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FibroMeter scores are predictive noninvasive markers of advanced and significant liver fibrosis in patients with chronic viral hepatitis or metabolic dysfunction-associated steatotic liver disease

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Abstract

Background FibroMeter and FibroMeter vibration-controlled transient elastography (FibroMeter VCTE) were assessed in a Greek cohort of patients with chronic viral hepatitis (CVH) B and C or metabolic dysfunction-associated steatotic liver disease (MASLD) to evaluate their accuracy in predicting advanced liver fibrosis against other well-validated noninvasive markers.

Methods Group 1: n=83 CVH and group 2: n=38 MASLD patients underwent liver biopsy and transient elastography (TE) on the same day as sera collection. FibroMeter scores APRI and FIB-4 were calculated in all 121 patients, while MASLD fibrosis score (MFS) was also calculated in group 2.

Results In CVH, FibroMeter VCTE performed equivalently to TE and better than the other markers in predicting advanced (≥F3) and significant (≥F2) fibrosis (area under the receiver operating characteristic curve [AUC] 0.887, P<0.001 for F3; AUC 0.766 P<0.001 for F2). FibroMeter Virus (cutoff 0.61) had lower sensitivity (20%) but performed equivalently to APRI and FIB-4. In MASLD, all markers but APRI performed equivalently in predicting advanced fibrosis. FibroMeter VCTE >0.2154 had the same sensitivity (100%) and specificity (81%) as TE (cutoff >7.1 kPa). FibroMeter MASLD >0.25 performed equivalently to MFS and FIB4, but with higher specificity (100%). Both FibroMeter and FibroMeter VCTE correlated with liver histology but not with liver enzymes.

Conclusions FibroMeter VCTE predicts accurately advanced fibrosis in CVH and MASLD, irrespectively of transaminase levels. FibroMeter Virus can be applied only as an alternative marker in CVH, while FibroMeter MASLD performs equally to TE and calculated scores (MFS, FIB-4) in predicting advanced fibrosis in MASLD patients.

Keywords FibroMeter, FibroMeter vibration-controlled transient elastography, chronic viral hepatitis, metabolic dysfunction-associated steatotic liver disease, liver fibrosis

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Introduction

The prognosis and management of liver disease is closely linked to the stage of liver fibrosis. Although liver biopsy still represents the reference procedure for the assessment of liver fibrosis, its potential complications, together with poor patients' acceptance, have led to a growing interest in the application of noninvasive procedures for liver stiffness evaluation [1-3].

Transient elastography (TE) is a surrogate marker for the estimation of liver fibrosis through liver stiffness measurements (LSM), with great accuracy for the diagnosis of advanced fibrosis (≥F3 Metavir) [4,5]. As a consequence, TE tends to replace liver biopsy in the estimation of liver fibrosis in chronic viral hepatitis B (CHB) and C (CHC), as well as in metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as nonalcoholic fatty liver disease

(NAFLD), where liver biopsy is not a prerequisite for diagnosis and treatment. The accuracy of TE in this setting has been well documented [6-10].

Moreover, a number of serum biomarkers, such as aspartate aminotransferase (AST) to platelet ratio index (APRI) and fibrosis-4 (FIB-4), have been shown to have some value in the estimation of liver fibrosis, especially in CHC and MASLD [11-13]. Accordingly, MASLD fibrosis score (MFS), a simple and easily accessible online score, has been proved useful for the determination of advanced disease in MASLD patients, who are at high risk for presenting advanced fibrosis and/or cirrhosis [14,15].

More recently, FibroMeter scores, a proprietary panel of serum biomarkers, as well as the FibroMeter vibration-controlled TE (FMVCTE) test, which combines LSM with FibroMeter values, were proved useful and accurate markers for liver fibrosis assessment, with comparable results to those from liver biopsy and TE in CHB and MASLD [5,16,17]. These scores, by including patients' clinical data, permit a more individualized approach to the estimation of liver fibrosis. Furthermore, we have recently shown that FibroMeter VCTE could predict advanced liver fibrosis (≥F3 Metavir) in autoimmune hepatitis and primary cholangitis with a comparable or even better diagnostic accuracy and specificity than TE, irrespectively of the biochemical activity of the disease [18].

Herein, we aimed to validate the diagnostic performance of FibroMeter Virus and FibroMeter MASLD for patients with CHB and C, and patients with MASLD, respectively, as well as FibroMeter VCTE compared to liver biopsy, in a well characterized Greek cohort of patients with chronic viral hepatitis (CVH) and MASLD. Apart from liver histology, other validated methods of fibrosis estimation, such as TE, APRI, FIB-4 and MFS, were also taken into account, in order to assess the usefulness and accuracy of multiple noninvasive markers in everyday clinical practice.

