather more information on the fibrosis-stage classification of the patients. Simple measures of the difference in spread of quantiles and interquartiles, as well box-plot representation, were provided. Receiver operating characteristic (ROC) curves were applied to identify the area under ROC curve (AUROC) of TE and ARFI able to discriminate the different classes of fibrosis. The best cutoff values were determined by Kolmogorov–Smirnov index, that is a natural generalization to continuous test of Youden index for binary test (23). Partial AUROC analysis for TE and ARFI has been calculated as previously proposed by McClish (24) and Jiang *et al.* (25).
3. *Analysis of agreement between the three devices:* ARFI, Metavir, and TE. Usual contingency tables representation were applied. Weight κ has been performed.
4. *Analysis of confounding variables*. Multivariate analysis (by SAS Statistical Packages, SAS, Toronto, Canada) was performed to evaluate whether elevated levels of BMI, ALT, or histological steatosis could influence the outcome of both ARFI and Fibroscan measurement.

RESULTS

Patient's features and histology

One hundred forty-six consecutive patients with CHC were evaluated. Seven patients were excluded for suboptimal liver biopsy (five patients) and coexisting alcohol abuse (two patients). One hundred thirty-nine consecutive patients (72 in Catania and 67 in Palermo) were enrolled in the study. **Table 1** shows the demographic, clinical, and histological characteristics of the patients. There were 83 (59.7%) male patients. Overall, the mean age was 55±12 years, with a mean age for females of 50.3±15 years and for males of 59±10 years. The mean value of BMI was 26±3 kg/h^2. The mean values of metabolic parameters (fasting glucose, total cholesterol, high-density lipoprotein, low-density lipoprotein-cholesterol, and triglycerides) are in normal ranges. The mean HCV-RNA plasma level was $1.65±3.31×10^6$ IU/ml. HCV genotype was 1a/b in 108 patients, 2 in 16 patients, and 3 in 15 patients. The distribution of Metavir histological stages among the 139 CHC patients was the following: F0 was reported in 13 patients (9.4%), F1 in 39 (28.0%), F2 in 33 (23.6%), and F3 in 24 (17.3%). Finally, 30 patients (22.1%) were classified as having cirrhosis (F4). Liver steatosis ≥30% was present in 14.8% of patients. ARFI and TE mean values were, respectively, 1.7±0.6 m/s and 10.3±8.6 kPa.

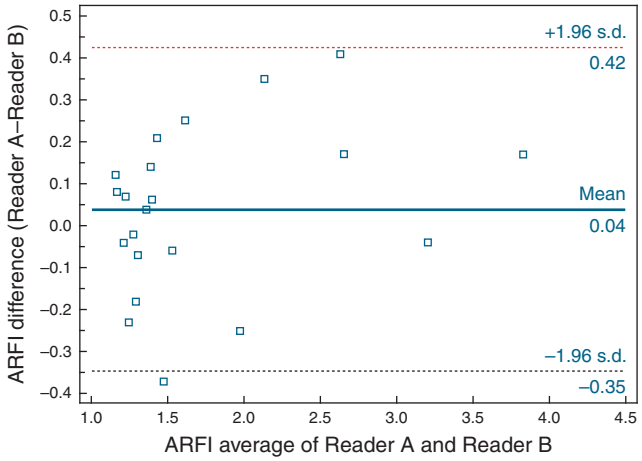
Analysis of TE and ARFI data on 139 patients with biopsy-proven CHC

TE was unreliable in nine patients (6.5%). No cases (0%) of invalid ARFI measurements were recorded ($P = 0.029$).

