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Relationship Among Fatty Liver, Specific and Multiple-Site Atherosclerosis, and 10-Year Framingham Score

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Despite a well-documented increase in the prevalence of subclinical atherosclerosis in patients with steatosis, the relationship among steatosis and atherosclerosis, specific atherosclerotic sites, multiple-site atherosclerosis, and cardiovascular risk prediction is incompletely understood. We studied the relationship among steatosis, atherosclerosis site, multiple-site atherosclerosis, coronary artery calcification (CAC), and 10-year Framingham Risk Score (FRS) in 2,554 patients with one or more cardiovascular risk factors (CVRF), free of cardiovascular events and other chronic liver diseases, and drinking less than 50 g alcohol/day. All patients underwent arterial ultrasound (carotid [CP] and femoral [FP] plaques defined as intima-media thickness (IMT) > 1.5 mm), coronary computed tomography scan (severe CAC if ≥ 100), 10-year FRS calculation, and steatosis detection by the fatty liver index (FLI, present if score ≥ 60). Patients with steatosis (36% of total) had higher prevalence of CP (50% versus 45%, $P = 0.004$) and higher CAC (181 ± 423 versus 114 ± 284, $P < 0.001$) but similar prevalence of FP (53% versus 50%, $P = 0.099$) than patients without steatosis. Steatosis was associated with carotid IMT and CAC, but not with FP, independent of age, diabetes, hypertension, and tobacco use ($P < 0.001$). Fifty-three percent of patients had at least 2-site atherosclerosis and steatosis was associated with at least 2-site atherosclerosis independent of age and CVRF (odds ratio = 1.21, 95% confidence interval 1.01-1.45, $P = 0.035$). Sixty-four percent of patients with steatosis had a FRS score of 10% or more. FLI was associated with FRS beyond the CVRF or the number of atherosclerosis sites ($P < 0.001$). Adding FLI to CVRF predicted an FRS greater than or equal to 10% better than CVRF alone (area under the receiver operating characteristic curve = 0.848 versus 0.768, $P < 0.001$). *Conclusion:* Steatosis is associated with carotid and coronary, but not femoral atherosclerosis, and with cardiovascular mortality risk. The multiple-site involvement and quantitative tonic relationship could reinforce the prediction of cardiovascular mortality or events over classical CVRF or imaging-based detection of atherosclerosis. (Hepatology 2019;69:1453-1463).

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The relationship between liver steatosis and early atherosclerosis markers such as carotid intima-media thickness (C-IMT) and coronary artery calcifications (CAC) has been investigated and is now supported by robust cross-sectional studies.[1,2] However, because of a significant overlap in clinical risk factors and pathogenic pathways (i.e., modified glucose homeostasis, abdominal obesity, insulin resistance, atherogenic dyslipidemia, and low-grade chronic inflammation),[3] it is difficult to determine whether liver fat independently contributes to atherosclerotic cardiovascular disease or the association is just a reflection of shared comorbidities. Few longitudinal studies have confirmed that patients with steatosis are more likely to develop CAC[4] or incident carotid plaques[5,6] over time, suggesting that steatosis is more than a marker

Abbreviations: ATS, atherosclerosis; AUROC, area under the receiver-operating characteristic curve; BMI, body mass index; CAC, coronary artery calcification; CI, confidence interval; C-IMT, carotid intima-media thickness; CP, carotid plaque; CT, computed tomography; CV, cardiovascular; CVRF, cardiovascular risk factor; FLI, fatty liver index; FP, femoral plaque; FRS, Framingham Score; GGT, gamma-glutamyltransferase; IMT, intima-media thickness; MESA, Multi-ethnic Study of Atherosclerosis; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; and ROC, receiver-operating characteristic.