Patients and methods

Patients

One hundred twenty-one consecutive patients with CVH (n=83; 43 CHB, 40 CHC) or MASLD (n=38) who were followed-up in our department and consented to undergo liver biopsy between October 2009 and December 2016 were retrospectively included in the study. Diagnosis of CHB, CHC and MASLD was based on well-established criteria for each disease [6-10]. On the same day as the liver biopsy, TE (FibroScan® 502, ECHOSENS, France) was performed blindly by 2 experienced hepatologists (KZ and NG), as described previously [18], along with fasting serum sampling.

Serum samples were stored at -80°C until testing for FibroMeter parameters, while a complete hematologic and biochemical workup was performed on the same day as the liver biopsy. Liver histology was evaluated by an experienced hepatopathologist (GK) who was aware of neither the patients' diagnoses nor their TE results. Exclusion criteria comprised the presence of active malignancy, overlapping chronic or acute liver disease (coexistence of CVH with autoimmune liver diseases, etc.), ascites, body mass index ≥40 kg/m^2, pregnancy or any implantable cardiac device.

The patients' characteristics at the time of the FibroMeter determination are shown in Table 1. All subjects provided written informed consent to participate in the study at the time of interview. The ethical committee of the General University Hospital of Larissa approved the study protocol, which conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as revised in Brazil in 2013, as reflected in *a priori* approval by the institution's human research committee (2258/21-3-2016).

Methods

FibroMeter Virus 3VG (Echosens, Paris, France) was calculated in CVH patients according to a patented formula, including age, sex, platelets (PLT), alpha-2-macroglobulin, alanine aminotransferase (ALT), urea, prothrombin index (PI), γ-glutamyl-transferase (γ-GT), and aspartate aminotransferase (AST). FibroMeter VCTE was calculated by a patented formula that includes age, sex, PLT, PI, AST, alpha-2-macroglobulin, γ-GT, and LSM. FibroMeter MASLD was calculated in MASLD patients based on a patented formula including age, body weight, glycemia, PLT, AST, ALT, and ferritin.

LSMs were performed using a FibroScan device powered by VCTE (Echosens) equipped with the standard M probe [19]. LSM results were expressed as the median (kPa) of all valid measurements, with interquartile range (IQR) and success rate. LSM was considered valid if there were 10 successful acquisitions with IQR/LSM <0.3 [19]. APRI, FIB-4 and MFS were calculated in all patients according to the published formulas [11,12,20]. Necroinflammatory activity and fibrosis stage were assessed using the Metavir score [21,22].

Statistical analysis

The SPSS 24 statistics software was used. Results were expressed as mean ± standard deviation or as median (range). Data were analyzed by *t*-test, Mann-Whitney *U*, χ^2, Fisher's exact test, Spearman's correlation coefficient (r)

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Table 1 Characteristics of patients with chronic liver diseases

Characteristics	CHB (n=43)	CHC (n=40)	MASLD (n=38)
Sex (F/M)	10/33	19/21	15/23
Age (years)	45 (19-72)	45 (25-67)	50 (16-69)
AST (U/L)	38 (14-78)	50 (18-154)	38 (19-126)
ALT (U/L)	46 (12-217)	64 (12-307)	65 (26-281)
γ-GT (U/L)	29 (5-208)	42 (13-359)	60 (18-235)
ALP (U/L)	76 (39-214)	70 (36-287)	72 (36-180)
Albumin (g/dL)	4.5 (3.4-5.3)	4.4 (3.7-5.3)	4.7 (2.7-5.2)
Platelets ($\times 10^9$/L)	191 (119-447)	220 (98-423)	219 (90-330)
Total bilirubin (mg/dL)	0.7 (0.3-9)	0.7 (0.3-5)	0.8 (0.2-1.8)
Under treatment (yes/no)	23/20	0/40	NA

CHB, chronic hepatitis B; CHC, chronic hepatitis C; MASLD, metabolic dysfunction associated steatotic liver disease; F/M, female/male; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, γ-glutamyl-transferase; ALP, alkaline phosphatase; NA, not applicable

and linear regression analysis. Kruskal-Wallis or ANOVA (depending on the homogeneity of variance test) were used for comparisons of LSM, FibroMeter Virus, FibroMeter VCTE, APRI, and FIB-4 values. The receiver operating characteristic (ROC) curve was used for the estimation of the area under the curve (AUC), sensitivity and specificity. The DeLong test using the Med Calc Software was used for comparisons between the AUCs. In addition, Youden's index was used for selecting the optimum cutoff point of the AUCs. A 2-sided *t*-test value of P<0.05 was considered as statistically significant. In addition, 95% confidence intervals (CI) were calculated using the Wilson procedure with a correction for continuity.