Table 1. Demographic, clinical, and histological features of 139 patients with chronic hepatitis C

Variables	Patients (139)
Sex—male (%)	83 (59.7)
Age (years)—mean ± s.d.	55 ± 12
BMI (kg/m^2)—mean ± s.d.	26 ± 3
ALT (UI)—mean ± s.d.	77.2 ± 33.0
AST (UI)—mean ± s.d.	66.6 ± 23.0 UI
GGT (UI)—mean ± s.d.	74.7 ± 45.0 UI
PLT×10^3/mmc—mean ± s.d.	157.500 ± 111.000/mmc
Blood glucose (mg/dl)—mean ± s.d.	101 ± 32 mg/dl
Total cholesterol (mg/dl)	171 ± 69
LDL	115.4 ± 34
HDL	42.1 ± 14
Triglycerides (mg/dl)	133.5 ± 55
HCV-RNA (UI/l)—mean ± s.d.	1.65 ± 3.31
HCV genotype	
1	108 (77.1%)
2	16 (11.4%)
3	15 (10.7%)
Metavir stage	
F0	13 (9.4%)
F1	39 (28.0%)
F2	33 (23.7%)
F3	24 (17.3%)
F4	30 (21.6%)
Liver steatosis score (%)	
0–<5	68 (49.1%)
5–<30	50 (36.1%)
≥30	21 (14.8%)
ARFI (m/s)—mean ± s.d.	1.7 ± 0.6
TE (kPa)—mean ± s.d.	10.3 ± 8.6

ALT, alanine aminotransferase; ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma glutamyltransferase; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PLT, platelets; TE, transient elastography.



Parameter	Coefficient	s.d.	*T* value	*P* value
Intercept:	–0.1091	0.1085	1.0054	0.3274
Slope:	0.08488	0.05759	1.4739	0.1569

Figure 1. Agreement between acoustic radiation force impulse (ARFI) data obtained by two different observers by Bland–Altman method.

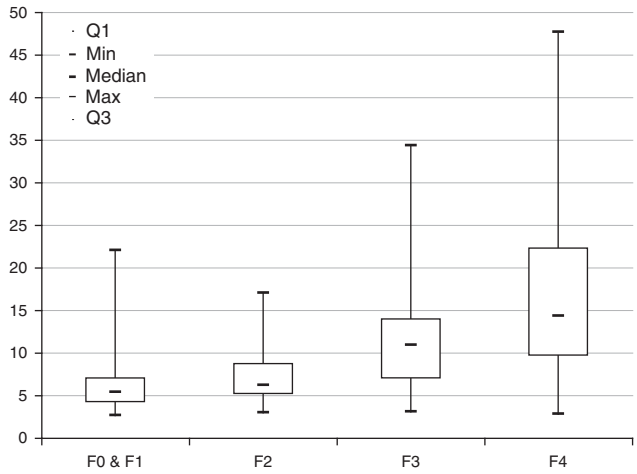


Figure 2. Distribution of transient elastography (TE) median values calculated for patients affected with chronic hepatitis C (CHC) at different fibrosis Metavir stages.

The mean values and the standard deviations of the ARFI value obtained from the two sonographers in the 21 patients were 1.76±0.78 m/s and 1.72±0.72 m/s.

By Bland–Altman method, there was no significant difference between the ARFI values of the 21 patients obtained from the two different sonographers (**Figure 1**).

Figure 2 shows the distribution of the observed TE median values calculated for patients affected with CHC at different Metavir fibrosis stages. The median values for CHC patients were 5.5 kPa for patients belonging to the F0–F1 group, 6.3 kPa for patients in the F2 group, 11 kPa for patients diagnosed as having a F3 liver fibrosis, and 14.4 kPa for patients belonging to the F4 group.

Given that according to our protocol, ARFI measurement consisted of 20 measurements for each patient, we built a scatterplot that reported the median values vs. the interquartile range (IQR) (**Figure 3**) either from healthy subjects or from CHC patients. It appeared that results from healthy subjects were concentrated in the lower left part of the first quadrant, where all ARFI measurements were ≤1 m/s. For these healthy subjects, medians ranged between 0.7 and 1 m/s, with the IQR lower than 0.7. Conversely, results from CHC patients showed medians ranging from 0.7 to 3.8 m/s, with IQR ranging between 0.2 and 2.4.