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of early atherosclerosis; conversely, cardiovascular risk factors (CVRFs) predict new onset fatty liver, suggesting a bidirectional relationship between liver fat and cardiovascular disease.[7]

Early detection of subclinical atherosclerosis is clinically relevant as it has predictive value for future cardiovascular events[8,9] and appears to improve cardiovascular risk prediction beyond classical risk factors.[10] More recent data suggest a quantitative relationship between early atherosclerosis and the risk of future cardiovascular events, depending not only on the localization but also on the number of the atherosclerotic sites involved.[11] Whether early atherosclerosis contributes to cardiovascular risk prediction in patients with nonalcoholic fatty liver disease (NAFLD) would be of particular interest because of a high prevalence and incidence of cardiovascular events in these patients.[12]

Previous data from longitudinal series[6] have shown that steatosis, assessed by the fatty liver index (FLI) score, predates the occurrence of early carotid atherosclerosis and its progression. To further reinforce the hypothesis of an independent contribution of steatosis to early atherosclerosis, we aimed to determine (1) the strength of the relationship between steatosis and specific atherosclerotic sites among carotid, femoral and coronary atherosclerosis; (2) whether a quantitative relationship exists with a higher probability of diffuse, multiple-site atherosclerosis in patients with steatosis; and (3) whether combining steatosis with early atherosclerosis will better predict cardiovascular risk than traditional CVRFs alone.

Materials and Methods

STUDY POPULATION

This is a retrospective, secondary analysis of a prospective cohort of consecutive patients between the ages of 18 and 75 years, seen in the Primary Cardiovascular Prevention Center at the Pitié-Salpêtrière Hospital from January 2010 to June 2016. This cohort was initially designed to focus on cardiovascular risk evaluation and prevention and on atherosclerosis imaging.[6,13-16] Therefore, included patients had to have at least one CVRF (e.g., age > 60 years in women and > 50 years in men; type 2 diabetes; dyslipidemia; high blood pressure; tobacco consumption) but no previous history of clinical cardiovascular events (e.g., myocardial infarction, coronary by-pass surgery or coronary angioplasty, stroke). For the purpose of this study we also excluded patients with excessive alcohol consumption (> 50 g/day in both men and women), any other identified cause of chronic liver disease including hepatitis B or C, and positive test for human immunodeficiency virus. All patients underwent a three-site evaluation of subclinical atherosclerosis lesions: carotid plaques (CP), femoral plaques (FP), and coronary calcium score. This was an observational, noninterventional study and all of the data were anonymously collected; the cohort was approved by the French National Commission for Data Protection and Liberties.

CLINICAL AND BIOLOGICAL EVALUATION

Clinical data were recorded for each patient the day of the medical visit: age, sex, past medical history, concomitant treatment, smoking status, and alcohol consumption; systolic and diastolic blood pressure and the anthropometric parameters were measured the same day. Blood samples were collected after overnight fasting. Type 2 diabetes was defined as fasting plasma glucose ≥ 5.6 mmol/L or self-reported treatment with hypoglycemic agents; high blood pressure was defined as systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmg Hg or treatment of previously diagnosed

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hypertension; dyslipidemia was defined as low-density lipoprotein ≥ 4.14 mmol/L or specific treatment for lipid disorders or high-density lipoprotein < 1.03 mmol/L in males or < 1.29 mmol/L in females. Current smoking was defined as smoking in the present or having smoked in the last year. Alcohol consumption was recorded based on self-reported frequency and amount of daily consumption: never, occasionally, regular—less than 50 g/day, and regular—more than 50 g/day.

Steatosis was diagnosed using the FLI, a well-validated surrogate of liver steatosis, when ≥ 60.[17] The formula for FLI was as follows: FLI = (exp[0.953 * $\log_e$ (triglycerides) + 0.139 * BMI + 0.718 * $\log_e$ (GGT + 0.053 * waist circumference - 15.745]) / (1 + exp[0.953 * $\log_e$ (triglycerides) + 0.139 * BMI + 0.718 * $\log_e$ (GGT) + 0.053 * waist circumference - 15.745]) * 100, where BMI is body mass index and GGT is gamma-glutamyltransferase.

The 10-year Framingham Score (FRS) was calculated using gender-specific score sheets[18] and was classified as low (< 10%), intermediate (10%-20%), and high (≥ 20%).

