Cardiovascular Diabetology

RESEARCH

Open Access

Metabolic dysfunction-associated steatotic liver disease (MASLD) biomarkers and progression of lower limb arterial calcification in patients with type 2 diabetes: a prospective cohort study

Damien Denimal[1,2*], Maharajah Ponnaiah[3], Franck Phan[3,4,5,6], Anne-Caroline Jeannin[4,5], Alban Redheuil[3,7,8], Joe-Elie Salem[9], Samia Boussouar[3,7,8], Pauline Paulstephenraj[3], Suzanne Laroche[4,5], Chloé Amouyal[3,4,5,6], Agnès Hartemann[3,4,5,6], Fabienne Foufelle[3,6] and Olivier Bourron[3,4,5,6]

Abstract

Background Studies have demonstrated that both lower limb arterial calcification and metabolic dysfunction-associated steatotic liver disease (MASLD) are linked to the development of peripheral artery disease. However, the potential relationship between MASLD biomarkers and progression of lower limb arterial calcification in individuals with type 2 diabetes (T2D) remains unclear. This study aimed to investigate whether the biomarkers of MASLD included in the FibroMax® panels are associated with the progression of lower limb arterial calcification in patients with T2D.

Methods The lower limb arterial calcification score (LLACS) was evaluated through computed tomography at baseline and after an average follow-up of 31.2 ± 3.7 months in a cohort of 150 patients with T2D. We also measured the serum biomarkers included in the FibroMax® panels (SteatoTest®, FibroTest®, NashTest®, ActiTest®). The predictive ability of these biomarkers of MASLD on LLACS progression was assessed through univariate and multivariate linear regression models, principal component regression analysis, as well as machine learning algorithms.

Results During the follow-up period, LLACS increased in 127 (85%) of the 150 patients with T2D. In univariate analysis, the annualized change in LLACS was positively and mainly correlated with baseline LLACS ($r = 0.860$, $p < 0.0001$), the FibroTest® score ($r = 0.304$, $p = 0.0002$), and age ($r = 0.275$, $p = 0.0006$), and negatively correlated with glomerular filtration rate ($r = -0.242$, $p = 0.003$). In multivariate analysis, the FibroTest® score remained independently associated with the annualized change in LLACS, after adjusting for baseline LLACS and risk factors for lower extremity artery disease (β coefficient [95% confidence interval]: 988 [284–1692], $p = 0.006$). This association persisted even after adjustment for variables selected by principal component analysis (β = 1029 [289–1768], $p = 0.007$). Two advanced

*Correspondence:
Damien Denimal
damien.denimal@u-bourgogne.fr

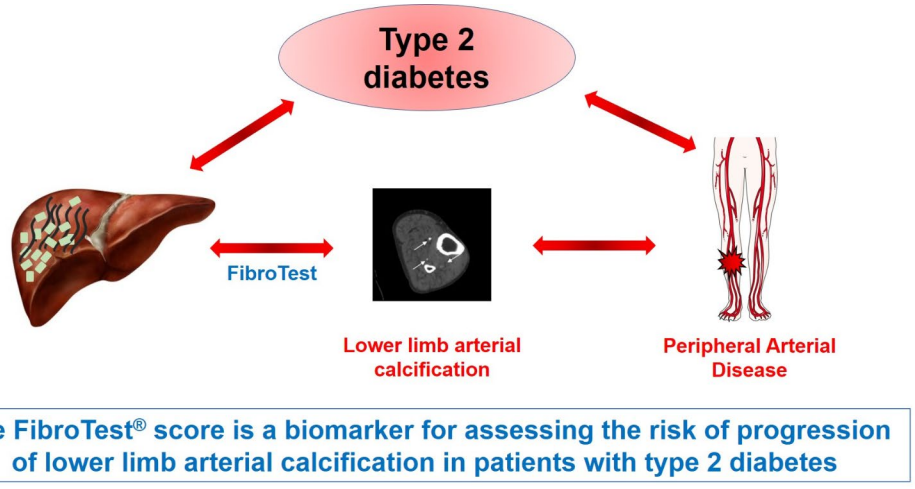
Full list of author information is available at the end of the article

machine learning models identified the FibroTest® score as the second most important predictor of annualized change in LLACS, following baseline LLACS.

Conclusions This study represents the first demonstration of an independent relationship between a non-invasive liver fibrosis test and the progression of lower limb arterial calcification in patients with T2D. Beyond its utility in assessing liver fibrosis, the FibroTest® could be a valuable and easy-to-use biomarker for predicting the risk of worsening lower limb arterial calcification.

Trial registration: ClinicalTrials.gov identifier NCT02431234.

Graphical abstract



Keywords Peripheral arterial disease, Type 2 diabetes mellitus, Liver fibrosis, Nonalcoholic fatty liver disease

Background

Patients with type 2 diabetes (T2D) have an increased risk of developing lower extremity artery disease, commonly known as peripheral artery disease (PAD) [1]. Lower limb arterial calcification plays a pivotal role in the development of PAD, particularly in T2D patients [1, 2]. Lower limb arterial calcification is independently and significantly associated with arterial thrombosis, occlusive arterial disease and lower limb amputation [3–9]. In addition, lower limb arterial calcification is associated with cardiovascular events and mortality [5, 10, 11]. Interest has therefore grown around this long-ignored complication of diabetes, for which no treatment is currently available.

Nonalcoholic fatty liver disease (NAFLD), recently renamed metabolic dysfunction-associated steatotic liver disease (MASLD) [12], is highly prevalent in patients with T2D [13]. Some recent evidence suggests a potential link between MASLD and PAD in cohorts of T2D and/or obese patients [14, 15]. In an observational prospective study involving 7,771 adults, with less than 18% of patients with diabetes, MASLD at baseline was even associated with a 67% higher risk of developing PAD [16].

Although both MASLD and lower limb arterial calcification are associated with PAD and share common pathophysiological mechanisms, such as insulin resistance, lipotoxicity, hepatokines, and inflammation, it remains unclear whether MASLD biomarkers are linked to the severity and progression of lower limb arterial calcification. A recent study in 77 patients with T2D identified an association between lower limb arterial calcification and the fatty liver index, a biomarker indicative of liver steatosis [17]. However, the cross-sectional design of this previous study precluded an assessment of the progression of lower limb arterial calcification over time. Furthermore, the study exclusively focused on a biomarker of liver steatosis and did not explore biomarkers representing other stages of MASLD, such as liver fibrosis or steatohepatitis.

Therefore, the primary aim of the present study was to assess whether the serum biomarkers of MASLD included in the FibroMax panels are associated with the progression over time of lower limb arterial calcification in patients with T2D. FibroMax® comprises four non-invasive biomarker panels designed to evaluate various aspects of liver pathology. The SteatoTest® score assesses the severity of liver steatosis, while the ActiTest® and NashTest® scores evaluate the levels of liver necrosis and inflammation. The FibroTest® score specifically measures the extent of liver fibrosis.

Methods

Study design and follow-up

DIACART (for "Arterial Calcification in the Diabetes") is a prospective monocentric cohort study. The recruitment period extended from February to October 2014, in which 198 people with T2D consulting at the Diabetology or Cardiology Departments of the Pitié-Salpêtrière Hospital (Paris, France) were enrolled. Inclusion criteria were T2D associated with at least one of the following factors: coronary artery disease or age > 50 years for men and > 60 years for women. Exclusion criteria were: an estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 m^2, immunodeficiency, acute infectious or inflammatory disease or type 1 diabetes. The study was approved by our institutional ethics committee (*Comité de Protection des Personnes* "Ile de France VI", Paris, France) and registered in ClinicalTrials.gov (NCT02431234). All patients were informed of the study objectives and procedures. Participants gave their written informed consent for participation prior to inclusion.

At the inclusion visit and after a mean follow-up of 31.2 ± 3.7 months (median: 30.7 months; range: 25.9–42.2 months), all patients had a clinical evaluation, laboratory blood tests, and helicoidal computerized tomography scans. Peripheral neuropathy was evaluated by the neuropathy disability score with values ≥ 6 considered abnormal [18]. Their medical records were reviewed to check the clinical information and the concomitant treatments.

For the sample size calculation, it was assumed that the correlation coefficient between a score included in the FibroMax® panels and the progression of lower limb arterial calcification in patients with T2D is 0.250. Based on these assumptions, a minimum of 123 patients was required to demonstrate a significant association between a score included in the FibroMax panels and the progression of lower limb arterial calcification, with an alpha risk of 5% and a statistical power of 80%.