Figure 4 shows the distribution of the observed ARFI median values calculated for healthy subjects and for patients affected with

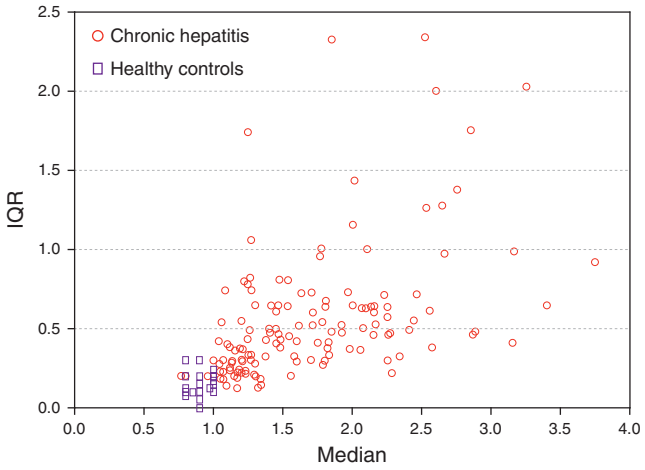


Figure 3. Scatterplot distribution of acoustic radiation force impulse (ARFI) velocity values (median vs. interquartile range (IQR)) among healthy controls (represented as squares) and chronic hepatitis C (CHC) patients (represented as circles).

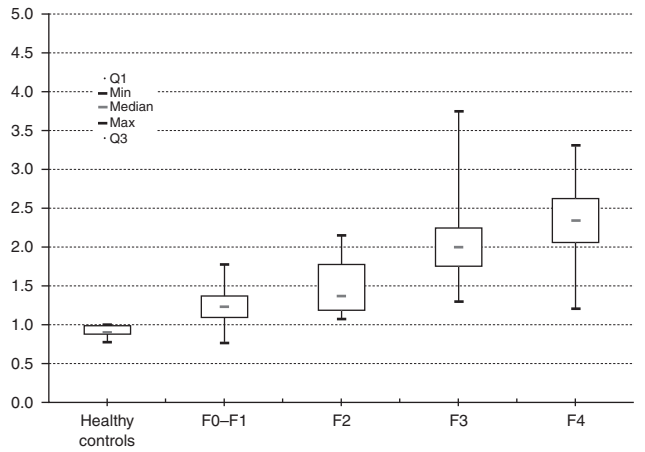


Figure 4. Distribution of acoustic radiation force impulse (ARFI) median values calculated for healthy controls and for patients affected with chronic hepatitis C (CHC) at different fibrosis Metavir stages.

CHC at different Metavir fibrosis stages. The median value for the 31 healthy subjects was 0.99 m/s, while the median values for CHC patients were 1.31 m/s for patients belonging to the F0–F1 group; 1.45 m/s for patients in the F2 group; 1.99 m/s for patients diagnosed as having a F3 liver fibrosis; and 2.34 m/s for patients belonging to the F4 group (analysis of variance, $P<0.001$). Interestingly, the spread of the distribution increased progressively from one extreme to the other, given that the IQR in the healthy subjects group was 0.12, while it was 0.57 in the F4 group.

Analysis of diagnostic performance between TE or ARFI and Metavir stage

ROC curve analysis identified a TE value ≥6.5 kPa (AUROC: 0.78; s.e.: 0.04; 95% CI: 0.70–0.85) and an ARFI value ≥1.3 m/s (AUROC: 0.86; s.e.: 0.03; 95% CI: 0.79–0.91) as the best cutoff for predicting fibrosis ≥F2 (**Figure 5a**). For severe fibrosis (**Figure 5b**), the best cutoff value for TE was 8.8 kPa (AUROC: 0.83; s.e.: 0.04; 95% CI: 0.75–0.89) and for ARFI, the best cutoff value was 1.7 m/s (AUROC: 0.94; s.e.: 0.02; 95% CI: 0.89–0.97). For predicting cirrhosis (**Figure 5c**), TE had its best cutoff at ≥11 kPa (AUROC: 0.80; s.e.: 0.05; 95% CI: 0.72–0.86), and ARFI at ≥2.0 m/s (AUROC: 0.89; s.e.: 0.04; 95% CI: 0.83–0.94) (**Table 2**).

