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Multiparametric ultrasound evaluation of liver fibrosis, steatosis, and viscosity in patients with chronic liver disease

Iulia Minciuna[1,2], Madalina Taru[1,2], Andreea Fodor[1], Oana Nicoara Farcau[1,2], Petra Fischer[1,2], Corina Radu[1,2], Monica Lupsor-Platon[1,2], Horia Stefanescu[1], Bogdan Procopet[1,2]

[1]Regional Institute of Gastroenterology and Hepatology "O. Fodor", [2]"Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Abstract

Aims: This study assessed the effectiveness of three ultrasound-based techniques (2D-SWE.PLUS, Att.PLUS, Vi.PLUS) for non-invasive evaluation of liver fibrosis, steatosis, and inflammation in chronic liver disease (CLD) patients. **Materials and methods:** Involving 209 consecutive compensated CLD patients, the study compared these ultrasound methods from Aixplorer with standard Vibration-controlled Transient Elastography (VCTE) and Controlled Attenuation Parameter (CAP) from Fibroscan, alongside non-invasive serological markers. **Results:** High validity rates were observed in measurements: 99% for VCTE, 89% for 2D-SWE.PLUS/Vi.PLUS, and 96.6% for Att.PLUS. 2D-SWE.PLUS showed a strong correlation with VCTE (R=0.91) and excelled at a lower Stability Index (80%), with optimal cut-offs for moderate and severe fibrosis at 8 kPa and 10 kPa, respectively. 2D-SWE.PLUS was superior to Fib4, eLIFT, APRI, BARD, and NFS in detecting advanced CLD. Att.PLUS moderately correlated with CAP (R=0.47) for steatosis grades, while Viscosity was highly effective in identifying significant fibrosis (AUC=0.87) but less so for inflammation. **Conclusions:** 2D-SWE.PLUS demonstrated superior diagnostic precision in liver fibrosis, exceeding other non-invasive markers. Att.PLUS was relatively accurate for liver steatosis, and viscosity more effectively indicated fibrosis stages than inflammation in CLD patients.

Keywords: Multiparametric assessment; fibrosis; steatosis; viscosity; ultrasound

Introduction

Persistent liver injury, regardless of the etiology, often leads to ongoing necro-inflammation, contributing to increased collagen accumulation, which is a crucial aspect of wound healing and liver regeneration [1]. Chronic injury promotes fibrosis, increasing the risk of advanced chronic liver disease (ACLD), liver failure, and hepatocellular carcinoma (HCC). Therefore, liver fibrosis remains the pivotal prognostic factor for morbidity and mortality in chronic liver disease (CLD).

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Corresponding author: Iulia Minciuna, MD
Regional Institute of Gastroenterology and Hepatology "O. Fodor", 19-21 Croitorilor str., 400394, Cluj-Napoca, Romania
Phone: 0264 596 414
E-mail: iuliabreaban@yahoo.com

While liver biopsy is the gold standard for assessing liver fibrosis and disease activity, it has some limitations regarding its invasiveness, reduced patient acceptance, potential complications, and sampling variability [2]. These limitations have been partly overcome by developing noninvasive tests for assessing liver fibrosis, necro-inflammatory activity, and steatosis.

In recent years, elastographic ultrasound techniques have advanced, enabling the staging of liver fibrosis with the ability to assess liver stiffness across a volume at least 100 times larger than a biopsy sample [3]. Two-dimensional share wave elastography (2D-SWE) is a novel technique utilizing focused ultrasonic beams to generate shear waves in liver tissue, that are captured in real-time by high frame-rate ultrasound imaging, producing a 2D quantitative map of liver tissue stiffness [4]. With the added benefit of B-mode guidance and the use of shear waves with broader bandwidths, 2D-SWE improves the separation of fibrosis stages [4].

A pivotal step in ultrasound-based technologies is conducting a multiparametric assessment, concurrently evaluating liver steatosis and necro-inflammatory activity.

The assessment of liver steatosis should be performed in every CLD patient as it is a characteristic feature of two of the most prevalent CLD (metabolic-associated steatotic liver disease-MASLD and alcohol related liver disease-ALD) and a significant prognostic factor in the remaining [5]. The Att.PLUS module, embedded in the Aixplorer MACH 30, measures the decrease in ultrasound wave amplitude propagating throughout a selected region of interest (ROI) within a tissue as a function of frequency. Additionally, the system allows the evaluation of liver viscosity by measuring liver shear weave dispersion, with preliminary studies suggesting its potential in reflecting necro-inflammatory activity in the liver and facilitating early identification of liver fibrosis [6].

