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ELASTOGRAPHY POINT QUANTIFICATION IN THE EVALUATION OF LIVER
FIBROSIS IN NAFLD PATIENTS

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease. Several non-invasive approaches to stage liver fibrosis in patients with NAFLD are being developed. Elastography Point Quantification (ElastPQ) is a non-invasive method to assess liver fibrosis measuring liver stiffness.

Aim: We evaluated the diagnostic performance of ElastPQ for identifying different degrees of fibrosis in patients with NAFLD. Furthermore, we compared ElastPQ with other non-invasive tests. We also identify the best liver stiffness cut offs for every stage of fibrosis.

Patients and methods: ElastPQ was performed in a training cohort of consecutive patients with biopsy-proved NAFLD, liver serum tests and Transient Elastography (TE). The diagnostic performance of ElastPQ was evaluated using AUROC analysis and compared with TE, Fibrosis-4 score (FIB-4), NAFLD fibrosis score (NFS) and AST/ALT (AAR). Then ElastPQ was performed in a validation cohort with biopsy-proven NAFLD in order to validate the resulting best cuts off.

Results: Overall, 106 patients with NAFLD were enrolled. The median stiffness values using ElastPQ was 4.69 kPa (2.82-29.86). The mean liver stiffness value divided for category of fibrosis stage were: 4.18 kPa in mild

fibrosis (F0-1), 4.49 kPa in significant fibrosis (F2), 6.89 kPa in advanced fibrosis (F3) e 12.14 kPa in cirrhosis (F4). In multivariate analysis, liver stiffness was associated only with the fibrosis stage (ß=2.987; p<0.001). The AUCs for the association with significant (≥F2), advanced fibrosis (≥F3) and cirrhosis (F4) were 0.783 (95%IC 0.693-0.857), 0.855 (95%IC 0.773-0.916) and 0.897 (95%IC 0.822-0.947), respectively. Diagnostic performance of ElastPQ compared to TE, resulted non-inferior in evaluating significant fibrosis (p=0.956), advanced fibrosis (p=0.171) and cirrhosis (p=0.773). ElastPQ had a higher diagnostic performance compared to FIB-4, NFS and AAR. The best fibrosis cuts off identified in the training cohort (80 patients enrolled) were 5.22 kPa, 6.3 kPa and 9.61 kPa respectively for fibrosis >F2, >F3 and F4.

Conclusions: ElastPQ is a promising imaging technique for evaluation of liver fibrosis and may represent a valuable technique in the evaluation of advanced liver fibrosis and an excellent tool in the diagnosis of cirrhosis in NAFLD patients.

Introduction

As a result of the growing obesity epidemic, nonalcoholic fatty liver disease (NAFLD) has become a major public health issue. NAFLD currently represents the leading cause of chronic liver disease in Western countries (1).

NAFLD is increasingly recognized as the liver disease component of metabolic syndrome (1). It is defined as the presence of 5% of hepatic steatosis, in the absence of competing liver disease etiologies, such as chronic viral hepatitis or autoimmune hepatitis, use of medications and other chronic liver diseases that induce steatosis (2) [Table 1].

NAFLD encompasses a spectrum of histopathological conditions, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis, with risk of hepatocellular carcinoma (3).

NASH has been recognized as one of the leading causes of cirrhosis in adults in the United States (1) and NASH-related cirrhosis is currently the second indication for liver transplants in the United States (4,5).

Clinically, NAFLD patients tend to be obese, with insulin resistance and/or type 2 diabetes, dyslipidemia, hypertriglyceridemia, and hypertension, which are all risk factors for cardiovascular diseases.

Table 1. Secondary causes of hepatic steatosis

Macrovesicular	*Microvesicular*
Alcohol abuse	Reye Syndrome
Hepatitis C (genotipe 3)	HELLP Syndrome
Wilson disease	Acute fatty liver disease in pregnancy
Lypodystrophy	Drugs: valproic acid, antivirals
Fasting	Genetic disorders of metabolism: Wolman's disease, lecithin-cholesterol acyl-transferase deficiency
Parenteral nutrition	
Abetalipoproteinemia	
Drugs:amyodarone,metotrexate,tamoxifen, corticosteroids	

The development and progression of liver fibrosis are the most important predictors of disease outcomes in NAFLD patients (2, 6-8).

Younossi et al. (9) and Ekstedt et al. (10) observed that the presence of advanced fibrosis (stage 3–4) was associated with an increased risk for overall and liver-related mortality. Within these studies, the risk of mortality was numerically but not statistically higher in NAFLD patients with early non-advanced fibrosis (stage 1–2). In contrast, Angulo et al. (11) observed that both advanced and non-advanced fibrosis were associated with an increased risk for mortality.

Accordingly, the early identification of the presence of fibrosis and the degree of fibrosis turns out to be mandatory.

Traditionally, liver biopsy has been used for the assessment of liver fibrosis in chronic liver disease (12). However, liver biopsy is limited by its

invasiveness, with potential severe complications in up to 1% of cases, sampling error, since the specimen represents roughly only 1/50 000 of the liver volume, and the inter- and intraobserver variability at microscopic evaluation (13).

In recent years, liver biopsy has been largely replaced by non invasive tests for the assessment of liver fibrosis (14), including ultrasound elastographic methods which have been an intense field of research (13).

It is also becoming increasingly clear that the best cut-off values of the different elastography techniques used to evaluate the presence and severity of liver fibrosis depend upon the etiology of the underlying liver disease, and upon the prevalence of the condition under study in the target population (13).

Among the elastographic methods, Transient elastography(TE) has been shown to be an excellent tool for the assesment of liver fibrosis. However it has its own limitations, such as failed or unreliable examinations, expecially in obese patients (15).

A systematic review of TE in patients with NAFLD involved 9 studies and 1047 patients (16). TE was excellent in diagnosing F3 fibrosis (85% sensitivity, 82% specificity) and cirrhosis (92% sensitivity, 92% specificity), but had only moderate accuracy for F2 fibrosis (79% sensitivity, 75% specificity) (16). Recomendation from EFSUMB guideline is that TE can be used to exclude cirrhosis in NAFLD patients (13) and it has been incorporated in several major guidelines on NAFLD (1, 17-18).