The performance of ARFI for the diagnosis of significant and severe fibrosis was significantly better than the performance of TE by pairwise comparison of ROC curves ($P=0.024$ and $P=0.002$, respectively) (**Figure 5a** and **b**). ARFI performance was not statistically significantly higher than TE performances for the diagnosis of cirrhosis ($P=0.09$) (**Figure 5c**). Using the partial AUROC analysis, ARFI performance was significantly better that TE for all stages of fibrosis (**Figures 5** and **6**).

Using the cutoff of 6.5 kPa of TE for the diagnosis of significant fibrosis (Metavir≥2), 8.8 kPa for the diagnosis of severe fibrosis, and the cutoff of 11 kPa for the diagnosis of cirrhosis (Metavir = 4), we evaluated the concordance rate of TE vs. the histological diagnosis of liver fibrosis. An average concordance rate of 45.4% was found. Weighted $\kappa=0.638$ (**Table 3**).

We performed the same analysis using the two ARFI cutoffs of 1.3 m/s for the diagnosis of significant fibrosis, 1.7 m/s for the diagnosis of severe fibrosis, and 2.0 m/s for the diagnosis of cirrhosis. The cumulative rate of concordance was 54.7%, and the highest concordance value was obtained for the diagnosis of mild fibrosis. Weighted $\kappa=0.786$ (**Table 3**).

Figure 5 shows the agreement and disagreement rates of ARFI and TE according to Metavir stage.

Analysis of confounding variables

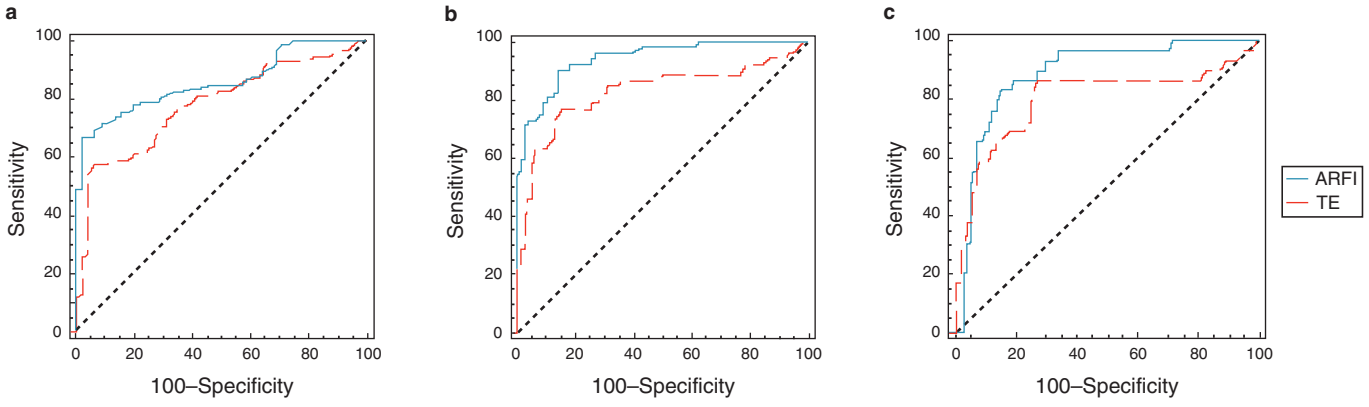
By univariate and multivariate linear regression analyses, ARFI values were not associated with BMI, ALT, grading, and histological steatosis levels. ARFI results were not significant either globally ($F=1.12$, $P=0.347$), or considering the individual risk factors (ALT: $t=1.72$, $P=0.0903$; BMI: $t=-0.01$, $P=0.992$; steatosis: $t=0.66$, $P=0.511$; and histological grading score: $t=1.76$, $P=0.0987$).

When we applied the same regression model to Fibroscan values, a significant association with ALT levels was observed ($t=4.46$, $P=0.027$), while histological grading score, BMI, and histological steatosis had no significant influence on Fibroscan results (histological grading: $t=1.99$, $P=0.075$; BMI: $t=-0.11$, $P=0.914$; and steatosis: $t=-1.20$, $P=0.235$). The global analysis, as well, was not significant ($F=2.16$, $P=0.101$).