Imaging for lower limb arterial calcification score

The lower limb arterial calcification score (LLACS) was obtained after scanning with a 128-slice multidetector dual-source computed tomography scanner (SOMATOM Definition Flash, Siemens Healthineers Healthcare™, Erlangen, Germany) without contrast, from the bottom of the patella down to the ankle. Three-millimeter cross-sectional slices were analyzed. The analysis was performed by radiologists who were blinded to the results of MASLD biomarkers and clinical examination, using a commercially available software package (Heartbeat CaScore, Philips Healthcare™, Eindhoven, Netherlands). Calcified areas along the below-knee arteries with a cross-sectional area ≥ 1 mm^2 and with a density ≥ 130 Hounsfield units were identified automatically. LLACS, determined according to the method described by Agatston et al. [19], was obtained separately for each of the main below-knee arteries (distal popliteal, anterior tibial, posterior tibial and peroneal arteries) and then added up to obtain the total calcification score [expressed in Agatston units (AU)], which was used in statistical analysis. LLACS progression was calculated as LLACS at the end of follow-up minus LLACS at baseline (i.e. "absolute change in LLACS") divided by follow-up duration in years ("annualized change in LLACS").

Laboratory evaluations and FibroMax® panels

Blood samples were collected after overnight fasting for the measurement of routine biochemistry diagnostic tests. Routine analytical procedures were performed according to the manufacturer instructions as previously described [20]. eGFR was calculated according to the MDRD formula.

FibroMax® (BioPredictive™, Paris, France), evaluated at the end of follow-up, corresponds to a combination of four non-invasive panels of biomarkers designed to assess liver steatosis (SteatoTest®), necrosis and inflammation (ActiTest® and NashTest®) and fibrosis (FibroTest®) [21]. FibroTest® includes serum alpha-2 macroglobulin, apolipoprotein-A1, haptoglobin, total bilirubin and gamma-glutamyltranspeptidase, adjusted for age and sex. Fibrosis severity is categorized as no (F0), minimal (F1), moderate (F2), advanced (F3) or severe (F4) fibrosis. ActiTest®, which includes the same components as FibroTest® plus alanine-aminotransferase, assesses inflammatory activity, categorized as none (A0), minimal (A1), moderate (A2) or severe (A3). SteatoTest® includes the same six components as ActiTest® plus body mass index (BMI), serum total cholesterol, triglycerides and fasting glucose, adjusted for age and sex. It is categorized as no (S0), minimal (S1), moderate (S2), and marked or severe (S3) steatosis. Lastly, NashTest® includes the same parameters as FibroTest plus alanine-aminotransferase, aspartate-aminotransferase, serum total cholesterol, triglycerides, and fasting glycemia, adjusted for age and sex. NashTest® severity is classified as minimal (N1), moderate (N2) or severe metabolic dysfunction-associated steatohepatitis (N3). The scores for all of the FibroMax® panels range from 0.00 to 1.00. The laboratory was blinded to LLACS values and clinical data.

Serum fetuin-A and interleukin-6 were measured by ELISA as previously described [20]. Plasma sphingolipids (23 ceramides, 8 dihydroceramides, and 7 sphingosines) were quantified by liquid chromatography coupled with tandem mass spectrometry, as previously described [22].

The triglyceride-glucose index (TyG index) was calculated as followed: ln(fasting triglycerides [mg/dL]*fasting plasma glucose [mg/dL]/2). The homeostasic model assessment of insulin resistance (HOMA-IR) was

calculated as fasting insulin (mU/L) x fasting plasma glucose (nmol/L)/22.5.

Statistical analysis

Data are shown as means ± standard deviation (SD) or medians [1st–3rd quartiles] for continuous variables and percentages (frequency) for categorical variables. The skewness of each continuous variable was assessed using Pearson's first skewness coefficient, and values were log10 transformed to improve normality if necessary.

Trends between several categories were assessed with the Cochran-Armitage trend test for proportions and the Jonckheere-Terpstra trend test for continuous variables using bootstrapping (10,000 iterations). The Mann–Whitney U test was used to compare two groups.

For the univariate correlation analysis, Spearman correlation coefficients (rho) were determined for continuous variables, and point-biserial correlation coefficients (r_{pbi}) were used to assess the association between continuous variables and categorical variables.

Multivariate analyses were conducted using multivariate linear regression based on the least-squares method. The collinearity between independent variables was checked and ruled out when variance inflation factor was below the threshold of four. Principal component regression, which is a combination of principal component analysis and multiple linear regression, was performed to identify independent variables through parallel analysis, followed by subsequent multiple linear regression using the selected variables. Additionally, ANCOVA analysis was employed to examine the impact of categorical variables on the dependent variable. An interaction term ("categorical variable*FibroTest® score") was incorporated to assess whether the relationship between the FibroTest® score and LLACS was consistent across subgroups. Bias-corrected and accelerated bootstrapped subgroup comparisons were performed using a post-hoc Tukey test following the one-way ANCOVA, based on 1,000 successful replicates. Prior to performing ANCOVA analysis, the assumptions of homogeneity of regression slopes and equality of variances were checked. Lastly, two advanced machine learning models (namely, Random Forest and XGBoost) were employed to validate the prognostic value of variables for predicting LLACS progression.

To identify potential explanatory variables influencing the relationship between MASLD biomarkers and LLACS, we conducted a machine learning approach utilizing the least absolute shrinkage and selection operator (LASSO) regression with extended Bayesian information criterion (EBIC). All potential explanatory variables were selected based on their pathophysiological relevance, and all were included in the same LASSO-EBIC analysis.

Statistical calculations were performed using GraphPad Prism (version 9.5.0) and JASP (version 0.18.3). A two-tailed probability level of 0.05 was considered as statistically significant. A Benjamini–Hochberg procedure was applied for controlling false positives in multiple testing, using a false discovery rate of 5%.

Results

A total of 198 patients were initially enrolled in the DIACART study. During the study follow-up period, 18 patients were lost to follow-up, 11 patients died prior to the second LLACS evaluation, and FibroMax® measurements were unavailable for 19 patients. Consequently, the final study population for analysis comprised 150 evaluable patients, exceeding the predefined minimal sample size of 123.

Patient characteristics

Table 1 shows the main clinical and biochemical characteristics of the 150 patients with T2D. At baseline, patients were predominantly middle aged, overweight, male, with a long duration of diabetes and a relatively good glycemic control. Less than 25% of the patients had retinopathy, while 35% had signs of peripheral neuropathy. Nearly a third of the patients had nephropathy but the eGFR (73 ± 19 mL/min/1.73 m^2) was relatively preserved as expected since an eGFR lower than 30 mL/min/1.73 m^2 was an exclusion criterion. Most of the patients were active or past smokers and had hypertension. They often took statins, antiplatelet therapies, β-blockers and renin-angiotensin system inhibitors. Diabetes was often treated with metformin, insulin and sulfonylurea. More than three-quarters of the patients (n = 112) had a SteatoTest stage ≥ S1, while 60.7% (n = 91) had a FibroTest stage ≥ F1.

Lower limb arterial calcifications at baseline and FibroMax® panels

The median LLACS at baseline was 503 [interquartile range: 44–2224] AU. We investigated the association between the degree of calcification in below-knee arteries at baseline and FibroMax® panels. In univariate regression analysis (Table 2), LLACS at baseline was positively associated with male gender ($r_{pbi} = 0.344$, $p < 0.0001$), beta-blockers use ($r_{pbi} = 0.282$, $p = 0.0005$), age (rho = 0.285, $p = 0.0004$), and the FibroTest® score (rho = 0.271, $p = 0.0008$), and negatively with eGFR (rho = – 0.241, $p = 0.003$). Subgroups analyses revealed that age, sex, BMI, diabetes duration, beta-blocker use, and eGFR categories had no significant impact on the association between the FibroTest® score and LLACS at baseline (Additional file 1).