Point shear wave elastography (pSWE) is an acoustic radiation force impulse (ARFI)-based technique and it is a relatively new non-invasive tool

for assessing liver fibrosis (19). It uses a short-duration, high-intensity acoustic pulse to displace tissue perpendicular to the tissue surface. The transducer then detects tissue displacement within a focal spot along the radiation force, and tissue stifness can be obtained. In pSWE, shear waves perpendicular to the longitudinal waves are measured.   pSWE can be incorporated into an ultrasound system with B-mode, also allowing direct anatomical visualisation to select a specific area, avoiding large vessels or biliry sistem (20).

Although recente studies have shown ARFI-based techniques to be promising with similar accuracy as TE, very few studies compared the accuracy of TE and pSWe, in particular in NAFLD patients.

A systematic review of 7 studies for a total of 723 patients who underwent SWS measurements with VTQ® technique to evaluate the diagnostic efficacy of pSWE in patients with NAFLD was recently published (21). The summary sensitivity was 80.2 % for detecting significant fibrosis (13).

Given the burden of NAFLD disease in terms of risk for death from all causes and the importance of identifying patients with fibrosis and especially the degree of fibrosis with new non-invasive techniques, we evaluate the diagnostic accuracy of a point sheare waves quantifcation elastography tecniques, ElastPQ, in a group of NAFLD patients.

The primary aim of this study was to identify the best cut offs for every stage of liver fibrosis and to evaluate diagnostic accuracy of ElastPQ as a non invasive tool for staging liver fibrosis in patients with NAFLD, using liver biopsy as a standard of reference. Our secondary aim was to

compare the diagnostic accuracy of ElastPQ with fibroscan and other non invasive markers (FIB-4, NFS, AAR) for the staging of fibrosis in NAFLD-related chronic liver disease using liver histology as the reference standard.

Methods

This is a prospective study that include all consecutive adult patients with NAFLD who were scheduled for a liver biopsy in two italian tertiary centers for the management of liver diseases (Univesity Hospital of Bologna and city Hospital of Faenza).

This is a two step study, with a Phase A, the training study, and a Phase B, the validation study (Figure 1). In Phase A, patients were enrolled between October 2012 and Dicember 2019 at the Univesity Hospital of Bologna. In phase B, patients were enrolled from January 2018 to July 2021 at the city Hospital of Faenza.

In the training cohort we studied the diagnostic performance of ElastPQ in the evaluation of liver fibrosis, correlating it to histology as a gold standard of reference and comparing it to other non-invasive techniques.

Moreover, we identified the best cut-offs for each degree of fibrosis, and we validate them using the validation cohort.

The study was approved by the local ethics committee (code 025/2013/0Sper) and informed consent was obtained from all participanting subjects.

Figure 1. Study design

A) <u>Training Study</u>
patients with suspected NASH
undergoing liver biopsy, abdominal US, serum markers and
elastography evaluation (TE and <u>ElastPQ)</u>,
(Policlinico S.Orsola-Malpighi Hospital)

<u>End Points:</u>
- To determinate diagnostic performance of ElastPQ in the evaluation
of liver fibrosis
compared to liver biopsy and other methods
- Identification of best <u>cut off </u>for every stage of fibrosis

B) <u>Validation Study:</u>
patient with hystological diagnosis of NASH, undergoing <u>ElastPQ</u> evaluation
(Ospedale degli Infermi Faenza)

<u>End Points:</u>
- Validation of previous data
- Application of best cut off

The diagnosis of NAFLD was defined as an excessive accumulation of triglycerides in the liver (≥5% of hepatocytes on liver biopsy), usually evidenced by a hyper-reflective pattern on ultrasound, in the presence of alcohol consumption <30 g per day for males and <20 g per day for females and exclusion of other causes of fatty liver, such as the use of medications that can cause fatty liver, viral hepatitis, autoimmune hepatitis or other causes of chronic liver disease. The diagnosis of NASH required the simultaneous presence of steatosis, ballooning and lobular inflammation. Data on clinical parameters such as age, gender and body mass index (BMI) were collected for all patients at the time of biopsy. The operator who performed the elastography measurements was blinded to the results of the biopsy and other non-invasive tests.

Demographic, anthropometric, clinical and laboratory data were recorded using a standard protocol.

Liver biopsy and histopathological examination

The histological evaluation of fibrosis was used in our study as a reference standard for the analysis. The biopsy was performed by an expert operator (C.S.) in the training study and (FG. F.) in the validation study, in accordance with the Menghini method, using a semi-automatic 16-gauge needle (BIOMOL®, 2008-HS Hospital Service S.p.A., Rome, Italy). Biopsy sampling was performed in the right liver lobe with an intercostal approach. The samples were analyzed by the referring pathologist of each hospital, with more than 10 years of experience, also blinded to the

results of non-invasive tests. All biopsy specimens were evaluated according to the NAFLD Activity Score (NAS score). The degree of steatosis was defined according to Kleiner and disease activity was quantified using the NAFLD activity score (NAS).

Serum indices of hepatic fibrosis

All patients underwent blood sampling for platelet counts (PLT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (γGT) and albumin after an overnight fast. Fibrosis-4 (FIB4), NAFLD fibrosis score (NFS) and AST/ALT ratio (AAR) were calculated.

These three algorithms were calculated as follows:

FIB4 = age (years) x AST (IU/L) / [PLT (10^9/L) x $\sqrt{}$ALT (IU/L)]

NFS = -1.675 + 0.037 x age (years) + 0.094 x BMI (kg/m^2) + 1.13 x fasting hyperglycemia/diabetes (yes = 1, no = 0) + 0.99 x AST/ALT ratio - 0.013 x PLT (10^9/L) + 0.66 x albumin (g/dL)

AAR = AST/ALT

Transient elastography

TE was performed with FibroScan (Echosens, Paris, France) by a doctor (F.C.) with experience of at least 500 examinations. Measurement of hepatic stiffness was performed with an intercostal approach in blocked inspiration, with the patient in the supine position, and the right arm

behind the head. This technique measures the propagation speed of low frequency (50 Hz) elastic waves mechanically generated through a 4 cm long and 1 cm diameter hepatic parenchyma cylinder. The measurement depth was between 25 and 65 mm with the M probe and between 35 and 75 mm with the XL probe (the latter available in this study only from January 2017).