DISCUSSION

In this prospective study, we found that ARFI imaging for the non-invasive measurement of liver fibrosis have a good yield and is reproducible and accurate for staging of both intermediated and advanced classes of liver fibrosis in a cohort of 139 consecutive patients with CHC enrolled in two tertiary centers.

Differently from TE, ARFI allowed sampling from many different areas of the liver parenchyma. Thus, it was more representative,



Partial AUC standardization proposed by McClish and Jiang

Metavir F2	ARFI	TE	Metavir F3	ARFI	TE	Metavir F4	ARFI	TE
AUC (0–0.20)	0.142	0.103	AUC (0–0.20)	0.156	0.120	AUC (0–0.20)	0.115	0.097
Partial area index	0.709	0.517	Partial area index	0.782	0.598	Partial area index	0.577	0.484
z test: 5.604	*P*-value (95%): 0.01047		*z* test: 9.591	*P*-value (95%): < 0.000001		*z* test: 3.844	*P*-value (95%): 0.00006	

Figure 5. Comparison between transient elastography (TE) and acoustic radiation force impulse (ARFI) performances for the diagnosis of: significant liver fibrosis (Metavir stage ≥2) (**a**), severe fibrosis (Metavir stage ≥3) (**b**), and cirrhosis (Metavir stage = 4) (**c**). AUC, area under the curve; ROC, receiver operating characteristic.

Table 2. Performances of TE and ARFI in 139 patients with chronic hepatitis C according to different classes

	AUROC	Cutoff	s.e.	95% CI	Sensitivity (%)	Specificity (%)	PPV	NPV	LR+	LR–
F≥2 (prevalence 62.9%)										
TE (kPa)	0.78	6.5	0.04	0.70–0.85	71	71	82	56	2.4	0.4
ARFI (m/s)	0.86	1.3	0.03	0.79–0.91	81	70	81	68	2.5	0.3
F≥3 (prevalence 38.8%)										
TE (kPa)	0.83	8.8	0.04	0.75–0.89	77	85	77	85	5.0	0.3
ARFI (m/s)	0.94	1.7	0.02	0.89–0.97	91	86	80	94	6.4	0.1
F≥4 (prevalence 21.6%)										
TE (kPa)	0.80	11.0	0.05	0.72–0.86	70	82	53	90	3.9	0.4
ARFI (m/s)	0.89	2.0	0.04	0.83–0.94	83	86	63	95	6.1	0.2

ARFI, acoustic radiation force impulse; AUROC, area under receiver operating characteristic curve; CI, confidence interval; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; TE, transient elastography.

and measured better than TE, the heterogeneity of liver fibrosis. We observed that ARFI had a significantly higher discriminatory capacity than TE for intermediate stages of fibrosis. In fact, the comparison between TE and ARFI AUROCs achieved statistical significance for both significant and severe fibrosis and even though by pairwise comparison analysis, a significant difference between the two tools for the diagnosis of cirrhosis was not found, the partial AUROC analysis found that the ARFI performances is significantly higher in all three stages of fibrosis. However, the large area of overlap between stage 3 and cirrhosis, observed for both TE and ARFI, does not allow, in single patients, for discrimination with high accuracy between the two stages of fibrosis.

The mean feature of a non-invasive tests of liver fibrosis is the ability to discriminate between two adjacent stages of fibrosis. For this reason, differently from the previously published pilot study (18), we have included only patients with a histological diagnosis of liver fibrosis performed up to 6 months before the non-invasive estimation of liver fibrosis and on a liver specimen longer than

15 mm. In this way, we have tried to minimize the biopsy sampling error and its effects on the performances evaluation of ARFI and TE.

Although Sporea *et al.* (26) did not find any significant differences between the performances of TE and ARFI, similarly to Goertz *et al.* (27) our data provided further evidence that ARFI has a better diagnostic accuracy for the diagnosis of severe fibrosis than of cirrhosis.

We are aware that TE performance observed in this study was lower than the mean AUROC assessed by meta-analysis (11); however, our TE performance (AUROC: 0.80) was comparable to the value reported by another Italian study (28) and the large heterogeneity of TE performances for the diagnosis of cirrhosis in the meta-analysis was also explained by the country where the studies were performed.