Results

Group 1: Viral hepatitis patients

In view of the small number of patients in each CVH group (CHB and CHC), and taking into account the current literature, which has shown a similar diagnostic accuracy of both FibroScan and FibroMeter scores for both CHB and C [6-8,23], we considered all CVH patients as a single group. Regarding liver histology 9/83 (10.8%, 95%CI 5-20%) had F0, 30/83 (36.1%, 95%CI 26-47%) F1, 18/83 (21.7%, 95%CI 13.7-32.3%) F2, 14/83 (16.9%, 95%CI 9.8-27%) F3, and 12/83 (14.5%, 95%CI 8-24%) F4 (Table 2). Histological activity, LSM, FibroMeter Virus, FibroMeter VCTE, APRI, and FIB-4 are shown in Table 2. LSM, FibroMeter Virus, FibroMeter VCTE, APRI and FIB-4 were significantly correlated with histological staging (r=0.651, P<0.001; r=0.366, P=0.001; r=0.625, P<0.001; r=0.330, P=0.002; r=0.333, P=0.002, respectively).

The ROC curves for all markers were significantly better than chance in predicting ≥F3 (LSM: AUC 0.897, P<0.001; FibroMeter Virus: AUC 0.699, P=0.004; FibroMeter VCTE: AUC 0.887, P<0.001; APRI: AUC 0.682, P=0.008; FIB-4: AUC 0.674, P=0.011) (Fig. 1A). The AUCs for LSM and FibroMeter VCTE performed equivalently and significantly better than all the other 3 markers (P<0.05 for all comparisons; data not shown). The AUCs for FibroMeter Virus, APRI and FIB-4 did not differ from each other (P>0.05 for all comparisons).

According to Youden's index, the threshold of LSM for the prediction of advanced fibrosis was >10kPa—sensitivity: 84.6% (95%CI 65-95%), specificity: 85.9% (95%CI 74.2-93.7%) (Fig. 1B), with positive predictive value (PPV) 71%, NPV 92.3% and diagnostic accuracy 84.3%—while the published cutoff (>9.5kPa) [23] had approximately the same sensitivity and specificity (84.6%, 95%CI 65-95% and 82.5% 95%CI 70-91%, respectively) [4,6,7,24-26]. FibroMeter VCTE >0.43 had sensitivity 88.5% (95%CI 70-97.6%), specificity 79% (95%CI 66-88.6%), PPV 64%, NPV 93.6% and diagnostic accuracy 80.7% (Fig. 1C), while the published cutoff >0.715 [3] had lower sensitivity (46%, 95%CI 26.6-66.6%), but higher specificity (96.5%, 95%CI 87.9-99.5%), in our cohort. In addition, FibroMeter Virus >0.37 had sensitivity 80.8% (95%CI 60.6-93.4%) and specificity 61.4% (95%CI 47.6-74%), PPV 48.8%, NPV 87.5% and diagnostic accuracy 66.3% (Fig. 1D). When the published cutoffs for severe fibrosis were taken into account, FibroMeter Virus >0.61 had a sensitivity and specificity of 20% (95%CI 4.3-48%) and 79.4% (95%CI 69.9-88.2%), APRI >0.7 26.7% (95%CI 7.8-55%) and 70.6% (95%CI 58.3-81%) and FIB-4>1.45 40% (95%CI 12.2-73.7%), and 63.6% (95%CI 45-79.6%), respectively [12,16,22,27-30].

The AUCs for predicting fibrosis ≥F2 were better than chance for all 5 markers (LSM: AUC 0.805, P<0.001; FibroMeter VCTE: AUC 0.766, P<0.001, FibroMeter Virus: AUC 0.631, P=0.04; APRI: 0.632, P=0.039; FIB-4: AUC 0.632, P=0.038). The AUCs for LSM and FibroMeter VCTE performed equally and significantly better than the other 3 markers (P<0.05 for all comparisons; data not shown).

The threshold of LSM for ≥F2 stage was >8.1 kPa, with sensitivity 68.2% (95%CI 52.4-81.4%) and specificity 84.6%

Table 2 Histological findings (according to Metavir score) and median values of LSM, FibroMeter Virus, FibroMeter VCTE, FibroMeter MASLD score, APRI, FIB-4 and MASLD score of the 2 groups of patients included in the study