By subdividing the cohort according to FibroTest® stages (Table 3), LLACS at baseline significantly increased with the FibroTest® categories (p-value for trend = 0.050). The association between the FibroTest®

Table 1 Participant characteristics (n = 150)

Characteristics	
General characteristics at baseline	
Age, y	64.0 ± 8.3
Gender, % male (n)	78.0% (117)
Diabetes duration, y	16.4 ± 9.2
Smoking (active or past), % (n)	65% (97)
Hypertension	85% (128)
Dyslipidemia	91% (137)
Retinopathy	24% (36)
Peripheral neuropathy	35% (52)
BMI, kg/m^2	28.7 [25.2 to 32.7]
Systolic blood pressure, mmHg	135 ± 17
Diastolic blood pressure, mmHg	76 ± 9
Insulin use, % (n)	51% (77)
Metformin use, % (n)	79% (119)
Sulfonylurea use, % (n)	45% (68)
GLP-1 receptor agonist, % (n)	7% (10)
Statin use, % (n)	89% (133)
Ezetimibe use, % (n)	15% (23)
Antiplatelet use, % (n)	82% (123)
ARB or ACEi use, % (n)	79% (119)
Beta-blocker use, % (n)	65% (97)
Biological characteristics at baseline	
Fasting glycemia, mmol/L	9.15 ± 3.20
HbA1c, %	7.7 [6.9 to 8.3]
eGFR, mL/min/1.73m^2	73 ± 19
Albuminuria, mg/mmol creat	2.3 [0.9 to 11.7]
Triglyceridemia, mmol/L	1.66 [1.12 to 2.41]
Total cholesterolemia, mmol/L	3.96 ± 0.90
LDL-cholesterolemia, mmol/L	2.07 ± 0.71
HDL-cholesterolemia, mmol/L	1.06 [0.88 to 1.27]
AST, IU/L	26 [21 to 31]
ALT, IU/L	25 [18 to 32]
AST-to-ALT ratio	1.08 [0.84 to 1.25]
GGT, IU/L	33 [24 to 50]
hsCRP, mg/L	1.24 [0.65–3.04]
IL-6, pg/mL	3.2 [2.10–4.80]
Below-knee arterial calcification	
LLACS at baseline, AU	503 [44 to 2224]
LLACS at the end of follow-up, AU	1242 [132 to 4112]
Absolute change in LLACS, AU	+ 547 [+ 53 to + 1761]
Annualized change in LLACS, AU/year	+ 215 [+ 21 to + 688]
FibroMax® panels	
SteatoTest® score	0.544 ± 0.206
S0, % (n)	24.3% (36)
S1, % (n)	29.1% (43)
S2, % (n)	18.9% (28)
S3, % (n)	27.7% (41)
FibroTest® score	0.376 ± 0.213
F0, % (n)	39.3% (59)
F1, % (n)	27.3% (41)
F2, % (n)	14.0% (21)
F3, % (n)	13.3% (20)
F4, % (n)	6.0% (9)

Table 1 (continued)

Characteristics	
NashTest® score	0.50 [0.25 to 0.50]
N0, % (n)	33% (50)
N1, % (n)	52% (78)
N2, % (n)	15% (22)
ActiTest® score	0.12 [0.07 to 0.18]
A0, % (n)	90% (135)
A1, % (n)	6% (9)
A2-A3, % (n)	4% (6)

Data are presented as median [1st to 3rd quartiles], mean ± standard deviation or percentage, as appropriate. ACEi, angiotensin converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin receptor blockers; AST, aspartate aminotransferase; AU, Agatston unit; BMI, body mass index; eGFR, estimated glomerular filtration rate; GGT, gamma glutamyltranspeptidase; GLP: glucagon-like peptide; LLACS, lower limb arterial calcification score

score and baseline LLACS did not remain significant after adjustment for sex, age, eGFR, and beta-blocker use (unstandardized β = 1821 [95% confidence interval (CI): − 2926–6569], $p = 0.45$). No association was found between LLACS at baseline and the SteatoTest®, ActiTest® or NashTest® scores ($p > 0.05$ for all) (Table 2).

Lower limb arterial calcifications at the end of follow-up and FibroMax® panels

The median LLACS at the end of follow-up was 1242 [interquartile range: 132–4112] AU. In univariate regression analysis (Table 2), LLACS at the end of follow-up was positively associated with baseline LLACS (rho = 0.968, $p < 0.0001$), the FibroTest® score (rho = 0.309, $p = 0.0001$), and age (rho = 0.273, $p = 0.0007$), and negatively with eGFR (rho = − 0.249, $p = 0.002$). Subgroups analyses revealed that age, sex, diabetes duration, and eGFR categories had no significant impact on the association between the FibroTest® score and LLACS at the end of follow-up (Additional file 1). BMI lower than 25 kg/m^2 significantly strengthened the association between the FibroTest® score and the LLACS at the end of follow-up (p for interaction = 0.008; size effect $\omega^2 = 0.038$).

By subdividing the cohort according to FibroTest stages (Table 3), LLACS at the end of follow-up significantly increased with the FibroTest categories (p-value for trend = 0.042). The association between the FibroTest® score and LLACS at the end of follow-up remained significant after adjustment for baseline LLACS, age, and eGFR (unstandardized β = 1863 [95% CI 364–3362], $p = 0.015$). No association was found between LLACS at the end of follow-up and the SteatoTest®, ActiTest® or NashTest® scores ($p > 0.05$ for all) (Table 2).

Progression of lower limb arterial calcifications and FibroMax® panels

During the follow-up period (mean ± SD: 31.2 ± 3.7 months, range: 25.9–42.2 months), LLACS increased in 127 (85%) of the 150 patients with T2D. In the entire cohort, LLACS significantly rose from 503 AU [44–2224] (median [1st–3rd quartiles]) at baseline to 1242 AU [132–4112] at the end of follow-up ($p < 0.0001$).

In univariate analysis (Table 2), the annualized change in LLACS was positively correlated with baseline LLACS (r = 0.860, $p < 0.0001$), the FibroTest® score (r = 0.304, $p = 0.0002$), age (r = 0.275, $p = 0.0006$), albuminuria (r = 0.226, $p = 0.006$), and the AST-to-ALT ratio (r = 0.261, $p = 0.001$), while a negative correlation was found with eGFR (r = − 0.242, $p = 0.003$). No significant associations were observed between the annualized change in LLACS and follow-up duration, SteatoTest®, ActiTest®, or NashTest® scores ($p > 0.05$ for all).

The FibroTest® score was significantly associated with the annualized change in LLACS, independent of sex, age, BMI, diabetes duration, beta-blockers use, eGFR, and albuminuria categories (Table 4). BMI below 25 kg/m^2 was the only variable that significantly strengthened the association between the FibroTest® score and the annualized change in LLACS (p for interaction = 0.011; size effect $\omega^2 = 0.034$). However, bootstrapped comparison revealed no significant differences in annualized change in LLACS between the two BMI subgroups after controlling for the FibroTest® score ($p = 0.27$).

Stratification of the cohort based on FibroTest® stages demonstrated a significant association with both absolute and annualized changes in LLACS increasing progressively across higher FibroTest® categories (p-values for trend = 0.030 and 0.023, respectively; Table 3). Annualized change in LLACS was greater in patients with a FibroTest® stage ≥ F1 compared to those with a stage F0 (889 [229–2063] vs. 243 AU [14–881], $p = 0.002$), in patients with a FibroTest® stage ≥ F2 compared to those with a stage < F2 (1096 [302–2134] vs. 397 AU [20–1594], $p = 0.002$), and in patients with a FibroTest® stage ≥ F3 compared to stage < F3 (1424 [427–3858] vs. 454 AU [33–1626], $p = 0.003$). Among the 23 patients with no LLACS progression during the follow-up, 21 (91%) had stages F0 or F1 (p-value for trend = 0.037).

In the multivariate analysis (Table 5), the FibroTest® score remained independently associated with the annualized change in LLACS after adjusting for variables

Table 2 Univariate correlations between LLACS and patient characteristics at baseline