This study aims to prospectively evaluate the feasibility and the performance of three new ultrasound-based techniques (ShearWave Elastography, Attenuation Plane-wave UltraSound, and Viscosity Plane-wave UltraSound) for the noninvasive assessment of liver fibrosis, steatosis, and inflammation, using Vibration-controlled transient elastography (VCTE) with Controlled Attenuation Parameter (CAP) as reference method. Moreover, we further evaluate if valid measurements could be achieved at different stability indexes and using a reduced number of measurements.

Material and methods

Study population

All consecutive adult patients with CLD who had undergone an abdominal ultrasound and liver stiffness evaluation using VCTE between October 2020 and January 2021 in our department were prospectively included. Patients with decompensated liver disease, liver cancer, obstructive jaundice, heart failure resulting in liver congestion, and aminotransferase levels more than five times the upper limit of normal were excluded.

Ultrasound-based measurements were performed in all patients in the same session, using ShearWave Elastography (2D-SWE.PLUS), Attenuation Plane-wave Ultrasound (Att.PLUS), Viscosity Plane-wave Ultrasound (Vi.PLUS) from Aixplorer MACH® 30 system (Supersonic Imagine, Aix-en-Provence, France), with VCTE and CAP (FibroScan, EchoSens, Paris, France) serving as the reference method. This research received approval from the Ethics Committee of the Regional Institute of Gastroenterology and Hepatology "Prof. Dr. Octavian Fodor", Cluj-Napoca and adhered to the World Medical Association Declaration of Helsinki, with all patients providing written informed consent.

The elastography and ultrasound examinations were performed by three physicians (HS, MLP, BP) with at least five years of experience blinded to each other's findings and the patient's medical information.

ShearWave PLUS Elastography

The 2D-SWE.PLUS measurements were conducted using the Aixplorer Mach 30 ultrasound system's C6-1X convex transducer and its inbuilt UltraFast™ software. Patients, in a fasting and supine position, underwent right liver lobe assessment using intercostal approach. After identifying the best acoustic window, the shear-wave measurement box was located at least 1.5 cm beneath the liver capsule, steering clear of large vessels, bile ducts, and rib shadows, over an area of uniform parenchyma. When the image had stabilized for 3 seconds, measurements were taken during neutral respiratory apnea. An adequate Share wave elastography (SWE) map was created, followed by image acquisition and the positioning of the Q-box within a region of consistent elasticity at a depth of 3–5 cm. Stability Index (SI) was computed, and the median value from five measurements represented liver stiffness (LS) in kilopascals (kPa). A measurement reliability criterion was set with an interquartile range to median ratio (IQR/M) below 30%.

Viscosity

As Vi.PLUS was combined with the SWE mode, acquisitions were concurrently carried out with the SWE measurements following the same protocol (fig 1).

Attenuation Plane-Wave Ultrasound

Operating in B-mode on a live image, Att.PLUS utilized the same ultrasound transducer and acquisition protocol as 2D-SWE. Reliable measurements based on median value from 5 assessments, had an IQR/M below

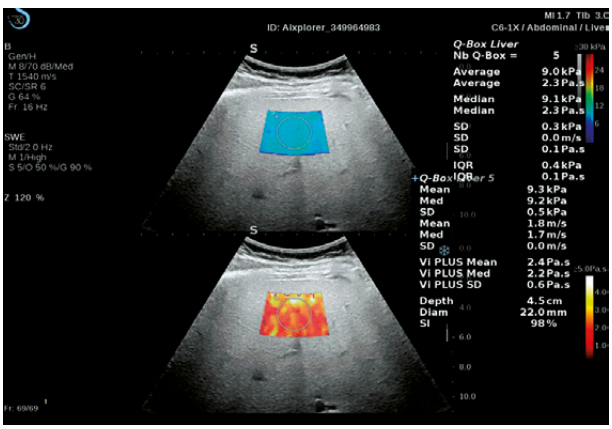

Fig 1. Illustration of a ShearWave PLUS Elastography (2D-SWE.PLUS) and Vi.PLUS measurement performed in a patient with liver steatosis.

30%. In addition, we compared the predictive performance of Att.PLUS of 3, 5, or 7 measurements.