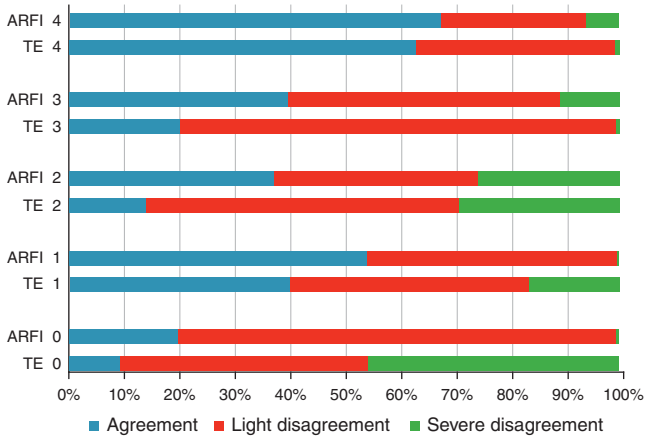


Figure 6. Rate of agreement of acoustic radiation force impulse/transient elastography (ARFI/TE) according to Metavir stage (%).

Another important clinical advantages of this new device is that ARFI is not influenced by clinical, biochemical, and histological variables. In fact, as previously described (14), we noticed a significant influence of ALT elevation on TE results, whereas ARFI elastography could be performed successfully independently of BMI, ALT level, histological grading, and steatosis. As previously shown for TE (10), the evaluation of ARFI interobserver agreement showed that it is repeatable, with good agreement between different observers, even at a single measurement level.

Mounting data suggest that fibrosis is spread heterogeneously within the liver during the progression of chronic hepatic disease. Huwart *et al.* (29) and Venkatesh *et al.* (30), by analyzing hepatic fibrosis with magnetic resonance elastography, showed that heterogeneity of hepatic elasticity and viscosity increased with an increase in fibrosis stage. Romero-Gomez *et al.* (31), using Fibro-CT in CHC, also found a strong heterogeneity of fibrosis in advanced disease.

For this reason, even liver biopsies that sample 1/50,000 of the liver parenchyma cannot always be adequate for assessing liver fibrosis (sampling error). TE is able to evaluate a larger area of live parenchyma compared with liver biopsy, though it usually measures liver stiffness of a single area of parenchyma.

The important advantage of ARFI is that it gives an absolute velocitometric value (m/s) within a restricted area of the liver, but is able to sample from many different areas of the liver parenchyma. The liver volume examined by 20 ARFI measurements is equivalent to a single TE result but, differently from TE, it offers 20 separate values of viscoelasticity from different parts of the hepatic parenchyma, and is thus more representative. By means of 20 ARFI measurements distributed in the right lobe of CHC patients, we found a variability of

Table 3. Analysis of concordance of ARFI and TE vs. Metavir stage

	F1	F2	F3	F4	Total	Concordance rate (%)
F1						
ARFI (m/s) < 1.3	**34**	14	0	1	49	69.4
TE (kPa) < 6.5	**31**	18	3	4	56	55.3
F2						
1.3≤ARFI (m/s) < 1.7	16	**9**	3	1	29	31.0
6.5≤TE (kPa) < 8.8	11	**6**	5	0	22	27.3
F3						
1.7≤ARFI (m/s) < 2.0	2	8	**8**	3	21	38.1
8.8≤TE (kPa) < 11.0	1	6	**2**	5	14	14.3
F4						
ARFI (m/s)≥2	0	2	13	**25**	40	62.5
TE (kPa)≥11.0	2	3	13	**20**	38	52.6
Cumulative concordance						
ARFI (m/s)		Weighted κ=0.786			76/139	54.7%
TE (kPa)		Weighted κ=0.638			59/130	45.4%

ARFI, acoustic radiation force impulse; TE, transient elastography.
Bold values are concordance between ARFI and TE with liver biopsy.

the values that progress according to the stage of fibrosis. We believe that this variability may be the expression of spotty and progressive structural disorders introduced by the fibrosis, and by progressive amounts of pathologic collagen type II and IV. Within the liver of CHC patients, stiffness is spotty, so that measurement should take into account not just a central tendency measure (the median) as obtained by TE, but also a measure of heterogeneity (the IQR).