The results were expressed as the median of the total valid measurements and expressed in kilopascals (kPa). The success rate was calculated as the ratio of valid measurements to the total of measurements. TE was considered reliable when it met the following criteria: 10 valid measurements, success rate> 60%, and interquartile range (IQR) <30% of the median. We considered invalid measurements that did not meet the above criteria (unreliable) or if there was a total absence of valid (non-executable) measurements.

Elastography Point Quantification (ElastPQ)

In this study a "Point Share Wave Elastography" (pSWE) was used, with built-in Philips iU22 ultrasound software, Bothell, USA with C5-1 convex probe (1-5 MHz). The measurement of hepatic stiffness with the ElastPQ technique was performed by a single operator (C.S.) in the training study, and (FG.F) in the validation study, both with more than 5 years of experience in elastography. The measurements are carried out at the level of the right hepatic lobe, intercostally, with the patient in supine decubitus, right arm in maximum abduction and in blocked spontaneous

breathing. Using a real-time B-mode image, the operator selects an area of the parenchyma free of blood vessels and biliary structures at least 1.5 cm below the hepatic capsule: once the chosen point has been identified, the operator positions the Region of Interest (ROI) of 0.5 x 1 cm. The maximum achievable depth of the ROI is approximately 7 cm from the liver capsule. Using the software provided by the company (version 6.3.2.2), the machine calculates hepatic stiffness expressed in kiloPascals (kPa). Each patient was subjected to at least 10 measurements and the mean value, the median and the standard deviation were then calculated. We have chosen to use the median value as a reference. The company providing the software did not provide information on the quality criteria of the exam, we considered as invalid those exams in which it was not possible to obtain at least 10 measurements.

Statistical analysis

Results were expressed as median (range) for continuous variables and as frequency and percentage for categorical variables. Median values for each grade of fibrosis were compared using the Mann-Whitney test. The correlations between the results of ElastPQ, TE, FIB4, NFS, AAR and histology were analyzed by calculating the Spearman correlation coefficient (r). We considered a strong correlation if r was between 0.7 and 1.0, good if r between 0.4 and 0.7, weak if r between 0.4 and 0. The influence of clinical, biological and histological parameters on hepatic stiffness values measured with ElastPQ were evaluated using multiple

regression (phasing out) analysis. The variables that influenced stiffness with p <0.1 in the univariate analysis were included in the model.

Receiver Operating Characteristics (ROC) curves have been constructed for ElastPQ, TE, FIB4, NFS and AAR. The area under the ROC curves (AUROC) and the 95% confidence intervals (95% CI) of the values of the AUROC curves were then calculated for each degree of histological fibrosis. We considered excellent AUROC values> 0.9, good between 0.8 and 0.9, acceptable between 0.7 and 0.8. The method of DeLong et al. was used to evaluate significant differences between the various AUROCs. A p value <0.05 was considered significant.

The best cut-offs were calculated for ElastPQ in the evaluation of fibrosis F2, F3 and F4 having accuracy with 95% confidence intervals (95% CI). The respective diagnostic accuracy parameters were then calculated for each value, such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (VPN) and positive (LR+) and negative (LR-) likelihood ratio, assuming as gold standard liver biopsy. All analyzes were performed using SPSS for Windows (Statistical Package for the Social Sciences, version 21.0, Armonk, New York, NY, USA).

Results

Training cohort study

Between October 2012 and December 2019, 120 patients with NAFLD underwent blood tests, ElastPQ, TE and diffuse liver biopsy at the University Hospital of Bologna. Of these, 14 were excluded: the liver biopsy did not meet the required qualitative criteria in 2 patients, hepatic steatosis was <5% in 10 patients and concomitant causes of liver damage were found in 2 patients.

In 106 (88.3%) patients the diagnosis of NAFLD was confirmed histologically (66 men and 40 women) with a median age of 52 (18-76) years and were enrolled in the training cohort. Patient characteristics are shown in Table 2.

43.4% of the patients were obese with a similar percentage of patients being overweight. Diabetes and arterial hypertension were found in 35.8% e 38.7% of patients respectively.

About 36% of patients had fibrosis ≥F3 (moderate fibrosis was present in 30.2% and cirrhosis in 5.7%). Two out of three patients (66%) had a steatosis <33%, a third about a steatosis between 33-65% (29.2%), while only a small part (4.7%) had a steatosis >66%. A histological diagnosis of NASH was made in 90 (85%) patients.

Table 2. Baseline characteristics of NAFLD patients in the training cohort undergoing measurement of hepatic stiffness with ElastPQ

Variable	*Training cohort (n=106)*
Age	52 (18-76)
Male	66 (62.3%)
BMI	29.5 (19.4-44.6)
BMI stage:	
• Normal weight	14 (13.2%)
• Over weight	46 (43.4%)
• Obese	46 (43.4%)
Hypertension	41 (38.7%)
Diabetes	38 (35.8%)
AST (U/l)	38 (12-154)
ALT (U/l)	44.5 (11-350)
gGT (U/l)	66 (13-704)
Platelets(10^9/l)	231 (77-534)
Steatosis degree:	
▪ S1 (5-33%)	70 (66%)
▪ S2 (34-66%)	31 (29.2%)
▪ S3 (>66%)	5 (4.7%)
Lobular inflammation:	
• no inflammation	3 (2.8%)
• <2 foci per 200 x filed	74 (69.8%)
• 2-4 foci per 200 x field	38 (26.4%)
• >4 foci per 200 x filed	1 (0.9%)
Balooning epatocellulare:	
▪ No	15 (14.2%)
▪ few	52 (49.1%)
▪ very	39 (36.8%)
NAS score:	
▪ 1	1 (0.9%)
▪ 2	10 (9.4%)
▪ 3	30 (28.3%)
▪ 4	34 (32.1%)
▪ 5	20 (18.9%)
▪ 6	11 (10.4%)
NAS stage:	
• F0 (no fibrosis)	6 (5.7%)
• F1 (perisinusoidal or periportal fibrosis)	46 (43.4%)
• F2 (perisinusoidal and portal/periportal fibrosis)	16 (15.1%)
• F3 (bridging fibrosis)	32 (30.2%)
• F4 (cirrhosis)	6 (5.7%)
NASH/NAFLD	90 (84.9%)/16 (15.1%)