Hepatorenal Index Measurement (HRI)

Analyzing the mean brightness of gray-scale pixels within two selected ROIs in the liver and kidney, HRI calculated brightness ratios (HRI). This calculation was done at different depth levels, averaging three HRI values.

Transient Elastography and Controlled Attenuation Parameter

The FibroScan was utilized to carry out VCTE along with CAP measurements as previously detailed [5]. Depending on the patient, either the Standard M probe (3.5 Hz frequency) or for obese patients with body mass index (BMI) ≥30kg/m2), the XL probe (2.5 Hz frequency) was chosen, with the assistance of the device's integrated automatic probe selection software. Reliable results, representing the median of 10 valid measurements with an IQR/M below 30%, were given in kPa.

In this study, the defined thresholds were as follows: F1: <7 kPa; F2: 7-10 kPa [7]; compensated advanced liver disease (cACLD): F3-F4 ≥10 kPa [8]. For evaluating steatosis, the thresholds used were: S1: 260 dB/m, S2: 285 dB/m, S3: 294 dB/m [9].

Non-invasive serological markers

We used the following cut-off values of the non-invasive fibrosis markers to predict cACLD: 3.25 for Fib4 (Fibrosis 4 score; assessing age, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), platelet count), 8 for eLIFT (Easy Liver Fibrosis Test; assessing age, gender, GOT, Gamma-glutamyl Transferase (GGT), platelets, prothrombin time), 1.5 for APRI (GOT to platelet ratio index), 0.676 for NFS (NAFLD fibrosis score; assessing age, BMI, GOT, GPT, platelet count, albumin, impaired fasting glucose/ diabetes), and 2 for BARD (BMI, aspartate aminotransferase/ alanine aminotransferase ratio, type 2 diabetes mellitus score) [10-14]. The formulas used to calculate the scores were included in the Supplementary file.

Statistical analysis

The statistical analysis was performed using R 4.1.1 software.

Quantitative variables were expressed as medians with extreme values (range) and compared using Student's t-test or Wilcoxon test as appropriate. Qualitative variables were expressed as numbers and percentages and compared using Chi-square or Fisher's exact tests, as appropriate.

Linear regression analysis was used to evaluate the correlation between LSMs obtained by VCTE and 2D-SWE.PLUS and between the values obtained using Att. PLUS and CAP.

Att.PLUS, 2D-SWE.PLUS and viscosity diagnostic performance were assessed using the concordance statistic (c-statistic), equivalent to the area under the receiver operating characteristic (ROC) curve. Optimal cut-off values that maximized the diagnostic accuracy were determined from the AUROC curve analysis. 95% confidence intervals (CI) were calculated for each predictive test, with a p-value below 0.05 considered statistically significant.

Results

Characteristics of the included population

Among 237 consecutive patients undergoing multiparametric measurements, 28 patients were excluded from the study due to ascitic decompensation. Table I exhibits the baseline characteristics of the 209 included patients, stratified by the presence of cACLD.

In the overall study population, the underlying liver disease etiology was viral in 102 cases (54.3%), followed by ALD in 48 cases (25.5%), and MASLD in 28 cases (14.9%). Most patients had mild or moderate fibrosis (n=107, 51.2%), with a median liver stiffness value of 9.8 kPa (2.5-75) by VCTE and of 10.4 kPa (3.3-76.7) by 2D-SWE.PLUS and with a median liver viscosity value of 2.2 Pa.s (1.1-5). According to CAP measurements, 64.1% (n=134) of patients had no or mild steatosis, with a median CAP value of 266 dB/m (100, 400), a median Att.PLUS value of 0.41 dB/cm/MHz (0.16-0.77) and a median HRI value of 1.19 (0.6-2.79).

Feasibility

Valid LSMs were obtained in 207 (99%) patients by VCTE, 186 (89%) patients by 2D-SWE.PLUS/Vi.PLUS, and 202 (96.65%) patients by Att.PLUS. Failure to acquire valid 2D-SWE. PLUS was due to an inhomogeneous filling of the color map (no or little signal) or due to unreliable measurements.

A valid liver stiffness measurement could be achieved significantly more times by VCTE than by SWE, p<0.001. Neither liver function nor BMI correlated with the measurement's feasibility assessed by SWE.

The median value of the SI in our population was 91% (12, 99). Accurate measurements of different stages of liver fibrosis could be achieved even at lower levels of the SI, >80%, with good accuracy of >90.2% and an AUC of 0.95 (95%CI: 0.92-0.99).