Variables	LLACS at baseline		LLACS at the end of follow-up		Annualized change in LLACS	
	rho/r_{pbi}	*p* value	rho/r_{pbi}	*p* value	rho/r_{pbi}	*p* value
LLACS at baseline	N.A	N.A	**0.968**	**< 0.0001**	**0.860**	**< 0.0001**
Age	**0.285**	**0.0004**	**0.273**	**0.0007**	**0.275**	**0.0006**
Gender (male)	**0.344**	**< 0.0001**	0.164	0.05	0.084	0.31
Diabetes duration	0.049	0.55	0.076	0.38	0.094	0.25
Smoking (active or past)	0.126	0.12	−0.077	0.35	−0.092	0.26
Hypertension	0.166	0.04	−0.004	0.97	0.007	0.92
Dyslipidemia	−0.061	0.46	−0.007	0.94	−0.065	0.43
Retinopathy	0.197	0.02	0.163	0.05	0.006	0.94
Neuropathy	0.101	0.18	0.024	0.77	−0.002	0.99
BMI	−0.021	0.80	−0.011	0.89	−0.011	0.90
SBP	0.140	0.09	0.156	0.06	0.155	0.06
DBP	−0.077	0.35	−0.091	0.27	−0.116	0.16
Insulin use	0.013	0.88	0.11	0.17	−0.084	0.31
Metformin use	0.017	0.84	−0.147	0.07	−0.028	0.73
Sulfonylurea use	−0.011	0.90	−0.074	0.37	−0.090	0.27
GLP-1 receptor agonist	−0.121	0.14	−0.033	0.69	−0.004	0.97
Statin use	0.028	0.73	0.002	0.98	0.083	0.31
Ezetimibe use	0.144	0.08	0.029	0.73	0.004	0.96
Antiplatelet use	0.167	0.04	0.151	0.07	0.128	0.12
ARB or ACEi use	−0.024	0.77	0.105	0.20	0.087	0.29
Beta-blocker use	**0.282**	**0.0005**	0.138	0.09	**0.222**	**0.006**
Fasting glycemia	−0.032	0.70	−0.014	0.87	0.001	0.99
HbA1c	−0.118	0.15	−0.127	0.12	−0.151	0.07
eGFR	**−0.241**	**0.003**	**−0.249**	**0.002**	**−0.242**	**0.003**
Albuminuria	0.127	0.12	0.191	0.02	**0.226**	**0.006**
Triglyceridemia	−0.118	0.15	−0.086	0.29	−0.027	0.74
Total cholesterolemia	−0.191	0.02	−0.176	0.03	−0.175	0.03
LDL-cholesterolemia	−0.119	0.15	−0.121	0.14	−0.151	0.07
HDL-cholesterolemia	−0.000	0.99	0.010	0.91	−0.006	0.95
AST	−0.014	0.87	0.110	0.18	0.109	0.18
ALT	−0.073	0.37	−0.075	0.36	−0.119	0.15
AST-to-ALT ratio	0.183	0.025	0.209	0.010	**0.260**	**0.001**
GGT	−0.008	0.92	0.043	0.60	0.054	0.51
FibroTest® score	**0.271**	**0.0008**	**0.309**	**0.0001**	**0.304**	**0.0002**
SteatoTest® score	−0.124	0.13	−0.098	0.24	−0.068	0.41
NashTest® score	−0.070	0.39	−0.052	0.53	−0.042	0.61
ActiTest® score	0.034	0.68	0.046	0.57	0.009	0.92
hsCRP, mg/L	0.069	0.40	−0.044	0.60	−0.050	0.55
IL-6, pg/mL	0.045	0.58	0.019	0.82	0.008	0.91

Rho and r_{pbi} represent the Spearman and point-biserial correlation coefficients, respectively. LLACS was log-transformed for point-biserial analysis. The correlations remaining significant after Benjamini–Hochberg correction to control the false discovery rate are reported in bold

ACEi, angiotensin converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin receptor blockers; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GGT, gamma glutamyltransferase; GLP, glucagon-like peptide; LLACS, lower limb arterial calcification score; N.A., not applicable; SBP, systolic blood pressure

significantly associated with the annualized change in LLACS in the univariate analysis, including baseline LLACS (Model 1, unstandardized β = 798 [95% CI: 125–1471], $p = 0.020$). In addition, the FibroTest score exhibited an association with annualized change in LLACS, independent of the risk factors for lower extremity artery disease outlined in the European Society of Cardiology guidelines [23], namely age, smoking, hypertension, diabetes duration, total cholesterolemia, and HDL-cholesterolemia (Model 2, β = 988 [284–1692], $p = 0.006$). The FibroTest® score remained also associated with the annualized change in LLACS, independently of variables previously identified in patients with T2D as being associated with lower limb arterial calcification (Model 3,

Table 3 LLACS according to FibroTest® categories

Characteristic	Total	FibroTest® stages				*p* value for trend
		F0	*F1*	*F2*	*F3-F4*	
All patients						
Participants, n (%)	150	59 (39%)	41 (27%)	21 (14%)	29 (19%)	
LLACS at baseline, AU	503 [44 to 2224]	181 [0 to 1449]	434 [102 to 2311]	923 [349 to 2196]	1199 [287 to 3998]	**0.050**
LLACS at the end of follow-up, AU	1242 [132 to 4112]	421 [20 to 3382]	1298 [95 to 4169]	1462 [609 to 3275]	2707 [845 to 7400]	**0.042**
Absolute change in LLACS, AU	547 [53 to 1761]	243 [14 to 881]	685 [49 to 2037]	644 [311 to 1650]	1424 [427 to 3858]	**0.030**
Annualized change in LLACS, AU/year	215 [21 to 688]	90 [5.4 to 382]	291 [19 to 781]	248 [125 to 633]	465 [140 to 1674]	**0.023**
Patients with no progression of LLACS						
Participants, n (%)	23	13 (57%)	8 (35%)	0 (0%)	2 (9%)	**0.037**

Data are presented as median [1st to 3rd quartiles] or number (percentage), as appropriate. Individuals with F3 or F4 stages were grouped together to reach a significant number of subjects. P-values in bold indicate statistical significance. AU, Agatston unit; LLACS, lower limb arterial calcification score

Table 4 Subgroup analysis of the association between the FibroTest® score and the annualized change in LLACS

Subgroup Cutoff for categorization	n	FibroTest® score		Interaction	Post-hoc comparison	
	< / ≥ cutoff	F	*p* value	*p* value	Mean difference (95% CI)	*p* value
Age, y						
60	32/118	10.5	0.001	0.76	− 135 (− 355 to 138)	0.48
67 (median)	74/76	10.3	0.002	0.11	− 83 (− 301 to 318)	0.59
Gender						
Female / male	34/116	10.9	0.001	0.74	− 77 (− 345 to 1101)	0.75
BMI, kg/m²						
25.0	31/119	11.5	0.0009	0.011	193 (− 138 to 589)	0.27
28.7 (median)	75/75	12.0	0.0007	0.32	− 88.5 (− 443 to 133)	0.48
30.0	90/60	11.9	0.0007	0.54	− 73.5 (− 602 to 157)	0.57
Diabetes duration, y						
10	41/109	11.9	0.0007	0.96	− 124 (− 411 to 188)	0.44
14 (median)	67/83	11.7	0.0008	0.35	− 129 (− 469 to 82)	0.34
20	99/51	12.0	0.0007	0.81	− 182 (− 826 to 50)	0.18
Beta-blocker users						
No/yes	53/97	8.1	0.005	0.23	− 309 (− 576 to − 101)	0.05
eGFR, mL/min/1.73 m²						
60	40/110	11.3	0.001	0.62	50 (− 234 to 355)	0.74
77 (median)	73/77	10.8	0.001	0.59	40 (− 192 to 390)	0.78
90	119/31	10.4	0.002	0.35	264 (75 to 519)	0.13
Albuminuria, mg/mmol creat						
3	86/62	10.2	0.001	0.80	− 188 (− 596 to 49)	0.18
2.3 (median)	74/74	10.3	0.001	0.81	− 164 (− 509 to 106)	0.26
30	131/17	11.3	0.001	0.79	− 18 (− 466 to 383)	0.99

F represents the F-statistic value for the FibroTest® score in the one-way ANCOVA using the annualized change in LLACS as dependent variable and the categorical variable as fixed factor. The test for interaction evaluates whether the relationships between the FibroTest® score and the annualized change in LLACS are similar in each subgroup. The mean difference between subgroups is based on the median of the bootstrap distribution. BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate

β = 868 [199–1538], $p = 0.011$) [20]. Lastly, the FibroTest® score remained independently associated with the annualized change in LLACS in a fully-adjusted multivariate model combining baseline LLACS, all variables included models 1, 2, and 3, and medications known to modulate cardiovascular risk and/or lower limb arterial calcification in patients with lower extremity artery disease [23–25] (Model 4, β = 841 [114–1567], $p = 0.024$). Unlike the FibroTest® score, the AST-to-ALT ratio did not remain significantly associated with the annualized change in LLACS regardless the multivariate model considered (e.g., β = 1096, $p = 0.35$ in the fully-adjusted model 4). In all multivariate models, baseline LLACS was strongly and independently associated with the annualized change in LLACS ($p < 0.0001$).