Factor associated with liver stiffness

In the univariate analysis, the factors associated with liver stiffness measured with ElastPQ were age (r = 0.293), BMI (r = 0.214), AST (r = 0.311), ALT (r = 0.177), PLT (r = -0.223), lobular inflammation (r = 0.179), hepatocellular ballooning (r = 0.160), NAS score (r = 0.132) and stage of fibrosis (r = 0.533). In multivariate analysis, only the stage of fibrosis was independently associated with hepatic stiffness measured with ElastPQ (B = 2.978, p <0.001) as show in the Table 3.

Table 3. Factors associated with liver stiffness

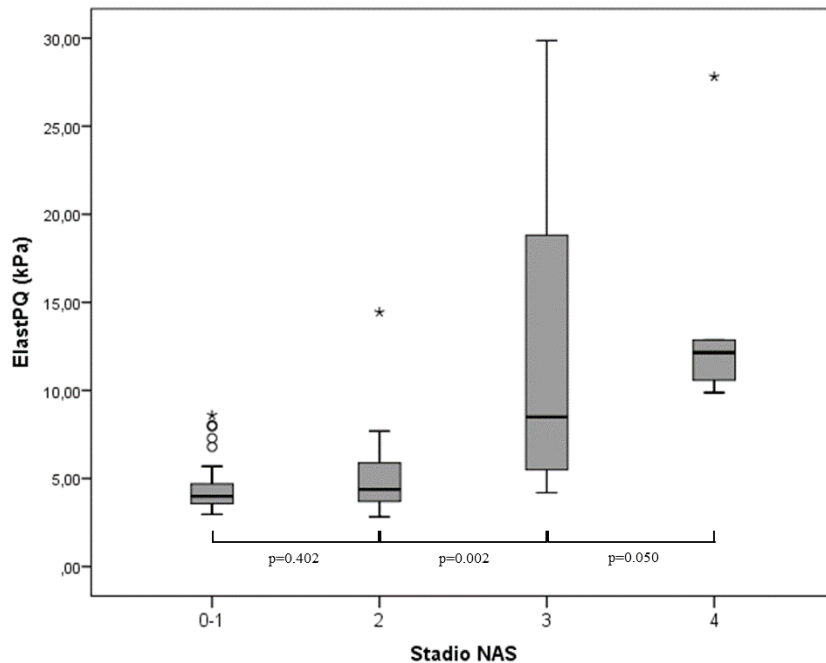| Variable	Univariate		Multivariate		
	ρ	p	B	Standard deviation error	p
Age	0.293	0.001			
Sex	0.109	0.133			
BMI	0.214	0.014			
AST	0.311	0.001			
ALT	0.177	0.035			
PLT	-0.223	0.011			
Steatosis degree	-0.062	0.264			
Lobular inflammtion	0.179	0.033			
Ballooning	0.160	0.051			
NAS score	0.132	0.090			
NAS Stage	0.533	<0.001	2.978	0.464	<0.001

Liver stiffness measurement

The median of hepatic stiffness values by ElastPQ was 4.69 kPa (2.82-29.86). Stiffness increases with increasing stage of histological fibrosis: 4.18 kPa (2.97-13.23) in F0-1, 4.49 kPa (2.82-14.44) in F2, 6.89 kPa (3.8-29.86) in F3 and 12.14 kPa (9.87- 27.81) [Table 4 and Figure 2].

Table 4. Fibrosis degree according NAS stage

NAS Stage	N	Median	Range
F0-1	52	4.15	2.97-13.23
F2	16	4.49	2.82-14.44
F3	32	6.89	3.8-29.86
F4	6	12.14	9.87-27.81

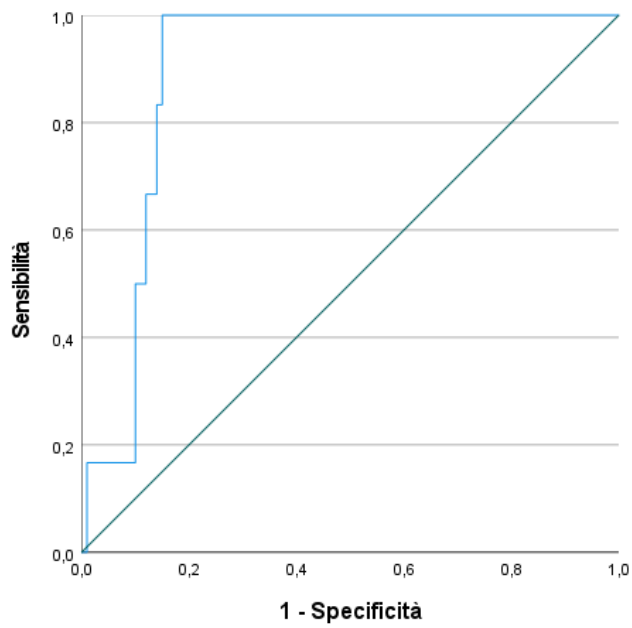**Figure 2. Boxplot of ElastoPQ measurements according to the stage of fibrosis**