When adjusting for the number of Att.PLUS measurements, CAP value was significantly correlated with Att.PLUS value (p<0.001). Specifically, in this prediction model, a 1-unit increase in Att.PLUS value led to a 256.91 dB/m (95%CI: 188.08–325.73) increase in CAP

Table I. Characteristics of the study population

Variable	Overall population n=209	cACLD n=102	Non-cACLD n=107
Sex (male)	105 (50.2)	60 (58.8)	45 (42.1)
Age (years)	59 (18-87)	61 (32-87)	58 (18-79)
BMI	27.68 (15.57- 42.24)	29.4 (19.10- 42.24)	26.3 (15.60- 40.9)
Etiology			
ALD	48 (25.5)	41 (40.2)	7 (6.5)
Viral	102 (54.3)	38 (37.3)	64 (59.8)
MAFLD	28 (14.9)	10 (9.8)	18 (16.8)
Cholestatic liver disease	10 (5.3)	5 (4.9)	5 (4.7)
Fibroscan: Liver stiffness (kPa)	9.8 (2.5- 75)	25.1 (10-75)	5.1 (2.5- 9.9)
Fibroscan probe: M	193 (92.3)	90 (88.2)	103 (96.2)
Fibroscan: Fibrosis stage			
F0-F1 (<7 kPa)	78 (37.3)	0	78 (72.9)
F2 (7-10 kPa)	29 (13.9)	0	29 (27.1)
F3-F4 (>10 kPa)	102 (48.8)	102 (100)	0
CAP value (db/m)	266 (100- 400)	267.5 (100- 400)	263 (100-400)
CAP: Steatosis grade			
No steatosis	100 (47.8)	47 (46.1)	53 (49.5)
S1 (260-284 db/m)	34 (16.3)	15 (14.7)	19 (17.8)
S2 (285-294 db/m)	9 (4.3)	5 (4.9)	4 (3.7)
S3 (≥295 db/m)	66 (31.6)	35 (34.3)	31 (29.0)
Number of HRI measurements	3 (1-4)	3 (1-4)	3 (1-4)
HRI value	1.19 (0.6- 2.79)	1.19 (0.68- 2.29)	1.19 (0.6- 2.79)
Number of Att.PLUS measurements	5 (1-7)	5 (2-7)	5 (1-7)
Att.PLUS value (dB/cm/MHz)	0.41 (0.16- 0.77)	0.41 (0.16- 0.77)	0.42 (0.21- 0.73)
2D-SWE.PLUS value (kPa)	10.4 (3.3- 76.7)	22.6 (6.5- 76.7)	6.3 (3.3- 37.7)
Viscosity value (Pa.s)	2.2 (1.1- 5)	2.7 (1.2- 5)	1.8 (1.1- 3.0)

Data are presented as number (%) or median (min, max). BMI- body mass index, cACLD - compensated advanced chronic liver disease, MASLD - metabolic associated steatotic liver disease, ALD - alcohol related liver disease, COPD - Chronic obstructive pulmonary disease, HRI - hepatorenal index, 2D-SWE.PLUS - Shear Wave Elastography, CAP - Controlled Attenuation Parameter, F - fibrosis stage, S – steatosis grade

value. However, incorporating the number of Att.PLUS measurements into the model did not enhance its predictive performance.

Fibrosis

In the overall group, 2D-SWE.PLUS value had an excellent correlation with VCTE value, R=0.913 ($p<0.001$), particularly in the cACLD subgroup. The correlation increased from R=0.56 to R=0.84 as the estimated fibrosis stage was higher. Adjusting for estimated steatosis grade maintained a good correlation between 2D-SWE.PLUS and VCTE values.

The AUC for predicting moderate fibrosis was 0.95 (95%CI: 0.92-0.98), with an optimal cut-off value of 8 kPA and 90% accuracy. For cACLD diagnosis, the AUC was 0.97 (95%CI: 0.95-0.99), with an optimal cut-off value of 10 kPa and 92.8% accuracy. No factor related to liver function, etiology, comorbidities, BMI, age, or estimated degree of steatosis correlated with the failure to estimate fibrosis stage by SWE.