As complementary multivariate analysis, we conducted a principal component regression, with a principal component analysis as a first step to identify predictors of the

Table 5 Multivariate analysis for annualized changes in LLACS

Variables	Without adjustment for LLACS (log) at baseline		With adjustment for LLACS (log) at baseline	
	β coefficient [95% CI]	*p* value	β coefficient [95% CI]	*p* value
Model 1				
FibroTest® score	**1000 [263 to 1738]**	**0.008**	**798 [125 to 1471]**	**0.020**
Age	10.2 [− 10.9 to 31.2]	0.34	− 2.35 [− 21.9 to 17.2]	0.82
Albuminuria (log)	60.6 [− 153 to 274]	0.56	22.7 [− 172 to 217]	0.82
eGFR	− 0.011 [− 9.5 to 9.5]	0.99	0.45 [− 8.17 to 9.10]	0.92
Model 2				
FibroTest® score	**1270 [503 to 2038]**	**0.001**	**988 [284 to 1692]**	**0.006**
HDL-cholesterolemia (log)	651 [− 367 to 1669]	0.21	457 [− 469 to 1384]	0.33
Total cholesterolemia	− 92.8 [− 259 to 73]	0.27	− 57.1 [− 208 to 94.2]	0.46
Smoking (active or past)	− 194 [− 503 to 116]	0.22	− 161 [− 442 to 121]	0.26
Diabetes duration	7.78 [− 8.18 to 23.8]	0.34	8.71 [− 5.78 to 23.2]	0.24
Hypertension	− 164 [− 577 to 249]	0.44	− 93.0 [− 469 to 283]	0.63
Age	3.95 [− 14.8 to 22.7]	0.68	− 8.28 [− 25.9 to 9.30]	0.35
Model 3				
FibroTest® score	**1100 [371 to 1828]**	**0.003**	**868 [199 to 1538]**	**0.011**
Triglyceridemia (log)	− 429 [− 1310 to 452]	0.34	− 151 [− 961 to 659]	0.71
Peripheral neuropathy	− 75.8 [− 386 to 234]	0.63	− 70.0 [− 962 to 659]	0.63
Age	9.28 [− 8.89 to 27.4]	0.31	− 2.87 [− 20.0 to 14.3]	0.74
Model 4				
FibroTest® score	**1121 [289 to 1954]**	**0.008**	**841 [114 to 1567]**	**0.024**
Beta-blocker use	**356 [1.37 to 710]**	**0.049**	259 [− 50 to 568]	0.10
Hypertension	− 418 [− 942 to 105]	0.12	− 333 [− 788 to 122]	0.15
Diabetes duration	11.0 [− 6.1 to 28.1]	0.20	10.2 [− 4.7 to 25.0]	0.18
ARB or ACEi use	256 [− 203 to 716]	0.27	85 [− 317 to 487]	0.67
HDL-cholesterolemia (log)	675 [− 417 to 1768]	0.22	804 [− 144 to 1753]	0.10
Smoking (active or past)	− 201 [− 522 to 121]	0.22	**− 318 [− 599 to − 37]**	**0.027**
Antiplatelet use	122 [− 301 to 546]	0.57	− 328 [− 719 to 63]	0.10
Age	6.2 [− 16.0 to 29.0]	0.59	− 8.8 [− 29.1 to 11.4]	0.39
Total cholesterolemia	− 40.1 [− 222 to 142]	0.66	− 5.5 [− 164 to 153]	0.95
Metformin use	97.3 [− 282 to 477]	0.61	130 [− 200 to 459]	0.44
eGFR	1.2 [− 8.8 to 11.1]	0.82	1.99 [− 6.67 to 10.6]	0.65
Albuminuria (log)	35.7 [− 190 to 262]	0.76	64.5 [− 132 to 261]	0.52
Statin use	− 15.1 [− 540 to 510]	0.95	− 126 [− 582 to 330]	0.59
Model 5				
FibroTest® score	**1029 [289 to 1768]**	**0.007**	**850 [150 to 1550]**	**0.017**
Age	8.7 [− 12.6 to 29.9]	0.42	− 2.0 [− 21.1 to 17.1]	0.71
Diabetes duration	8.0 [− 8.4 to 24.4]	0.34	7.9 [− 6.6 to 22.5]	0.75
Albuminuria (log)	48.7 [− 166 to 264]	0.66	30.8 [− 159 to 221]	0.84
eGFR	0.52 [− 9.0 to 10.1]	0.91	1.58 [− 6.88 to 10.1]	0.28

The model 1 included all quantitative variables significantly associated with the annualized change in LLACS in univariate regression after Benjamini–Hochberg correction (see Table 2). The model 2 included baseline risk factors for lower extremity artery disease according to European Society of Cardiology guidelines [23]. The model 3 incorporated variables previously identified in patients with T2D as being associated with lower limb arterial calcification in prior studies [9, 20]. The fully-adjusted model 4 corresponded to models 2 + 3 + 4 + medications known to modulate cardiovascular risk and/or lower limb arterial calcification in patients with lower extremity artery disease [23–25]. The model 5 included the variables selected using principal component regression (see Fig. 1). P-values in bold indicate statistical significance. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blockers; CI, confidence interval; eGFR, estimated glomerular filtration rate; LLACS, lower limb arterial calcification score

annualized change in LLACS among all the quantitative variables reported in Table 2. The parallel analysis led to the selection of six principal components, cumulatively representing 68% of the total variance. Figure 1 illustrates the loading plot of the two main principal components. Principal component regression revealed that the following six variables were significantly associated with the annualized change in LLACS: LLACS at baseline ($p < 0.0001$), FibroTest® score ($p < 0.0001$), age ($p = 0.001$), diabetes duration ($p = 0.03$), eGFR ($p = 0.003$), and albuminuria ($p = 0.002$). Subsequent multiple linear regression analysis, incorporating these six variables, showed

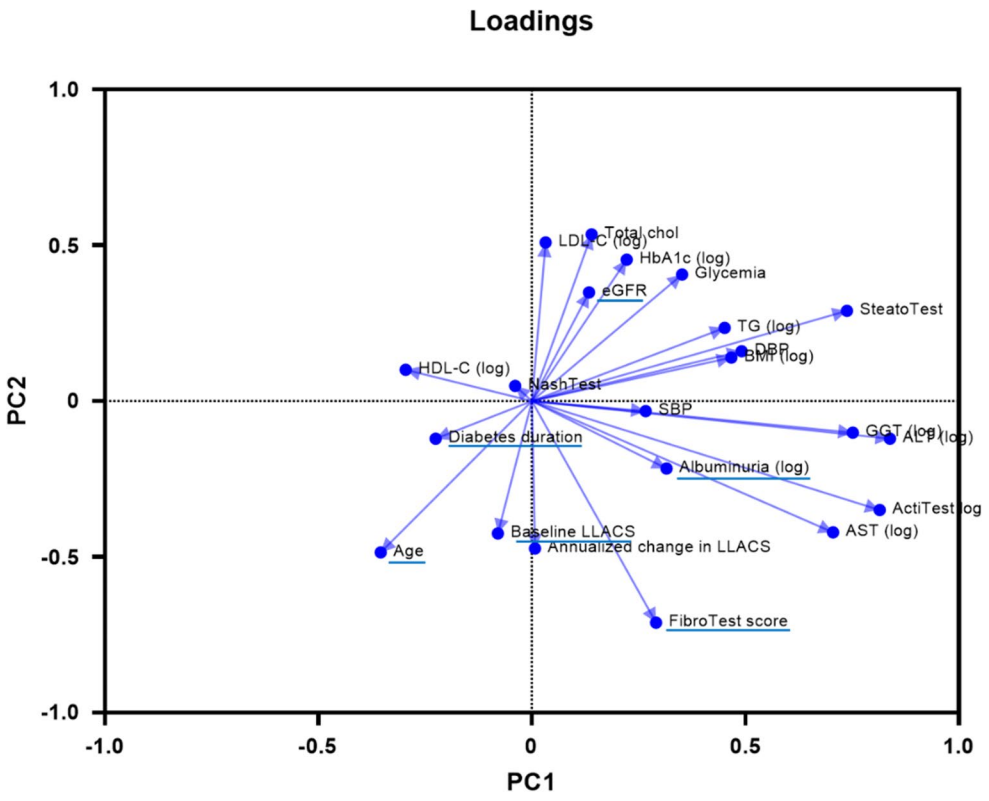


Fig. 1 Loading plot of the principal component analysis. The six underlined variables are those selected by the parallel analysis for the principal component regression. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GGT, gamma glutamyltranspeptidase; LLACS, lower limb arterial calcification score; SBP, systolic blood pressure; TG, triglycerides

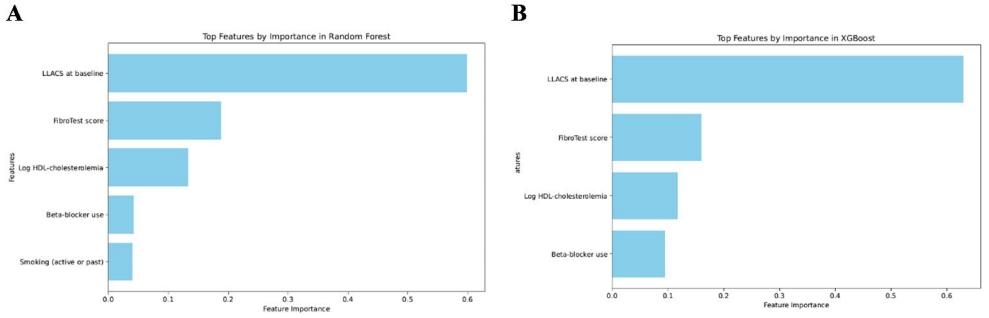


Fig. 2 Random Forest (**A**) and XGBoost (**B**) importance plots for predicting annualized changes in LLACS. Only the top four-five variables by importance that are predictive of LLACS progression are reported. eGFR, estimated glomerular filtration rate; LLACS, lower limb arterial calcification score

that the FibroTest® score was independently associated with the annualized change in LLACS, even after adjusting for baseline LLACS (Model 5 in Table 5, β = 850 [150–1550], $p = 0.017$).