Findings	CVH (n=83)	MASLD (n=38)	P-value
Fibrosis score			
≥F2 (F0-1 vs. F2-3-4)	39/ 44 (53%)	32/ 6 (16%)	
≥F3 (F0-1-2 vs. F3-4)	57/ 26 (31%)*,**	35/ 3 (8%)**	0.004
≥F4 (F0-1-2-3 vs. F4)	71/ 12 (14.5%)	36/ 2 (5.3%)	
Activity score			
A0-1	61 (73.5%)	30 (78.9%)	
A2	18 (21.7%)	5 (13.2%)	
A3	4 (4.8%)***	3 (7.9%)*	0.001
LSM (kPa)	7.6 (3.3-45.7)	6.2 (3.3-66.4)	NS
FibroMeter Virus	0.395 (0.045-0.855)	0.284 (0.061-0.635)	<0.001
FibroMeter VCTE	0.37 (0.035-0.999)	0.137 (0.033-0.999)	<0.001
FibroMeter MASLD score	NA	0.0672 (0.0007-0.993)	
APRI	0.47 (0.1-2.5)	0.43 (0.21-1.82)	0.009
FIB-4	1.12 (0.26-5.31)	1.1 (0.23-4.46)	<0.001
MASLD score	NA	-2.589 (-5.264-(-0.096))	

*P=0.009, **P=0.01, ***P=0.001

NA, not applicable; LSM, liver stiffness measurements; VCTE, vibration-controlled transient elastography; MASLD, metabolic dysfunction associated steatotic liver disease; APRI, aspartate aminotransferase to platelet ratio index; FIB-4, fibrosis-4; CVH, chronic viral hepatitis

(95%CI 69.5-94%), while the respective values for the published cutoff (>7.2 kPa) [7,24] were 75% (95%CI 59-87%) and 74.3% (95%CI 57.8-87%), and in a recent study a cutoff >7 kPa achieved an even higher sensitivity of 83-89% [31]. For FibroMeter VCTE the threshold was >0.408, with sensitivity 66% (95%CI 50-79.5%) and specificity 79.5% (95%CI 63.5-90.7%), while the respective values for the published cutoff (>0.384) [3] were 68.2% (95%CI 52.4-81.4%) and 74.4% (95%CI 57.8-87%).

LSM, FibroMeter VCTE, FibroMeter Virus, APRI and FIB-4 were negatively correlated with PLT (r=-0.274, P=0.012; r=-0.487, P<0.001; r=-0.547, P<0.001; r=-0.47, P<0.001; and r=-0.65, P<0.001; respectively) and albumin (r=-0.381, P<0.001; r=-0.35, P=0.001; r=0.245, P=0.026; r=-0.259, P=0.018; and r=-0.362, P=0.001; respectively).

LSM was correlated positively with AST, ALT and γ-GT levels, as well as histological activity, and negatively with albumin and PLT (P<0.05 for all comparisons; data not shown). FibroMeter VCTE and FibroMeter Virus were both positively correlated with AST, γ-GT and histological activity, and negatively with albumin and PLT (P<0.05 for all comparisons; data not shown). Linear regression analysis showed that albumin and histological stage, but also histological activity, could independently predict LSM (P=0.041, P<0.001 and P=0.047, respectively), while γ-GT, PLT, albumin and histological stage were independent predictors of FibroMeter VCTE (P=0.001, P<0.001, P=0.008 and P<0.001, respectively). In addition, linear regression revealed that only PLT and histological stage could independently predict FibroMeter Virus (P<0.001 and P=0.025, respectively).

Group 2: MASLD patients

According to liver biopsy 5/38 (13.2%, 95%CI 5.7-27%) had F0, 27/38 (71.1%, 95%CI 55-83%) F1, 3/38 (7.9%, 95%CI 2-22%) F2, 1/38 (2.6%, 95%CI 0.1-15%) F3, and 2/38 (5.3%, 95%CI 0.9-19%) F4 (Table 2). Histological activity, LSM, FibroMeter VCTE, FibroMeter MASLD, APRI, FIB-4 and MFS are shown in Table 2.

LSM, FibroMeter VCTE, FibroMeter MASLD, MFS, APRI and FIB-4 were significantly correlated with the histological staging (r=0.47, P=0.003; r=0.502, P=0.001; r=0.481, P=0.002; r=0.538, P<0.001; r=0.37, P=0.022; and r=0.489, P=0.002; respectively).

Since only 3 patients had F3-F4 further analysis was not performed. However, patients were divided in F0-F1 (n=32) and F2-F4 (n=6) groups in order to estimate the ability of the tests to discriminate non-significant from significant/advanced fibrosis. Apart from APRI, the ROC curves of all markers were significantly better than chance in predicting ≥F2 (LSM: AUC 0.906, P=0.002; FibroMeter VCTE: AUC 0.948, P=0.001; FibroMeter MASLD score: AUC 0.911, P=0.002; MFS: AUC 0.984, P<0.0001; APRI: AUC 0.719, P=0.093; and FIB-4:

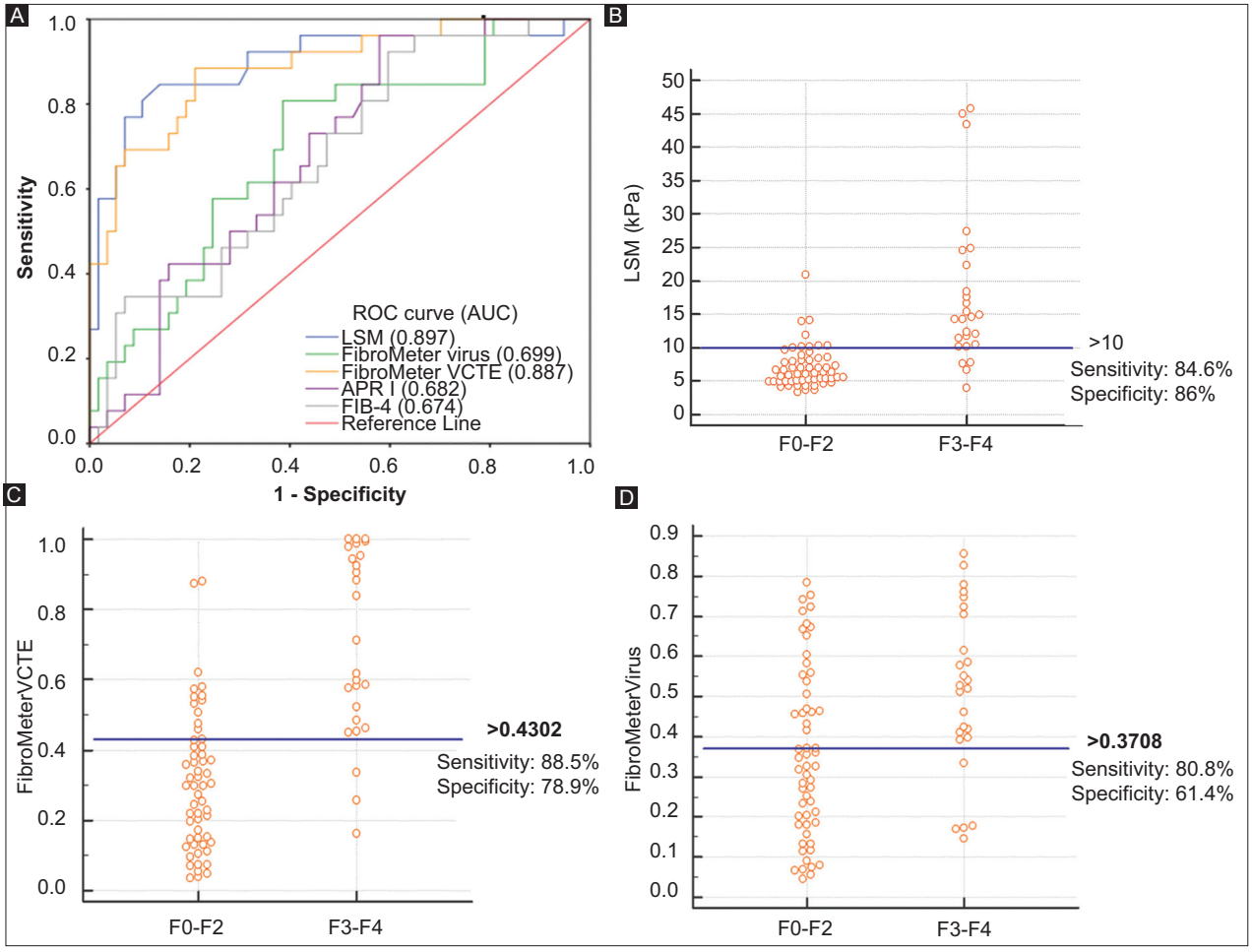

Figure 1 (A) Receiver operating characteristic curves for LSM, FibroMeter Virus, FibroMeter VCTE, APRI and FIB-4 in predicting significant fibrosis (F3-F4), in patients with CVH (group 1). (B) According to Youden's index, the threshold of LSM for the prediction of F3-F4 was >10 kPa: sensitivity 84.62% (95%CI 65-95%), specificity 85.9% (95%CI 74.2-93.7%). (C) According to Youden's index, the threshold of FibroMeter VCTE for the prediction of F3-F4 was >0.43: sensitivity 88.46% (95%CI 70-97.6%), specificity 78.95% (95%CI 66-88.6%). (D) According to Youden's index, the threshold of FibroMeter Virus for the prediction of F3-F4 was >0.37: sensitivity 80.8% (95%CI 60.6-93.4%), specificity 61.4% (95%CI 47.6-74%)
LSM, liver stiffness measurements; VCTE, vibration-controlled transient elastography; APRI, aspartate aminotransferase to platelet ratio index; FIB-4, fibrosis-4; CVH, chronic viral hepatitis; CI, confidence interval

AUC 0.943, P=0.001) (Fig. 2A). Apart from APRI, all AUCs performed equally in predicting ≥F2 fibrosis (P>0.05 for all comparisons).