Diagnostic performance of ElastPQ in determining fibrosis
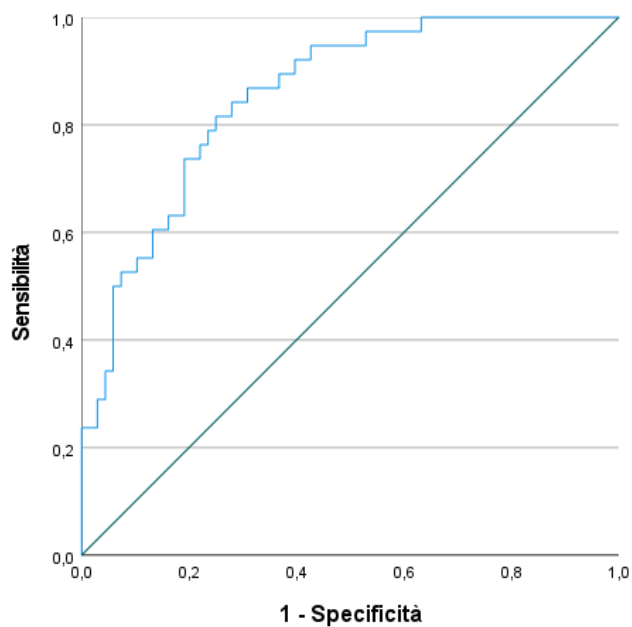
The diagnostic performance of ElastPQ according to AUROC values was acceptable for the diagnosis of significant fibrosis (≥F2) and good for the diagnosis of severe fibrosis (≥F3) and cirrhosis (F4) ranging between 0.855 and 0.897 (Figure 3).

Figure 3. Receiver-operating characteristic curve (AUROC) in training cohort for A. significant fibrosis (F≥2), B. advanced fibrosis (F≥3) and C. cirrhosis (F4)

A.

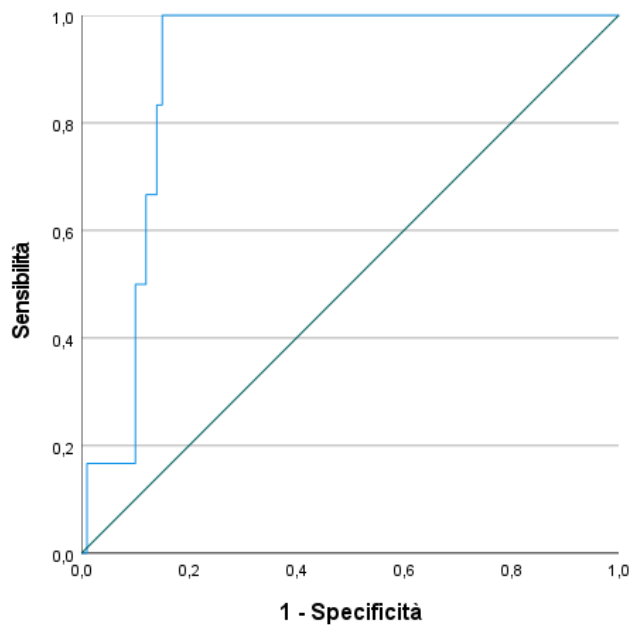**B.**

C.

Optimal ElastPQ cuf-offs for the diagnosis of fibrosis ≥F2, ≥F3 and cirrhosis are described in Table 5.

For fibrosis stages ≥F2, the best cut-off with an accuracy (95% CI) of 73.6 %, was 5.2kPa, with a sensitivity of 68.5 % and a specificity of 78.8%.

For fibrosis stages ≥F3, the best cut-off with an accuracy (95% CI) of 77.4%, was 6.3 kPa, with a sensitivity of 71.1% and a specificity of 80.9%.

For stages of F4 fibrosis, the best cut-off with an accuracy (95% CI) of 85.9%, was 9.61 kPa, with a sensitivity of 100% and a specificity of 85%.

Table 5. ElastPQ performance in the training cohort

Stadio fibrosi	AUROC (95% CI)	P value		Cut off	Sensibilità (95% CI)	Specificità (95% CI)	VPP (95% CI)	VPN (95% CI)	Accuracy (95% CI)	+LR	-LR
≥2	0.783 (0.693-0.857)	<0.001	Best cut off	5.22	68.5% (54.3-80.1)	78.8% (64.9-88.5)	77.1% (62.3-87.5)	70.7% (57.1-81.5)	73.6% (64.1-81.7)	3.24	0.40
			Sensibilità ≥90%	3.79	90.7% (79.7-96.9)	38.5% (25.3-53)	59.8% (48.3-70)	79.2% (57.3-92)		1.47	0.24
			Specificità ≥90%	7.29	44.4% (30.9-58.6)	90.4% (79-96.8)	82.8% (63.5-93.5)	61% (49.2-71.7)		4.62	0.61
≥3	0.855 (0.773-0.916)	<0.001	Best cut off	6.3	71.1% (53.9-84)	80.9% (69.2-89)	67.5 (50.8-80.9))	83.3% (71.7-91)	77.4% (68.2-84.9)	3.72	0.36
			Sensibilità ≥90%	4.5	92.1% (78.6-98.3)	60.3% (47.7-72)	56.5% (43.3-68.8)	93% (80.3-98.2)		2.32	0.13
			Specificità ≥90%	7.96	52.6% (35.8-69)	91.2% (81.8-96.7)	76.9% (55.9-90.3)	77.5% (66.5-85.8)		5.96	0.52
4	0.897 (0.822-0.947)	<0.001	Best cut off	9.61	100% (51.7-100)	85% (76.1-91.1)	28.5% (12.2-52.3)	100 (94.6-100)	85.9% (77.7-91.9)	6.67	0
			Sensibilità ≥90%	9.35	100% (54.1-100)	85% (76.5-91.4)	28.6% (12.2-52.3)	100% (94.6-100)		6.67	0
			Specificità ≥90%	12.16	50% (11.8-88.2)	90% (82.4-95.1)	23.1% (6.1-54)	96.8% (90.2-99.2)		5	0.56

Comparison of the diagnostic performance of ElastoPQ with TE and other non-invasive methods for assessing fibrosis

Comparisons of AUROC values between ElastPQ, TE, FIB4, NFS and AAR were performed on 96 patients (14 were excluded due to invalid TE values).

The median values of hepatic stiffness for each stage of fibrosis are shown in Table 6. A good correlation with the stage of fibrosis was shown for ElastPQ, TE, FIB4 and NFS (respectively r = 0.651, 0.616, 0.581 and 0.512) while it is found weak for AAR. ElastPQ and TE confirmed a strong correlation between them (r = 0.705).