In our investigation to validate the relationship between the FibroTest® score and LLACS progression, two advanced machine learning models (namely, Random Forest and XGBoost) were employed considering all variables used in the five models shown in Table 4. Both machine learning models demonstrated robust predictive capabilities, with R^2 scores of 0.723 and 0.709, respectively, indicating that they could explain more than 70% of the variance in annualized change in LLACS. The mean squared errors were 250 and 238 for the Random Forest and XGBoost, respectively, underscoring the accuracy of both models in predicting annualized change in LLACS. As shown in Fig. 2, the feature importance analysis revealed that the FibroTest® score emerged as the second most important predictor in both machine learning models, following baseline LLACS.

Preliminary study exploring the pathophysiological links between FibroTest® and LLACS progression

Insulin resistance, sphingolipids, hepatokines, and inflammation have been demonstrated to be involved in the pathogenesis of both MASLD and vascular calcification [26–28]. Therefore, we hypothesized that they could be potentially implied in the relationship between FibroTest® and LLACS progression. We measured plasma circulating sphingolipids, serum fetuin A (a hepatokine), high-sensitivity C-reactive protein, interleukin 6, and calculated insulin resistance indexes (TyG index and HOMA-IR). In the univariate analysis, only the sphingomyelins d18:1/18:0, d18:1/22:0, and d18:1/24:0 were significantly associated with both the annualized change in LLACS and the FibroTest® score after applying the Benjamini–Hochberg correction (Additional file 2). We subsequently performed a machine learning EBIC-LASSO analysis with all these variables to assess the interrelationship between these biomarkers, the annualized change in LLACS, and the FibroTest® score. As shown in the network plot (Additional file 3), none of these biomarkers appeared in the same cluster as the annualized change in LLACS and the FibroTest® score. In addition, introducing one to one these biomarkers in our multivariate models did not suppress the independent relationship between the FibroTest® score and the annualized change in LLACS (data not shown).

Since the relationship between MASLD and incident PAD has been shown to be BMI-dependent [16], we aimed to assess the impact of BMI on the association between the FibroTest® score and the annualized change in LLACS. The study population was grouped according to BMI < 25 (n = 31) or ≥ 25 kg/m^2 (n = 119), BMI < 28.7 or ≥ 28.7 (median) kg/m^2 (n = 75 in each subgroup), and BMI < 30 (n = 90) or ≥ 30 kg/m^2 (n = 60). Using ANCOVA analysis, the FibroTest® score remained associated with the annualized change in LLACS after adjustment for age, diabetes duration, eGFR and albuminuria, independently of the BMI category ($p = 0.007$, $p = 0.006$, and $p = 0.006$ for BMI thresholds of 25, 28.7, and 30 kg/m^2, respectively). Furthermore, none of the BMI categories significantly modified the strength of the association between the FibroTest® score and the annualized change in LLACS after adjusting for the aforementioned covariates (p for interaction = 0.09, 0.47, and 0.70 for BMI thresholds of 25, 28.7, and 30 kg/m^2, respectively).

Discussion

The present study is the first to demonstrate an independent association between a non-invasive liver fibrosis test, namely the FibroTest®, and the progression of lower limb arterial calcification over a mean period of 2.5 years in 150 people with T2D. Interestingly, this association was independent of the traditional risk factors for PAD progression, including age, hypertension, diabetes duration, smoking, and dyslipidemia. The FibroTest® was also associated with LLACS progression independently of factors driving arterial calcification, such as baseline arterial calcification, age, sex, neuropathy, triglycerides and statin use. Machine learning approaches also validated the prominent role of FibroTest® score in the prediction of LLACS progression. Our findings are of particular clinical significance due to the pivotal role of lower limb arterial calcification in PAD development and foot complications [29].

Previous studies have demonstrated that liver fibrosis is linked to atherosclerotic cardiovascular events [30–33]. In recent years, there has been growing interest in the use of liver fibrosis biomarkers to assess cardiovascular risk. A FibroTest® stage ≥ F2 was an independent predictor of cardiovascular events in 900 patients with T2D [34]. Non-invasive tests of liver fibrosis including the FibroTest® have been shown to be linked to coronary calcification and its progression [35–38]. Vascular calcification is implicated in diseases affecting coronary and carotid arteries but also in PAD [39]. Coronary arterial calcification and calcification of the arteries of the lower limbs share common pathophysiological mechanisms, but they also have their own specificities, so that it is not possible to generalize data from coronary arteries to the arteries of the legs. Our study aimed to determine factors associated with calcification progression in the below-knee arteries. Arterial calcification in this location is mainly medial artery calcification rather than intima calcification, which is more commonly observed in larger arteries like coronary, carotid and proximal lower limb arteries [8, 40]. We can therefore consider than our study is the first to investigate the relationship between MASLD biomarkers and the progression of medial artery calcification.

Our study highlights that, unlike steatosis and steatohepatitis, liver fibrosis may be strongly associated with lower limb arterial calcification in patients with T2D, and the more severe the fibrosis, the more the LLACS increases during follow-up. One hypothesis that could explain why there was only an association between lower limb arterial calcification and the FibroTest® among the four FibroMax® panels is that liver fibrosis is the final stage of liver disease, signifying an extended progression of lipotoxicity and both hepatic and systemic inflammation. This hypothesis is supported by the observation that advanced FibroTest® stages are associated with the highest values of LLACS progression.

We aimed to explore the pathophysiological links between FibroTest® and LLACS progression. Insulin resistance and inflammation are involved in the pathophysiology of both liver fibrosis and arterial calcification [26, 39, 41]. In addition, sphingolipids have been demonstrated

to be linked to MASLD and, with a lesser degree of evidence, to vascular calcification [22, 27, 42–44]. Lastly, dysregulated secretion of hepatokines in MASLD plays a role in pathways related to vascular diseases [45], and fetuin A has been shown to inhibit vascular calcification [46]. However, our network analysis suggested that insulin resistance, inflammation, circulating sphingolipids and fetuin A may not play a significant role in mediating the relationship between FibroTest® and LLACS progression. In fact, plasma sphingolipids have been demonstrated to be primarily linked to liver steatosis rather than liver fibrosis in T2D patients [22, 42]. Further investigations, including in vitro and in vivo mechanical studies are required to identify potential pathophysiological variables that could explain the association between liver fibrosis and progression in lower limb arterial calcification in patients with T2D.

The growing interest in arterial calcifications of the lower limbs in recent years stems from two observations in patients with severe PAD and diabetes [39]. Firstly, in the arteries below the knee, unlike the coronary arterial bed, atheromatous lesions are very rare, unlike medial arterial calcifications, which are very prevalent [8]. Secondly, chronic arterial thrombosis is strongly associated with these areas of arterial calcification, independently of the presence of atheromatous lesions [8]. These points could explain why, in the lower limbs, arterial calcification is strongly associated with arterial occlusion. Finally, the pathophysiology of arterial disease seems to differ between the arterial bed below the knee and the coronary arterial bed, where an increase in vascular calcification does not necessarily mean a worsening of vascular disease and may even reflect a stabilization of plaque. Indeed, atheroprotective statin therapy has been shown to be associated with increased progression of coronary artery calcification, whereas no association was observed between statin use and LLACS in the present study. A better understanding of the pathophysiology of arterial calcification and determination of the factors associated with its progression are therefore necessary to develop a specific treatment for arterial calcification of the lower limbs.