According to Youden's index the threshold of LSM for ≥F2 stage was >7.1 kPa (sensitivity 100%, 95%CI 54-100%; specificity 81.2%, 95%CI 63.6-93%) (Fig. 2B), while the published cutoff excluding the presence of advanced chronic liver disease (<7 kPa) [6,31,32] had a sensitivity of 96% (95%CI 93-98%). The respective threshold for FibroMeter VCTE was >0.2154 (sensitivity 100%, 95%CI 54-100%; specificity 81.2%, 95%CI 63.6-93%) (Fig. 2C), while the respective values for the published cutoff (>0.32) [33-35] were 66.7% (95%CI 22.3-95.6%) and 96.9% (95%CI 83.8-99.92%). The threshold for FibroMeter MASLD score was >0.2561 (sensitivity 87.5%, 95%CI 71-96.5%; specificity 100%, 95%CI 54-100%) (Fig. 2D); the respective values for the published cutoff (>0.611) [33] were 33% (95%CI 4.3-77.7%) and 90.6% (95%CI 75-98%)]. For the MFS the cutoff was >-1.031 (sensitivity 100%, 95%CI 54-100%); specificity 96.9%, 95%CI 83.8-99.9%), while the respective values using the published cutoff for ≥F2 fibrosis (>-1.1) [34] were 100% (95%CI 54-100%) and 93.8% (95%CI 79-99%). Finally, the threshold for FIB-4 was >1.53 (sensitivity 100%, 95%CI 54-100%; specificity 90.6%, 95%CI 75-98%), while the respective values for the published cutoff (>1.3) [35-38] were 100% (95%CI 54-100%) and 84.3% (95%CI 67-94.7%).

LSM, FibroMeter VCTE, FibroMeter MASLD, FIB-4 and MFS correlated negatively with PLT (r=-0.418, P=0.009;