For the diagnosis of significant fibrosis (≥F2), ElastPQ and TE have a good AUROC comparable between the two methods (0.810 versus 0.807, p = 0.956) while those of FIB4 and NFS (respectively 0.776 and 0.713) are acceptable, not higher than ElastPQ.

For the diagnosis of advanced fibrosis (≥F3), ElastPQ has an excellent AUROC (0.911) while that of TE, FIB4 and NFS which have a good AUROC (respectively 0.850, 0.861 and 0.803) is good, the first two not significantly higher than ElastPQ. The AUROC of ElastPQ was not inferior to TE, FIB4 and NFS even for the diagnosis of cirrhosis (Table 7).

Table 6. Median values and correlations with histology and ElastPQ in the training cohort
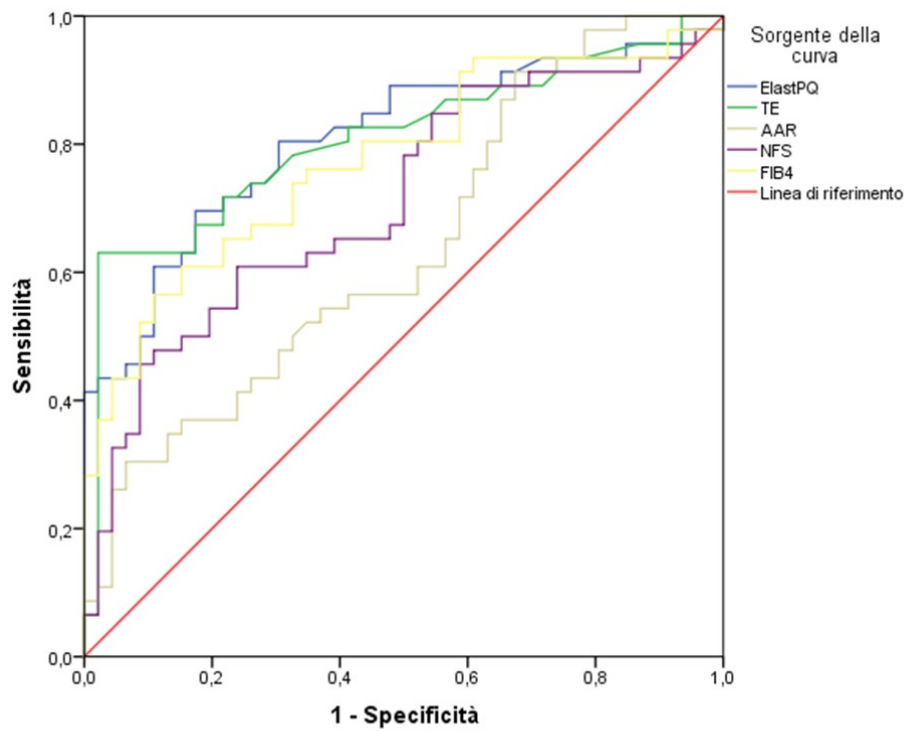
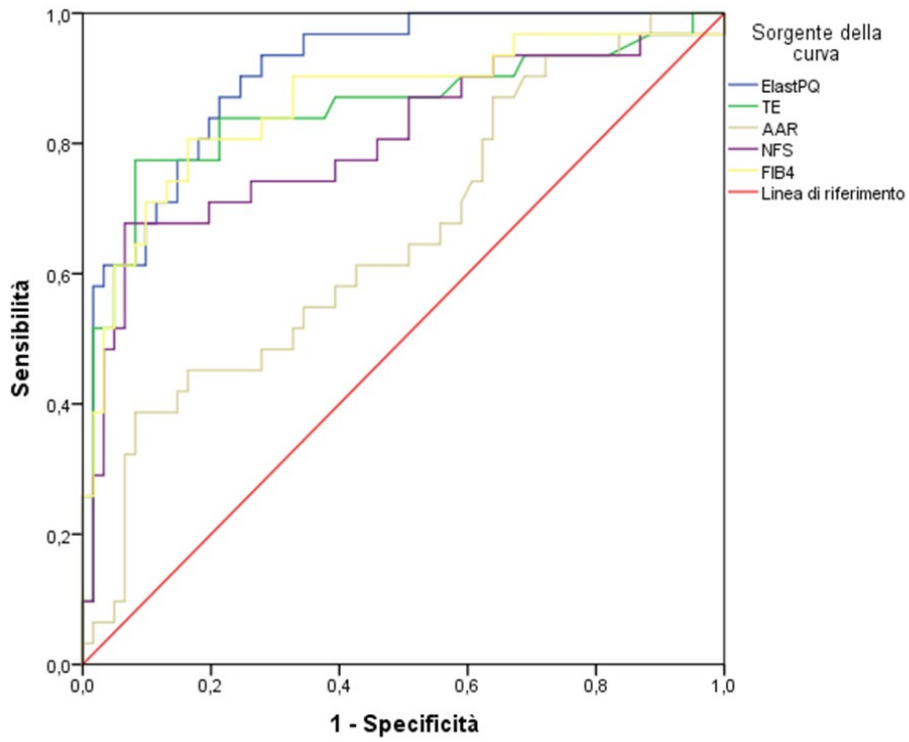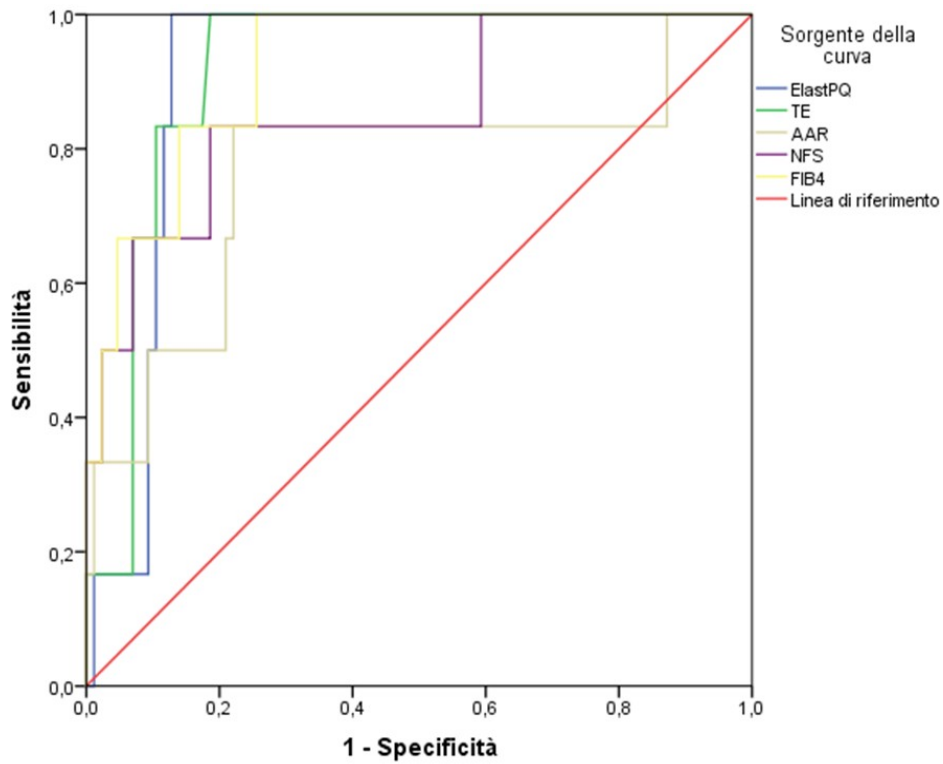|	Stadio NAS				Coefficiente di correlazione	
	Stadio 0-1	Stadio 2	Stadio 3	Stadio 4	vs biospia	vs ElastPQ
ElastPQ	4.03 (2.97-8.59)	4.38 (2.82-14.44)	8.49 (4.19-29.86)	12.14 (9.87-27.81)	0.651 (p<0.001)	
TE	6.1 (3.6-37.4)	7.5 (4.2-14.3)	12 (4.1-44.3)	25 (12-55.1)	0.616 (p<0.001)	0.705 (P<0.001)
FIB4	0.95 (0.27-2.43)	1.08 (0.34-3.03)	1.85 (0.19-4.06)	3.35 (1.8-5.67)	0.553 (p<0.001)	0.581 (p<0.001)
NFS	-2.382 (-4.956-1.285)	-2.221 (-4.77—0.901)	-0.187 (-0.698-1.514	1.052 (-2.233-3.226)	0.463 (p<0.001)	0.512 (p<0.001)
AAR	0.708 (0.3-1.5)	0.683 (0.5-1.8)	0.789 (0.4-1.4)	1.137 (0.5-1.9)	0.300 (p=0.004)	0.264 (p=0.011)