In general, devices that measure fibrosis on large volumes of liver or at multiple points inside the liver, such as elastographic imaging techniques or ARFI itself, clearly show the dishomogeneous features of fibrosis in the progression of chronic liver disease, and this should be taken into consideration when interpreting the results of non-invasive tests to measure liver fibrosis.

Moreover, in the comparison of TE, ARFI, and Metavir, we should not forget that these three methods measure in different scales. While TE analyzes liver volume that measures cm 4×1, that is, 100 times bigger than that explored by liver biopsy, and then works in macroscales, ARFI works within a radial plane of 0.5×0.4 cm^2 localized in an ROI of 0.5×1 cm^2, that is, in mesoscale. Therefore, in a dishomogeneous medium, where finite microscopic elements are not prevalent, the ARFI values, in theory, could better reflect the microscopic composition.

We should also remember that staging of histologic fibrosis by biopsy is not a quantitative measure of the amount of fibrosis in the liver, since distinction of stages is based on the morphology of fibrotic tissue. Thus, when measuring liver stiffness, it is not surprising to find considerable variations among patients with the same histologic fibrosis stage.

This study has limitations. First, the analysis was carried out in a relatively small number of patients, and it would be interesting to see if these results also hold true in larger groups of patients with CHC, and in patients with liver disease of other etiologies. Second, our study included a cohort of European, non-drinker patients with a low prevalence of obesity and cirrhosis, who were enrolled in a tertiary referral center for liver disease, limiting the broad application of the results. Third, we recalculated TE cutoff values in our population of CHC patients instead that use the cutoffs previously published. This allowed us to perform a better comparison between TE and ARFI where previously validated cutoff values are not still available. A further methodological limitation could reside in the accuracy of liver biopsy examination for assessing fibrosis, which is universally defined as "gold standard." However, it is widely known that liver biopsy should be considered a "reference standard," because of the limitations previously discussed.

Finally, TE and ARFI have been performed in different way as TE is a site-specific tool whereas ARFI is an average of 20 measures from different locations and the two are not directly comparable. However, the aim of the study was not only to compare the two methods but we are interested to assess if ARFI could represent a useful tool to evaluate the heterogeneous distribution of fibrosis in the liver.

In conclusion, we believe that there is sufficient evidence for considering ARFI more reliable, reproducible, and accurate than TE for staging liver fibrosis, and that it should be taken into consideration as a non-invasive method for the evaluation of liver fibrosis in CHC patients.

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CONFLICT OF INTEREST

Guarantor of the article: Vincenza Calvaruso, MD, PhD.

Specific author contributions: Designed the study, measured liver stiffness with ARFI elastography, and contributed to data acquisition: L. Rizzo; designed the study, contributed to data acquisition, responsible for writing the manuscript, and participated in statistical analysis: Vincenza Calvaruso; responsible for writing the manuscript and responsible for statistical analysis: C. Cammà; responsible for writing the manuscript and have seen and approved the final version: A. Craxì; measured liver stiffness with ARFI elastography and contributed to data acquisition: N. Alessi; participated in statistical analysis: M. Attanasio and L. L'abbate; participated in patient management and data collection: B. Cacopardo S. Petta, F. Fatuzzo, A. Montineri, A. Mazzola, G. Nunnari, F. Bronte, and V. Di Marco. All authors have seen and approved the final version of the manuscript.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Acoustic radiation force impulse (ARFI) is a potential useful tool for the non-invasive evaluation of liver fibrosis in chronic hepatitis C.
- ✓ ARFI diagnostic accuracy is comparable to that of transient elastography (TE).

WHAT IS NEW HERE

- ✓ Acoustic radiation force impulse (ARFI) has a good diagnostic accuracy for the diagnosis of significant fibrosis (≥F2), severe fibrosis (F3), and cirrhosis.
- ✓ ARFI value most likely represents the spatial heterogeneity of hepatic fibrosis in chronic hepatitis C.
- ✓ ARFI high-frequency pulse is not significantly diffracted by subcutaneous tissue.

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