Table II illustrates the correlation between different non-invasive markers of liver fibrosis and the two elastography methods. Our findings demonstrated that regardless of the underlying cause, VCTE values strongly correlated with those of 2D-SWE.PLUS. In ALD and the viral group, VCTE showed the strongest correlation with 2D-SWE.PLUS values, followed by the NFS and Fib4 values. In the MASLD group, VCTE showed the highest correlation with NFS, nearly the same as with 2D-SWE. PLUS, followed by e-LIFT and Fib4.

Using VCTE as the reference, we identified 2D-SWE. PLUS as having the best performance in identifying patients with cACLD, followed by Fib4 with an AUC of 0.74 (95%CI:0.67-0.81), e-LIFT with 0.68 (95%CI:0.61-0.75), APRI with 0.61 (95%CI:0.54-0.69), BARD with

Table II. The correlation between non-invasive markers of liver fibrosis, Fibroscan and 2D-SWE.PLUS values, stratified by etiology of liver disease.

			VCTE	2D-SWE.PLUS	BARD	NFS	APRI	FIB4	ELIFT
ALD	VCTE	Cc	1	0.836	-0.004	0.741	0.31	0.448	0.136
		p value	-	<0.001	0.98	0.002	0.046	0.003	0.48
	2D-SWE. PLUS	Cc	0.836	1	0.016	0.802	0.378	0.453	0.289
		p value	<0.001	-	0.927	<0.001	0.014	0.003	0.128
Viral	VCTE	Cc	1	0.899	0.405	0.713	0.642	0.696	0.696
		p value	-	<0.001	0.001	0.009	<0.001	<0.001	<0.001
	2D-SWE. PLUS	Cc	0.899	1	0.405	0.727	0.609	0.660	0.676
		p value	<0.001	-	0.001	0.007	<0.001	<0.001	<0.001
MASLD	VCTE	Cc	1	0.897	0.095	0.900	0.688	0.788	0.873
		p value	-	<0.001	0.69	0.037	<0.001	<0.001	0.002
	2D-SWE. PLUS	Cc	0.897	1	0.333	0.700	0.653	0.845	0.627
		p value	<0.001	-	0.151	0.188	0.001	<0.001	0.071

2D-SWE.PLUS- Shear Wave Elastography, MASLD- metabolic associated steatotic liver disease, ALD- alcohol related liver disease, Fib4- fibrosis 4 score, eLIFT- easy liver fibrosis test, APRI- AST to platelet ratio index, BARD- BMI, aspartate aminotransferase/alanine aminotransferase ratio, type 2 diabetes mellitus score, NFS- NAFLD fibrosis score, Cc- Correlation coefficient

0.56 (95%CI:0.48- 0.64) and NFS with 0.40 (95%CI: 0.49-0.65).

Steatosis: Att.PLUS and HRI analysis

In the overall group, CAP values moderately correlated with Att.PLUS (R=0.472, p<0.001), and weakly with the HRI median value (R=0.308, p<0.001).

Overall, Att.PLUS demonstrated moderate performance in predicting steatosis grade (AUC=0.76; 95%CI:0.69-0.82). Optimal Att.PLUS cut-off values for S1, S2, and S3 were 0.45 dB/cm/MHz, 0.5 dB/cm/MHz, and 0.55 dB/cm/MHz, respectively.

Across different fibrosis stages, moderate correlations existed between the CAP and Att.PLUS values in patients with mild or no fibrosis (R=0.48, p<0.001), moderate fibrosis (R=0.58, p<0.001), and cACLD, (R=0.47, p<0.001) (fig 2).

Att.PLUS moderately discriminated patients with different steatosis grades. No factors related to liver function, etiology, comorbidities, BMI, age, or estimated degree of fibrosis were associated with the failure to Att. PLUS's failure to determine steatosis grade.

Concerning etiology, Att.PLUS achieved the best performance in predicting different steatosis grades in the MASLD subgroup (AUC: 0.76 to 0.86), followed by the chronic viral hepatitis subgroup with a moderate performance (AUC: 0.66 to 0.81), and ALD subgroup with poor performance, (AUC: 0.66 to 0.7).