Table 7. Comparison of AUROCs among the various non-invasive methods in the training cohort

	Stage 01 vs 2-4			Stage 02 vs 3-4			Stage 03 vs 4		
Modality	AUROC (95% CI)	SE	vs ElastPQ P value	AUROC (95% CI)	SE	vs ElastPQ P value	AUROC (95% CI)	SE	vs ElastPQ P value
ElastPQ	0.810 (0.714-0.884)	0.0460		0.911 (0.833-0.960)	0.0295		0.909 (0.831-0.959)	0.0323	
TE	0.807 (0.712-0.882)	0.0475	0.9564	0.850 (0.761-0.916)	0.0499	0.1712	0.918 (0.842-0.965)	0.0337	0.7733
FIB4	0.776 (0.677-0.856)	0.0491	0.5752	0.861 (0.773-0.924)	0.0461	0.2747	0.922 (0.848-0.968)	0.0447	0.7855
NFS	0.713 (0.609-0.802)	0.0544	0.0726	0.803 (0.707-0.879)	0.0544	0.0418	0.855 (0.766-0.919)	0.0955	0.5620
AAR	0.644 (0.537-0.741)	0.0571	0.0115	0.652 (0.545-0.748)	0.0613	<0.0001	0.763 (0.663-0.845)	0.129	0.2798

Figure 3. Receiver-operating characteristic curve (AUROC) of ElastPQ, TE, FIB4, NFS and AAR for the diagnosis of (A) significant fibrosis (≥F2), (B) advanced fibrosis (≥F3), (C) cirrhosis (F4)

A

B

C

Validation cohort study

Between January 2018 and July 2021, 84 patients with NAFLD underwent blood tests, ElastPQ and diffuse liver biopsy at the city Hospital of Faenza. Of these, 4 were excluded since the biopsy did not meet the required qualitative criteria. The diagnosis of NAFLD was confirmed histologically in 80 patients (48 men and 32 women) and were therefore enrolled in the validation cohort. Patient characteristics are shown in Table 8.

The median age was 54 (19-79) years and 36.2% were obese. Diabetes and arterial hypertension were found in 32.5% e 41.37% of patients respectively. Based on liver biopsy, 33.8% of patients had fibrosis ≥F3 (moderate fibrosis was present in 22.5% and cirrhosis in 11.3%).

Training and validation cohorts were similar for demographic and clinical characteristics such as the presence of hypertension, diabetes, BMI degrees and stage of fibrosis.

Table 8. Comparison between the main features of the training cohort and the validation cohort