SUBCLINICAL ATHEROSCLEROSIS IMAGING

Both carotid and femoral ultrasound were performed using high-resolution B mode ultrasound technique (ACUSON Sequoia 512, Siemens Healthineers, Erlangen, Germany) according to previously described methods.[6] The carotid artery territory was examined for carotid plaques and C-IMT at the terminal portion of the common carotid, the bulb, and the proximal segment of internal and external carotid artery. The common femoral artery proximal to the bifurcation of the deep femoral artery was considered for the assessment of femoral plaques. Plaques were defined as focalized structures protruding 1.5 mm or more into the vessel lumen. All measurements were done by two trained physicians with an interobserver coefficient of variation for C-IMT of less than 3%.[19]

CAC was measured using a multidetector computed tomography (CT) scan (syngo.CT CaScoring, Siemens) and quantified by the previously described Agatston method.[20] A single radiologist performed the central reading of all CT scans. Significant CAC was defined by a score of more than 100 Agatston units.

Multiple-site atherosclerosis was defined by the presence of atherosclerotic lesions in more than one of the examined territories among carotid, femoral, or coronary artery.

STATISTICAL METHODS

All quantitative data were expressed as mean ± SD and compared using either Student *t* test or analysis of variance with Bonferroni correction for multiple comparisons. Qualitative data were expressed as a percentage and compared using the X^2 test. Linear or binary logistic regression models were used to determine the predictive value of liver steatosis for the presence of subclinical atherosclerosis at each individual site among carotid, coronary and femoral atherosclerosis, or for multiple-site (≥ 2) atherosclerosis. All multivariable models were adjusted for age as a continuous variable and included individual CVRFs that were significantly associated with individual or multiple-site atherosclerosis in univariate analysis. To avoid colinearity, variables included in the FLI calculation formula were not considered separately for multivariable models. The association of the presence of carotid, coronary, and femoral atherosclerosis with intermediate/high FRS was studied with logistic regression models, adjusted for age (as a linear variable) and traditional CV risk factors, to estimate potential increases of the area under the receiver-operating characteristic (ROC) curve (AUROC) that may result from adding one, two, or three atherosclerotic measurements and steatosis to the logistic prediction model. Then, the ROC curves were compared using the online DeLong test calculator (https://vassarstats.net/roc_comp.html). Of note, variables included in the FLI index are not part of the FRS.

We further calculated the Youden index (value with highest sensitivity and specificity) to determine the proportion of correctly classified patients with regard to their FRS, before and after introducing an estimate of steatosis by the FLI in both models, one with CVRFs and the other with individual atherosclerosis sites. This provides a measure of reclassification due to any additional input form the FLI in the respective model.

All statistical tests were two-sided and the significance level was set at $P < 0.05$. For multiple comparisons using Bonferroni correction, the significance level was set at $P < 0.01$. Statistical analyses were performed using SPPS 21 MacOS statistical software (IBM Corp., Chicago, IL).

Results

STUDY POPULATION

Among the 2,617 patients meeting the inclusion and exclusion criteria, 2,554 had concomitant

evaluation of CP and C-IMT, FP and CAC, and were included in this study. Seventy-eight percent of these patients had two or more CVRFs and 20% had a family history of cardiovascular disease. Most of the patients (85%) had dyslipidemia. Only a minority of patients (4%) had regular alcohol consumption between 40 and 50 g/day. Other characteristics of the study population are provided in Table 1.

RELATIONSHIP BETWEEN STEATOSIS AND SINGLE-SITE ATHEROSCLEROSIS (CAROTID, CORONARY, AND FEMORAL ATHEROSCLEROSIS)

Thirty-six percent (n = 930) of patients had steatosis. General characteristics of patients with and without steatosis are shown in Table 1.