Viscosity as a potential marker of liver inflammation and fibrosis

The viscosity correlated weakly with GOT (R=0.33, p<0.001) but not with GPT values (fig 3). No differences in viscosity mean values were observed between patients

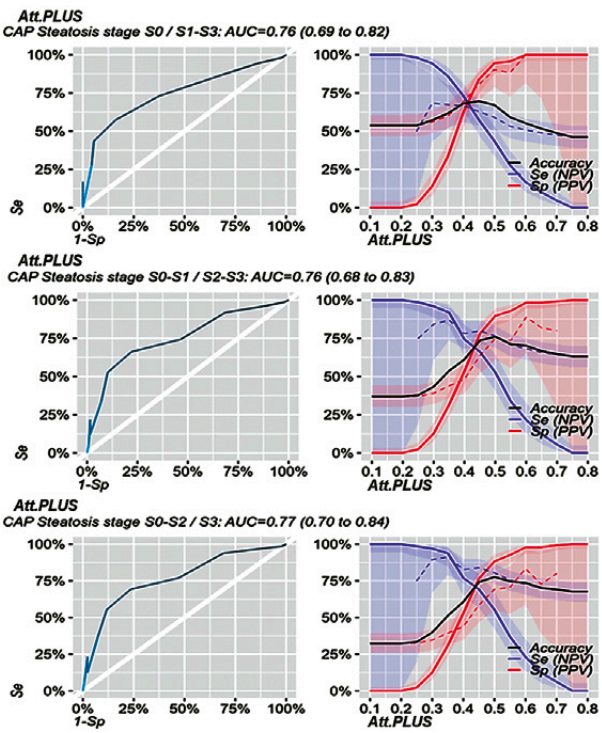

Fig 2. Performance of Att.PLUS measurement for predicting mild, moderate and severe steatosis. CAP - Controlled Attenuation Parameter, S-steatosis grade, AUC - area under the ROC curve

with GOT ≥100 U/l and those with lower levels (2.3 vs. 2.1, p>0.05). Only the platelet-to-lymphocyte ratio (PLR) significantly correlated with viscosity (R=0.358, p<0.001), but not CRP or neutrophil-to-lymphocyte ratio (NLR) (R=0.026, R=0.095, p>0.05).

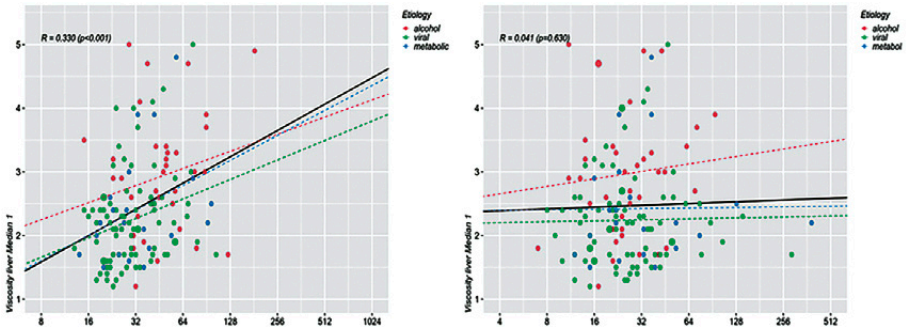

Fig 3. The correlation between Viscosity and GOT and GPT level in study population. GOT - glutamic-oxaloacetic transaminase, GPT - Glutamate Pyruvate Transaminase

In the study group, viscosity moderately correlated with the VCTE values (R=0.679, p<0.001) and with other non-invasive markers of liver fibrosis, including Fib-4, APRI, and e-LIFT (R=0.486, R=0.437, R=0.473, p< 0.001). However, viscosity poorly correlated with CAP values (R=0.209, p=0.004).

Discussion

In this observational, prospective study, we confirmed the non-inferior performance of SWE elastography to estimate liver fibrosis compared to VCTE. Moreover, the Att.PLUS module had relatively good accuracy in diagnosing and grading hepatic steatosis.

Different US elastography techniques have now become essential in the management of CLD patients and provide an adequate alternative to liver biopsy [15]. While VCTE has been widely accepted as an alternative to liver biopsy [16], 2D-SWE emerges as a novel and promising elasticity test method, offering two-dimensional elasticity images, assessing liver homogeneity and having the main advantage of being integrated in an ultrasound machine that grants the use of a variety of imaging modalities. This integration allows for a more comprehensive assessment of the area of interest, simplifying and shortening the diagnostic process and avoiding possibly harmful invasive procedures like liver biopsy and prolonged waiting times for MRI [4].

ShearWave PLUS is a new elastography software that is included in the Aixplorer Mach 30 ultrasound machine and incorporates a new quality criterion, the Stability Index tool. Notably, our study challenges the manufacturer's recommended SI threshold of >90%, demonstrating that reliable measurements of liver fibrosis stages can be achieved with high accuracy (>90%) even at a lower stability index (>80%). The success rate of 2D-SWE.PLUS, while high (89%), was somewhat lower than VCTE's or the one reported by Popa et al (95.8%) [17]. This could be possibly influenced by an increased number of overweight and obese patients in the study, a factor known to impact reliable results with 2D-SWE [18].