Variable	Total population (n=186)	Training cohort (n=106)	Validation cohort (n=80)	p
Age	53 (18-79)	52 (18-76)	54 (19-79)	0.456
Male	114 (61.3%)	66 (62.3%)	48 (60%)	0.763
BMI	29 (19.4-44.6)	29.5 (19.4-44.6)	28.5 (19.7-41)	0.183
BMI stage:				
• Normal weight	29 (15.6%)	14 (13.2%)	15 (18.8%)	
• Over weight	82 (44.1%)	46 (43.4%)	36 (45%)	0.472
• Obese	65 (40.3%)	46 (43.4%)	29 (36.2%)	
Hypertension	74 (39.8%)	41 (38.7%)	33 (41.3%)	0.763
Diabetes	64 (34.4%)	38 (35.8%)	26 (32.5%)	0.644
AST	38 (12-154)	38 (12-154)	38 (12-154)	0.789
ALT	45.5 (11-478)	44.5 (11-350)	46 (11-478)	0.882
gGT	64 (11-704)	66 (13-704)	63 (11-643)	0.812
Platelets	235 (77-534)	231 (77-534)	217 (77-397)	0.657
NAS stage:				
• F0 (no fibrosis)	15 (8.1%)	6 (5.7%)	9 (11.3%)	
• F1 (perisinusoidal or periportal fibrosis)	71 (38.2%)	46 (43.4%)	25 (31.3%)	
• F2 (perisinusoidal and portal/periportal fibrosis)	35 (18.8%)	16 (15.1%)	19 (23.8%)	0.088
• F3 (bridging fibrosis)	35 (26.9%)	32 (30.2%)	18 (22.5%)	
• F4 (cirrhosis	15 (8.1%)	6 (5.7%)	9 (11.3%)	

We applied in this cohort the best cut offs of liver stiffness identified in the training cohort. In the validation cohort, the accuracy of the best cut offs for F≥2 and F≥3 is comparable to that of the training cohort (respectively p = 0.219 and p = 0.406). That for F≥4 is even better (p = 0.006) (Table 9).

The results show that these cut-offs are reproducible and have a good performance.

Table 9. Performance of the best cut offs of ElastPQ applied in the validation cohort

Stadio fibrosi	Best Cut off	Sensibilità (95% CI)	Specificità (95% CI)	VPP (95% CI)	VPN (95% CI)	Accuracy (95% CI)	+LR	-LR
≥2	5.22	73.9% (58.6-85.2)	91.2% (75.2-97.7)	91.9% (77-97.9)	72.1% (56.1-84.2)	81.3% (71-89.1)	8.38	0.29
≥3	6.3	74.1% (53.4-88.1)	86.8% (74-94.1)	74.1% (53.4-88.1)	86.8% (74-94.1)	82.5% (72.4-90.1)	5.61	0.30
4	9.61	100% (62.9-100)	97.2% (89.3-99.5)	81.8% (47.7-96.8)	100% (93.4-100)	97.5% (91.3-99.7)	35.5	0

Discussion

In recent years, the number of ultrasound-based elastography techniques has increased rapidly and numerous Shear Wave Elastography techniques have been introduced by various companies. Regarding Philips ElastPQ technology, only a few studies have been published to date, often with limited series, mainly in cases of viral aetiology and without histology as a reference standard, due to the decreasing number of liver biopsies performed in many centers.

Although several studies have evaluated the diagnostic performance of ElastPQ in patients with primarily viral liver disease, our study is one of the few to evaluate the accuracy of ElastPQ specifically in patients with NAFLD and to compare it with TE and other serum markers of fibrosis using the liver biopsy as a reference standard.

Consistent with previous results, our data showed that liver stiffness measured with ElastPQ correlated directly and linearly with the stages of fibrosis. Using ElastPQ, steatosis, lobular inflammation and ballooning (assessed directly and indirectly by the NAS score) do not appear to significantly influence the liver stiffness values and only stage of fibrosis was independently associated with liver stiffness. This result can be considered positive because the impact of several confounding factors is often a limitation for most other diagnostic techniques. However, the lack of a link between these factors and ElastPQ can also be considered

negative in NAFLD screening, because some patients with NASH may have no fibrosis but have liver damage with lobular inflammation or hepatocyte ballooning. Therefore, ElastPQ may not help diagnose early-stage NASH without fibrosis.

Our results strongly support that ElastPQ has high diagnostic accuracy for the staging of hepatic fibrosis. As previously reported for other elastography techniques, in this prospective cohort of patients with NAFLD undergoing liver biopsy, the diagnostic accuracy of ElastPQ was better in differentiating between non-advanced and advanced fibrosis (≤F2 vs ≥F3) with an AUROC of approximately 85% and in discriminating the presence of cirrhosis (≤F3 vs F4) with an AUROC of approximately 89%, because patients with cirrhosis were few. Both represent an important clinical threshold correlated to a worse long-term outcome of this type of patient. Furthermore, drug therapies currently under development should be reserved, at least initially, for this category of patients.

ElastPQ, on the other hand, is less performing for minor degrees of fibrosis with an AUROC of 80% in the identification of significant fibrosis (≤F1 vs ≥F2). According to these results, this technique can be used in clinical practice as a valuable diagnostic tool for diagnosing advanced fibrosis and as an excellent tool for diagnosing cirrhosis

Furthermore, ElastPQ showed no inferior performance compared to the most used elastography technique in clinical practice (TE) for each stage

of fibrosis, suggesting that both methods can be used in the non-invasive work-up of patients.

The diagnostic performance of ElastPQ was also compared with that of serum indices of hepatic fibrosis. ElastPQ, like TE, generally performs better than FIB4, NFS in identifying each stage of hepatic fibrosis.

In conclusion, ElastoPQ is an accurate and reliable non-invasive method for staging liver fibrosis in patients with NAFLD. This technique provides similar diagnostic performance to TE in identifying all stages of fibrosis, but it has the advantage of being integrated with a conventional ultrasound device which would allow savings in time and cost. Furthermore, thanks to the use of B-mode imaging, it allows the measurement of hepatic stiffness in regions with sufficient acoustic signal and without artifacts.

ElastPQ could provide a measurement of stiffness in those patients where reliable data cannot be obtained using TE, particularly in those patients with high BMI. Finally, this technique does not seem to be influenced by confounding factors such as inflammation and/or the presence of steatosis unlike other methods, such as TE.

It's well known that the best cut-off values of the different elastography techniques used to evaluate the presence and severity of liver fibrosis depend upon the etiology of the underlying liver disease.

We identified the best cut offs for each degree of fibrosis in the training population and applied them to the validation population and they result to be reproducible in identifying degrees of fibrosis.

In the future, further prospective studies with larger patient cohorts are needed to better validate the cut offs obtained with ElastPQ for different stages of fibrosis and to assess their prognostic value in predicting clinically relevant outcomes, such as the development of portal hypertension, ascitic decompensation and mortality.

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