Our study has some limitations to underline. Firstly, our assessment of MASLD severity relied on serum biomarkers rather than histological evaluation of liver biopsies. However, the diagnostic accuracy of FibroTest® has been deemed satisfactory when compared to histological findings in individuals with T2D [47–49], establishing it as a non-invasive test recommended by clinical guidelines to rule out advanced fibrosis in MASLD patients [50, 51]. Additionally, liver stiffness measurement using imaging techniques was not performed. Nevertheless, previous studies have shown that the diagnosis performance of FibroTest® for fibrosis staging is comparable to that of liver stiffness measurement by vibration-controlled transient elastography or two-dimensional shear-wave elastography [47, 52–54]. Secondly, we could not compare the results obtained with FibroTest® to the non-patented tests FIB-4 (Fibrosis-4 index) or NAFLD fibrosis score since we did not collect platelet counts. Interestingly, a recent observation indicates that the FibroTest® outperforms FIB-4 in patients with T2D for diagnosing advanced fibrosis, suggesting that FibroTest® may hold particular significance in the future management of patients with T2D [49].

Conclusions

Our study is the first to present evidence of the independent relationship of a non-invasive test of liver fibrosis for the progression of lower limb arterial calcification in patients with T2D. Consequently, beyond its interest for the assessment of liver fibrosis in MASLD, the FibroTest® could be a valuable and easy-to-use biomarker to assess the risk of worsening lower limb arterial calcification. Further studies are needed to assess the relationship between FibroTest® and severe lower limb complications in patients with T2D, such as symptomatic PAD, lower limb amputations and arterial revascularization.

Abbreviations

BMI	Body mass index
eGFR	Estimated glomerular filtration rate
HOMA-IR	Homeostasic model assessment of insulin resistance
LASSO-EBIC	Least absolute shrinkage and selection operator regression with extended Bayesian information criterion
LLACS	Lower limb arterial calcification score
MASLD	Metabolic dysfunction-associated steatotic liver disease
NAFLD	Nonalcoholic fatty liver disease
PAD	Peripheral artery disease
T2D	Type 2 diabetes
TyG index	Triglyceride-glucose index

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12933-025-02705-9.

Additional file 1

Additional file 2

Additional file 3

Acknowledgements

The authors thank all the patients, investigators, nurses and CIC-1901 staff members involved in the DIACART study (particularly Ms Aida Zarhrate-Ghoul). We are grateful to Jean-Paul Pais-de-Barros (DiviOmics Platform, Dijon, France) and Marie Lhomme (ICAN, Paris, France) for lipidomic analysis, and to Suzanne Rankin (Dijon University Hospital, France) for the language revision of the manuscript.

Author contributions

DD, FF and OB were involved in the conception and design of the study. FP, A-CJ, J-ES, SL, CA, AH and OB recruited participants and collected the data. AR and SB performed cardiac imaging measurements. DD, MP, PP, and OB performed the data analysis. DD wrote the first draft of the manuscript, and all authors read, reviewed, and approved the final version. DD is the guarantor

of this work and, as such, had full access to all the data in the study and takes responsibility for its integrity and the accuracy of the data analysis.

Funding
DIACART study was funded by the French Ministry of Health grant (PHRC code project: P111105; N° ID RCB: 2013-A00896-39) and by the Lilly Company. The company was neither involved in the design of the study nor in data collection. The first author's presence at INSERM UMR_S 1166 was made feasible due to a local grant from the University Hospital of Dijon.

Availability of data and materials
The datasets used and/or analyzed during this current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study involving human participants have been performed in accordance with the Declaration of Helsinki and have been approved by our local ethics committee.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]INSERM U1231, Center for Translational and Molecular Medicine, Dijon, France
[2]Department of Clinical Biochemistry, CHU Dijon-Bourgogne, Dijon Bourgogne University Hospital, 2 rue Ducoudray, 21000 Dijon, France
[3]Institute of Cardiometabolism and Nutrition (ICAN), Paris, France
[4]Sorbonne Université, Paris, France
[5]Department of Diabetology, Assistance Publique-Hôpitaux de Paris (AP-HP), Pitié-Salpêtrière Hospital, 47-83 Boulevard de l'Hôpital, Paris, France
[6]INSERM UMR_S 1166, Sorbonne University, Team Metabolic Diseases, Diabetes and Co-Morbidities, Paris, France
[7]Laboratoire d'Imagerie Biomédicale INSERM_1146, CNRS_7371, Paris, France
[8]ICT Cardiovascular and Thoracic Imaging Unit, AP-HP, Pitié Salpêtrière University Hospital, Paris, France
[9]Department of Pharmacology, INSERM, AP-HP, CIC-1901, Pitié-Salpêtrière Hospital, Sorbonne Université, Paris, France

Received: 6 January 2025 / Accepted: 24 March 2025
Published online: 23 April 2025

References

1. American Diabetes Association. Peripheral arterial disease in people with diabetes. Diabetes Care. 2003;26:3333–41.
2. St HC. Medial arterial calcification: a significant and independent contributor of peripheral artery disease. Arterioscler Thromb Vasc Biol. 2022;42:253–60.
3. Criqui MH, Matsushita K, Aboyans V, Hess CN, Hicks CW, Kwan TW, et al. Lower extremity peripheral artery disease: contemporary epidemiology, management gaps, and future directions: a scientific statement from the American Heart Association. Circulation. 2021;144:e171–91.
4. Guzman RJ, Brinkley DM, Schumacher PM, Donahue RMJ, Beavers H, Qin X. Tibial artery calcification as a marker of amputation risk in patients with peripheral arterial disease. J Am Coll Cardiol. 2008;51:1967–74.
5. Everhart JE, Pettitt DJ, Knowler WC, Rose FA, Bennett PH. Medial arterial calcification and its association with mortality and complications of diabetes. Diabetologia. 1988;31:16–23.
6. Huang C-L, Wu I-H, Wu Y-W, Hwang J-J, Wang S-S, Chen W-J, et al. Association of lower extremity arterial calcification with amputation and mortality in patients with symptomatic peripheral artery disease. PLoS ONE. 2014;9:e90201.
7. Zettervall SL, Marshall AP, Fleser P, Guzman RJ. Association of arterial calcification with chronic limb ischemia in patients with peripheral artery disease. J Vasc Surg. 2018;67:507–13.
8. Narula N, Dannenberg AJ, Olin JW, Bhatt DL, Johnson KW, Nadkarni G, et al. Pathology of peripheral artery disease in patients with critical limb ischemia. J Am Coll Cardiol. 2018;72:2152–63.
9. Bourron O, Aubert CE, Liabeuf S, Cluzel P, Lajat-Kiss F, Dadon M, et al. Below-knee arterial calcification in type 2 diabetes: association with receptor activator of nuclear factor κB ligand, osteoprotegerin, and neuropathy. J Clin Endocrinol Metab. 2014;99:4250–8.
10. Ohtake T, Mitomo A, Yamano M, Shimizu T, Mochida Y, Ishioka K, et al. Impact of arterial calcification of the lower limbs on long-term clinical outcomes in patients on hemodialysis. J Clin Med. 2023;12:1299.
11. Chowdhury MM, Makris GC, Tarkin JM, Joshi FR, Hayes PD, Rudd James HF, et al. Lower limb arterial calcification (LLAC) scores in patients with symptomatic peripheral arterial disease are associated with increased cardiac mortality and morbidity. PLOS ONE. 2017;12:0182952.
12. Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol. 2023;79:1542–56.
13. Castera L, Laouenan C, Vallet-Pichard A, Vidal-Trécan T, Manchon P, Paradis V, et al. High prevalence of NASH and advanced fibrosis in type 2 diabetes: a prospective study of 330 outpatients undergoing liver biopsies for elevated ALT, using a low threshold. Diabetes Care. 2023;46:1354–62.
14. Zou Y, Li X, Wang C, Wang J, Wang F, Ma L, et al. Association between non-alcoholic fatty liver disease and peripheral artery disease in patients with type 2 diabetes. Intern Med J. 2017;47:1147–53.
15. Liu Y, Liu C, Shi X, Lin M, Yan B, Zeng X, et al. Correlations of non-alcoholic fatty liver disease and serum uric acid with subclinical atherosclerosis in obese Chinese adults. J Diabetes. 2017;9:586–95.
16. Song X-H, Liu B, Lei F, Liu Y-M, Zhang X, Chen Z, et al. The association between metabolic dysfunction-associated fatty liver disease and peripheral arterial disease in the Chinese population. Diabetes Metab Syndr Obes. 2023;16:373–84.
17. Mai P, Li Q, Li S, Wang C, Xu S, Zhang K, et al. The Association between fatty liver index and lower limb arterial calcification in patients with type 2 diabetes mellitus. Rev Cardiovasc Med. 2024;25:362.
18. Abbott CA, Carrington AL, Ashe H, Bath S, Every LC, Griffiths J, et al. The North-West Diabetes Foot Care Study: incidence of, and risk factors for, new diabetic foot ulceration in a community-based patient cohort. Diabet Med. 2002;19:377–84.
19. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827–32.
20. Bourron O, Phan F, Diallo MH, Hajage D, Aubert C-E, Carlier A, et al. Circulating Receptor Activator of Nuclear Factor kB Ligand and triglycerides are associated with progression of lower limb arterial calcification in type 2 diabetes: a prospective, observational cohort study. Cardiovasc Diabetol. 2020;19:140.
21. Poynard T, Lassailly G, Diaz E, Clement K, Caïazzo R, Tordjman J, et al. Performance of biomarkers FibroTest, ActiTest, SteatoTest, and NashTest in patients with severe obesity: meta analysis of individual patient data. PLoS ONE. 2012;7:e30325.
22. Carlier A, Phan F, Szpigel A, Hajduch E, Salem JE, Gautheron J, et al. Dihydroceramides in triglyceride-enriched VLDL are associated with nonalcoholic fatty liver disease severity in type 2 diabetes. Cell Rep Med. 2020;1:100154.
23. Aboyans V, Ricco J-B, Bartelink M-LEL, Björck M, Brodmann M, Cohnert T, et al. ESC Guidelines on the diagnosis and treatment of peripheral arterial diseases. Eur Heart J. 2017;2018(39):763–816.
24. Phadwal K, Koo E, Jones RA, Forsythe RO, Tang K, Tang Q, et al. Metformin protects against vascular calcification through the selective degradation of Runx2 by the p62 autophagy receptor. J Cell Physiol. 2022;237:4303–16.
25. Mary A, Hartemann A, Liabeuf S, Aubert CE, Kemel S, Salem JE, et al. Association between metformin use and below-the-knee arterial calcification score in type 2 diabetic patients. Cardiovasc Diabetol. 2017;16:24.
26. Ke Z, Huang R, Xu X, Liu W, Wang S, Zhang X, et al. Long-term high level of insulin resistance is associated with an increased prevalence of coronary artery calcification: The CARDIA Study. J Am Heart Assoc. 2023;12:e028985.
27. El Jamal A, Bougault C, Mebarek S, Magne D, Cuvillier O, Brizuela L. The role of sphingosine 1-phosphate metabolism in bone and joint pathologies and ectopic calcification. Bone. 2020;130:115087.