Correlation analysis between 2D-SWE.PLUS and VCTE values, with coefficients above 0.8, aligns with previous studies [17,19]. Furthermore, 2D-SWE.PLUS showed excellent diagnostic accuracy for liver fibrosis stage, similar to serologic non-invasive markers in the MASLD subgroup and superior in the ALD and viral subgroups, as previously outlined by Liu et al [20]. Additionally, irrespective of etiology, 2D-SWE.PLUS performed better than any other serological maker of liver fibrosis in identifying patients with cACLD, making it a valuable tool in everyday clinical practice.

In our study, using 2D-SWE.PLUS we identified 8 kPa as the best cut-off value to separate mild from moderate fibrosis and 10 kPa as the best cut-off value to identify cACLD patients. Previous studies found lower cut-off values for diagnosing F2 fibrosis (7 kPa [17], or 7.1 kPa [21]). The difference may be due to the method used to select the best cut-off value as previous studies focused on maximizing the Youden index, while our study aimed to maximize diagnostic accuracy.

Apart from liver fibrosis, which is considered the strongest predictor for long-term clinical outcomes in any CLD, steatosis and inflammation are emerging prognostic factors due to their contribution to disease progression, especially in the context of MASLD [15].

Although hepatic steatosis is characteristic of MASLD and ALD, it is also significantly expressed in HCV-infected patients, (prevalence of 40-80% [22]), and also in HBV-infected patients, (prevalence of up to 29.6% [23]). The presence of steatosis can contribute to fibrosis progression in HCV patients despite sustained virological response in a similar pattern as that observed in MASLD patients [24]. In chronic hepatitis B infection, the presence of liver steatosis is significantly associated with all-cause mortality and cancer, regardless of their baseline HBV viral load [25]. Therefore, developing new non-invasive methods of diag-

nosing and grading steatosis would clarify this unmet need.

In this study, Att.PLUS moderately correlated with CAP, R=0.47, similar to Popa et al results and to the performance of the attenuation software program from Hitachi [17,26]**.** Our identified cut-off values for diagnosing liver steatosis grades were lower than those reported in other studies. For instance, Farraioli's study used cut-off values of 0.63 dB/cm/MHz (258 dB/m on CAP) for detecting S>0 liver steatosis and 0.72 dB/cm/MHz (304 dB/m on CAP) for detecting S>1 steatosis [19]. The disparity in results could be attributed to the use of different cut-off values for defining S1 and S2 by CAP measurement in our study population, which were previously validated in a cohort of patients within our institution [5].

The study reveals a significant correlation between CAP and Att.PLUS values, but the number of Att.PLUS measurements does not enhance the predictive model. Our study demonstrated that performing only 3 measurements yielded the same feasible results as performing 5 measurements, an aspect with no previous consensus.

Recently, it has been shown that lobular inflammation grade significantly correlated with the shear wave dispersion slope, thus viscosity might also reflect the degree of liver inflammation [27]. In our cohort we have identified a weak correlation between viscosity, CAP values, and some markers of liver inflammation. However, in the absence of liver biopsy no firm conclusion can be drawn.

In accordance with Chen et al results, we have shown that viscosity may better represent liver fibrosis stage, but less accurate than 2D-SWE.PLUS [28]. Importantly, viscosity had excellent performance in predicting severe fibrosis across the study population.

The study's primary limitation is the absence of liver biopsy as a reference method, although the purpose of the study was to assess a new ultrasound-based technique in accordance with a noninvasive validated method. An additional limitation is the unbalanced distribution of estimated liver fibrosis stages, particularly underrepresenting moderate fibrosis, hindering the calculation of several cut-off values. Nowadays, from the clinical practice perspective it is more relevant to identify the patients with cACLD.

In **conclusion,** multiparametric ultrasound evaluation offers valuable information in a single analysis in CLD patients. Using VCTE as reference standard, 2D-SWE. PLUS exhibits excellent diagnostic accuracy for liver fibrosis, outperforming other non-invasive serological markers. Att.PLUS has a relatively good accuracy of liver steatosis, but more studies are still needed before definitive implementation in clinical practice.

Conflict of interest: none

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