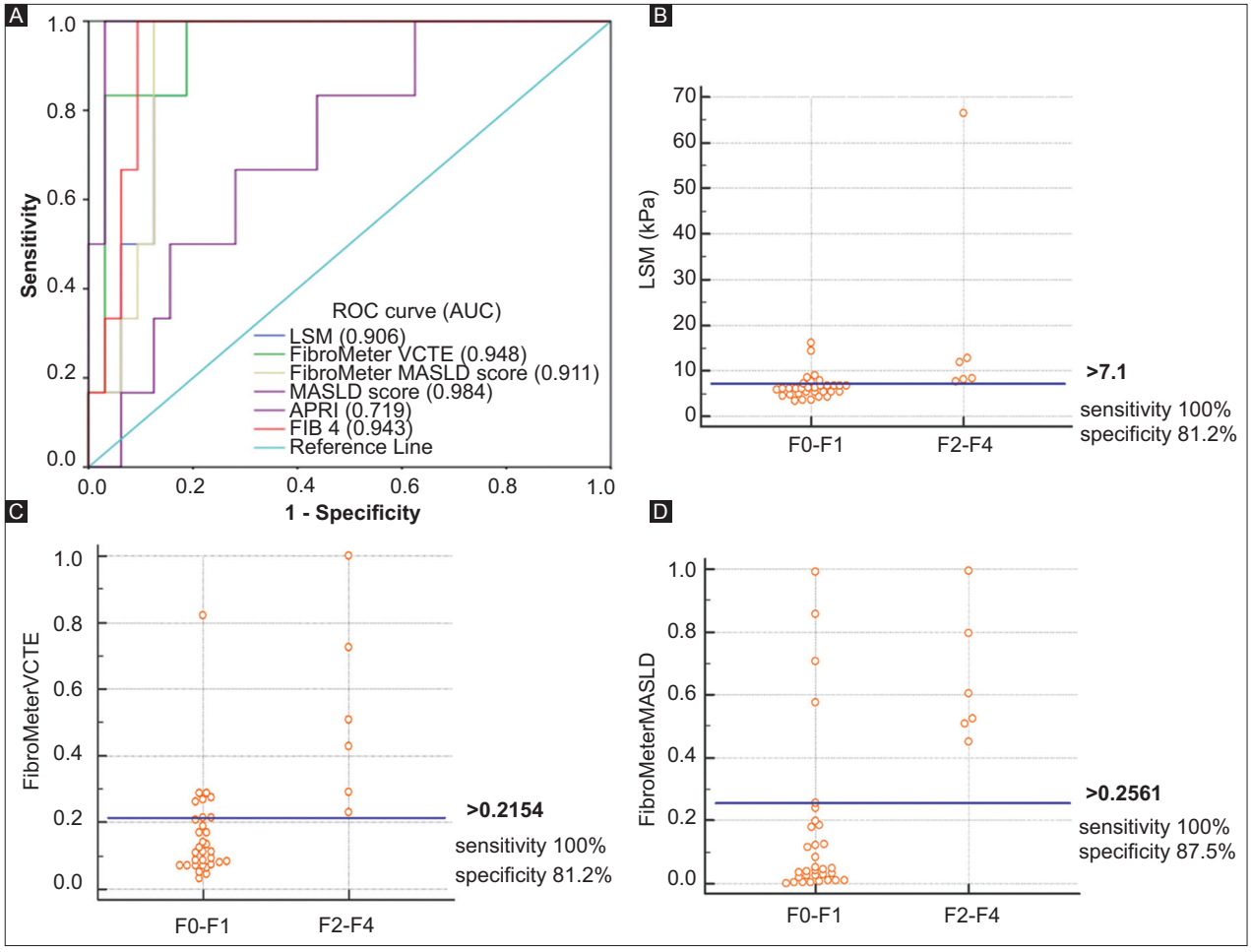

Figure 2 (A) Receiver operating characteristic curves for LSM, FibroMeter VCTE, FibroMeter MASLD score, APRI, FIB-4 in predicting moderate/severe fibrosis (F2-F4), in patients with MASLD (group 2). (B) According to Youden's index, the threshold of LSM for the prediction of F2-F4 was >7.1 kPa: sensitivity 100% (95%CI 54-100%), specificity 81.2% (95%CI 63.6-93%). (C) According to Youden's index, the threshold of FibroMeter VCTE for the prediction of F2-F4 was >0.2561: sensitivity 87.5% (95%CI 71-96.5%); specificity 100% (95%CI 54-100%). (D) According to Youden's index, the threshold of FibroMeter MASLD for the prediction of F2-F4 was >0.2561: sensitivity 87.5% (95%CI 71-96.5%); specificity 100% (95%CI 54-100%)

LSM, liver stiffness measurements; VCTE, vibration-controlled transient elastography; APRI, aspartate aminotransferase to platelet ratio index; FIB-4, fibrosis-4; MASLD, nonalcoholic fatty liver disease; CI, confidence interval

r=-0.589, P<0.001; r=-0.525, P=0.001; r=0.674, P<0.001; and r=-0.765, P<0.001; respectively). In addition, LMS was positively correlated with AST (r=0.323, P=0.048) and MFS correlated negatively with ALT (r=-0.342, P=0.035).

Discussion

In the present investigation we evaluated the diagnostic accuracy of FibroMeter and FibroMeter VCTE in comparison to TE, APRI, FIB-4 and MASLD fibrosis score in a well-characterized cohort of Greek patients who had CVH and MASLD with available liver histology. Our results indicate that: 1) in CVH, FibroMeter VCTE is an accurate marker for predicting advanced (≥F3) and significant (≥F2) fibrosis, with comparable sensitivity and specificity to those of TE, and far more accurate than APRI and FIB-4, which are freely available. Although the AUC for FibroMeter Virus was the same as that for APRI and FIB-4, it could serve as an alternative predictor, probably in combination with other noninvasive markers, in cases where LSM is not available. Furthermore, 2) in MASLD, FibroMeter VCTE is able to predict significant fibrosis (≥F2) with the same sensitivity and specificity as TE, while FibroMeter MASLD can be also used as an accurate diagnostic marker with even higher specificity, about 100%, performing equivalently to well-validated noninvasive markers (MFS, FIB-4 and MASLD fibrosis score).

In the field of CVH, previous studies have demonstrated the utility of noninvasive biomarkers for the assessment of liver fibrosis with, accuracy differing between studies [3,28,29,31,39-41]. Moreover, a recent study from Houot *et al* [42], reported the reliability of noninvasive biomarkers such as FibroMeter and FIB-4 in predicting significant liver-related events and deaths. Results from our study demonstrated that the combination of blood biomarkers with TE (FibroMeter VCTE) performs better than calculated scores (APRI and FIB-4) and equivalently to TE alone in determining both significant and advanced fibrosis. Moreover, our results showed that a lower cutoff for FibroMeter VCTE (>0.43) has an even higher sensitivity of 88% (compared to 46% in the current literature) in predicting advanced fibrosis (≥F3) in patients with CVH, having comparable sensitivity and specificity to TE [3,36].

Regarding FibroMeter Virus, our results showed that a cutoff of >0.61 has lower diagnostic accuracy and the lowest sensitivity (20%), compared to APRI and FIB-4, for the diagnosis of severe fibrosis in patients with CVH, but performs better in terms of specificity. These findings suggest that FibroMeter Virus could serve as an alternative marker for predicting fibrosis when histology and elastography are not available, and that the combination of FibroMeter Virus with other calculated markers, such as APRI and FIB-4, could increase their diagnostic accuracy, as has already been demonstrated by Chindamo *et al* [16].