28. Lorant DP, Grujicic M, Hoebaus C, Brix J-M, Hoellerl F, Schernthaner G, et al. Fetuin-A levels are increased in patients with type 2 diabetes and peripheral arterial disease. Diabetes Care. 2011;34:156–61.
29. Losurdo F, Ferraresi R, Ucci A, Zanetti A, Clerici G, Zambon A. Association of infrapopliteal medial arterial calcification with lower-limb amputations in high-risk patients: a systematic review and meta-analysis. Vasc Med. 2021;26:164–73.
30. Kim D, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. Hepatology. 2013;57:1357–65.
31. Namakchian M, Rabizadeh S, Seifouri S, Asadigandomani H, Bafrani MA, Seifouri K, et al. Fibrosis score 4 index has an independent relationship with coronary artery diseases in patients with metabolic-associated fatty liver disease. Diabetol Metab Syndr. 2023;15:57.
32. Ekstedt M, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology. 2015;61:1547–54.
33. Zeb I, Li D, Budoff MJ, Katz R, Lloyd-Jones D, Agatston A, et al. Nonalcoholic fatty liver disease and incident cardiac events. J Am Coll Cardiol. 2016;67:1965–6.
34. Perazzo H, Munteanu M, Ngo Y, Lebray P, Seurat N, Rutka F, et al. Prognostic value of liver fibrosis and steatosis biomarkers in type 2 diabetes and dyslipidaemia. Aliment Pharmacol Ther. 2014;40:1081–93.
35. Denimal D, Ponnaiah M, Jeannin A-C, Phan F, Hartemann A, Boussouar S, et al. Non-alcoholic fatty liver disease biomarkers estimate cardiovascular risk based on coronary artery calcium score in type 2 diabetes: a cross-sectional study with two independent cohorts. Cardiovasc Diabetol. 2024;23:69.
36. Lee J, Kim HS, Cho YK, Kim EH, Lee MJ, Bae IY, et al. Association between noninvasive assessment of liver fibrosis and coronary artery calcification progression in patients with nonalcoholic fatty liver disease. Sci Rep. 2020;10:18323.
37. Perdomo CM, Ezponda A, Núñez-Córdoba JM, Herrero JI, Bastarrika G, Frühbeck G, et al. Transient elastography and serum markers of liver fibrosis associate with epicardial adipose tissue and coronary artery calcium in NAFLD. Sci Rep. 2022;12:6564.
38. Park JG, Jung J, Verma KK, Kang MK, Madamba E, Lopez S, et al. Liver stiffness by magnetic resonance elastography is associated with increased risk of cardiovascular disease in patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2021;53:1030–7.
39. Davaine J-M, Denimal D, Treca P, Francon H, Phan F, Hartemann A, et al. Medial arterial calcification of the lower limbs in diabetes: Time for awareness? A short narrative review. Diabetes Metab. 2024;15:101586.
40. Kim TI, Guzman RJ. Medial artery calcification in peripheral artery disease. Front Cardiovasc Med. 2023;10:1093355.
41. Fujii H, Kawada N. The role of insulin resistance and diabetes in nonalcoholic fatty liver disease. Int J Mol Sci. 2020;21:3863.
42. Denimal D, Béland-Bonenfant S, Pais-de-Barros J-P, Rouland A, Bouillet B, Duvillard L, et al. Plasma ceramides are associated with MRI-based liver fat content but not with noninvasive scores of liver fibrosis in patients with type 2 diabetes. Cardiovasc Diabetol. 2023;22:310.
43. Liao L, Zhou Q, Song Y, Wu W, Yu H, Wang S, et al. Ceramide mediates Ox-LDL-induced human vascular smooth muscle cell calcification via p38 mitogen-activated protein kinase signaling. PLoS ONE. 2013;8:e82379.
44. Morris TG, Borland SJ, Clarke CJ, Wilson C, Hannun YA, Ohanian V, et al. Sphingosine 1-phosphate activation of ERM contributes to vascular calcification. J Lipid Res. 2018;59:69–78.
45. Stefan N, Schick F, Birkenfeld AL, Häring H-U, White MF. The role of hepatokines in NAFLD. Cell Metab. 2023;35:236–52.
46. Westenfeld R, Schäfer C, Krüger T, Haarmann C, Schurgers LJ, Reutelingsperger C, et al. Fetuin-A protects against atherosclerotic calcification in CKD. J Am Soc Nephrol. 2009;20:1264–74.
47. Poynard T, Paradis V, Mullaert J, Deckmyn O, Gault N, Marcault E, et al. Prospective external validation of a new non-invasive test for the diagnosis of non-alcoholic steatohepatitis in patients with type 2 diabetes. Aliment Pharmacol Ther. 2021;54:952–66.
48. Bril F, McPhaul MJ, Caulfield MP, Castille J-M, Poynard T, Soldevila-Pico C, et al. Performance of the SteatoTest, ActiTest, NashTest and FibroTest in a multiethnic cohort of patients with type 2 diabetes mellitus. J Investig Med. 2019;67:303–11.
49. Poynard T, Deckmyn O, Peta V, Paradis V, Gautier J-F, Brzustowski A, et al. Prospective direct comparison of non-invasive liver tests in outpatients with type 2 diabetes using intention-to-diagnose analysis. Aliment Pharmacol Ther. 2023;58:888–902.
50. European Association for the Study of the Liver Clinical Practice Guideline Panel. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis—2021 update. J Hepatol. 2021;75:659–89.
51. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL–EASD–EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. Diabetologia. 2016;59:1121–40.
52. Poynard T, Peta V, Deckmyn O, Pais R, Ngo Y, Charlotte F, et al. Performance of liver biomarkers, in patients at risk of nonalcoholic steato-hepatitis, according to presence of type-2 diabetes. Eur J Gastroenterol Hepatol. 2020;32:998–1007.
53. Caussy C, Vergès B, Leleu D, Duvillard L, Subtil F, Abichou-Klich A, et al. Screening for metabolic dysfunction-associated steatotic liver disease-related advanced fibrosis in Diabetology: A Prospective Multicenter Study. Diabetes Care. 2025;dc242075.
54. Meritsi A, Latsou D, Manesis E, Gatos I, Theotokas I, Zoumpoulis P, et al. Noninvasive, blood-based biomarkers as screening tools for hepatic fibrosis in people with type 2 diabetes. Clin Diabetes. 2022;40:327–38.

Publisher's Note