In MASLD patients, all markers but APRI were better than chance in predicting significant to advanced fibrosis (≥F2). However, no subgroup analysis for patients with F3 fibrosis stage was performed, in view of the small number of patients with severe fibrosis. Interestingly, FibroMeter VCTE performed equally to LSM in terms of sensitivity (100%) and specificity (81.3%). This is in agreement with previous studies, where TE and FibroMeter VCTE had the same diagnostic accuracy, but the use of FibroMeter VCTE in patients with advanced fibrosis increased the PPV for the diagnosis of advanced fibrosis (≥F3) [35]. However, although a cutoff for FibroMeter VCTE>0.215 had the highest diagnostic accuracy, its specificity was lower than that of MFS and FIB-4 (81.3% vs. 96% and 91%, respectively). This finding could be attributed to the fact that patients with significant and advanced fibrosis were analyzed together, and indicates that a combination of markers with a lower cutoff could reliably rule out advanced fibrosis [34,37,38].

As far as FibroMeter MASLD is concerned, recent studies have demonstrated a high diagnostic accuracy comparable to TE and even better compared to MFS and FIB-4 [32,33,37]. Accordingly, our results showed that a cutoff >0.25 for FibroMeter MASLD had higher diagnostic accuracy, and almost the same sensitivity and specificity compared to MFS and FIB-4 for predicting significant fibrosis, indicating that it could be applied as an accurate noninvasive marker in cases where LSM is not available for stratifying patients at increased risk of advanced fibrosis and/or cirrhosis.

Notably, all noninvasive markers were positively correlated with histological staging in both CVH and MASLD patient groups. However, unlike TE [36], neither FibroMeter VCTE nor FibroMeter Virus/FibroMeter MASLD correlated with liver enzymes, suggesting that these biomarkers could be used even in cases with marked elevation of transaminase levels. Moreover, among independent predictors only PLTs and albumin were able to affect the results of FibroMeter VCTE, while FibroMeter MASLD was positively correlated with glucose levels.

Our study had a number of limitations. First of all, the number of patients included was relatively small, primarily because this was a single-center study. Nevertheless, our results are comparable with those of previous studies based on larger cohorts [16,17,22,26,28,29,31,32], confirming our findings and indicating that our cohort was really well-characterized. In addition, because of the small number of patients we were not able to validate the studied markers for the prediction of advanced fibrosis in MASLD. However, our results for significant fibrosis were in agreement with recent publications in larger cohorts [31,32,35,38]. Another limitation is the retrospective character of the study. However, our data were collected prospectively, and the latest criteria published were used for the characterization and selection of our cohort. In addition, although previous studies have already validated the same scores in different cohorts, to the best of our knowledge this is the first study to validate and compare the efficacy of different noninvasive markers of liver fibrosis in a well-characterized Greek cohort and to support the higher accuracy of FibroMeter scores in MASLD.

In conclusion, FibroMeter VCTE could be used as an accurate noninvasive marker for predicting advanced cirrhosis in patients with CVH and MASLD. In addition, lower cutoffs for FibroMeter VCTE, in combination with TE and previously well validated markers such as MFS and FIB-4, could provide a more accurate approach to liver fibrosis assessment and increase the reliability of the results. In CVH, FibroMeter Virus could be used only as an alternative marker for predicting severe fibrosis when LSM is not available, while in MASLD patients, FibroMeter MASLD can predict advanced liver fibrosis equivalently to MFS and FIB-4.

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Summary Box

What is already known:

- Noninvasive biomarkers are tending to replace liver biopsy for the estimation of liver fibrosis in chronic viral hepatitis (CVH) B and C, and in metabolic dysfunction-associated steatotic liver disease (MASLD)
- Noninvasive biomarkers for the assessment of liver fibrosis in patients with CVH and MASLD have shown different accuracy between studies
- More recently, FibroMeter scores and FibroMeter vibration-controlled transient elastography (FibroMeter VCTE) measurements have been tested as noninvasive markers of liver fibrosis in CVH and MASLD

What the new findings are:

- In CVH, FibroMeter VCTE is a more accurate marker for predicting advanced (≥F3) and significant (≥F2) fibrosis than APRI and FIB-4; FibroMeter Virus has higher specificity, but lower sensitivity than APRI and FIB-4 for assessing severe fibrosis
- In MASLD, FibroMeter VCTE has higher diagnostic accuracy, but lower specificity than MFS and FIB-4 for predicting significant fibrosis; FibroMeter MAFLD has higher diagnostic accuracy than MFS and FIB-4 for assessing significant fibrosis
- FibroMeter VCTE and FibroMeter Virus/ FibroMeter MASLD were not correlated with the biochemical activity of CVH and MASLD

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