TABLE 1. Patient Characteristics According to the Presence of Hepatic Steatosis

	All (n = 2,554)	With Steatosis* (n = 930)	Without Steatosis† (n = 1,624)	*P*
Age, years (mean ± SD)	56 ± 11	57 ± 10	56 ± 12	0.59
Sex, male (%)	49	63	42	< 0.001
BMI, kg/m^2 (mean ± SD)	26 ± 4.5	30.6 ± 4.5	24 ± 3.1	< 0.001
Waist, cm (mean ± SD)	92 ± 14	104 ± 11	85 ± 9	< 0.001
SBP, mmHg (mean ± SD)	124 ±15	127 ± 14	121 ± 15	< 0.001
DBP, mmHg (mean ± SD)	71 ± 10	73 ± 10	70 ± 10	< 0.001
Fasting glucose, mmol/L (mean ± SD)	5.4 ± 1.2	5.8 ± 1.4	5.1 ± 0.90	< 0.001
HbA1c, % (mean ± SD)	5.9 ± 0.8	6.1 ± 0.88	5.8 ± 0.62	< 0.001
CT, mmol/L (mean ± SD)	5.9 ± 1.4	5.7 ± 1.5	5.9 ± 1.4	0.004
LDL, mmol/L (mean ± SD)	3.7 ± 1.4	3.5 ± 1.5	3.8 ± 1.2	< 0.001
HDL, mmol/L (mean ± SD)	1.38 ± 0.6	1.12 ± 0.7	1.5 ± 0.5	< 0.001
TG, mmol/L (mean ± SD)	1.7 ± 1.6	2.6 ± 2.3	1.2 ± 0.7	< 0.001
AST, IU/L (mean ± SD)	29 ± 10	31 ± 12	27 ± 7	< 0.001
ALT, IU/L (mean ± SD)	29 ± 20	38 ± 27	25 ± 12	< 0.001
GGT, IU/L (mean ± SD)	41 ± 54	64 ± 80	28 ± 22	< 0.001
CRP us, mg/L (mean ± SD)	2 ± 2.6	2.8 ± 2.7	1.6 ± 2.5	< 0.001
Lipid-lowering therapy (%)	51	53	50	0.139
Antihypertensive therapy (%)	37	49	29	< 0.001
Antidiabetic therapy (%)	12	21	8	< 0.001
CVRFs:				
Smoking status (%)				
Never/Former/Active	57/26/17	50/31/19	61/23/16	< 0.001
Dyslipidemia (%)	85	91	82	< 0.001
Type 2 diabetes (%)	14	24	9	< 0.001
Hypertension (%)	39	52	32	< 0.001
No CVRFs (%)				
(1/2/3/4/5)	22/38/27/11/2	12/34/34/17/3	27/40/24/8/1	< 0.001
Atherosclerosis				
C-IMT, mm (mean ± SD)	0.65 ± 0.14	0.66 ± 0.14	0.64 ± 0.14	< 0.001
CP (%)	47	50	45	0.004
FP (%)	51	53	50	0.099
CAC, Agatston units (mean ± SD)	141 ± 346	183 ± 425	117 ± 288	< 0.001
≥ 2-site atherosclerosis (%)	53	57	51	0.004
FRS, % (mean ± SD)	10 ± 8	14 ± 8	8 ± 6	< 0.001

*FLI ≥ 60.
†FLI < 60.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; C-IMT, carotid-intimla media thickness; CAC, coronary artery calcium score; CP, coronary plaque; CRP, C-reactive protein; CT, cholesterol; CVRF, cardiovascular risk factor; DBP, diastolic blood pressure; FP, femoral plaque; FRS, Framigham risk score; GGT, gamma-glutamyl transferase; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAD, pulmonary artery diastolic; SBP, systolic blood pressure; TG, triglycerides.

Carotid and Coronary Atherosclerosis

Patients with steatosis had significantly higher C-IMT (0.66 ± 0.14 versus 0.64 ± 0.14, $P < 0.001$) and CAC (183 ± 425 versus 117 ± 288, $P < 0.001$) than those without steatosis. The prevalence of carotid plaques was 50% in patients with steatosis versus 45% in those without ($P = 0.004$). Sixty-four percent of patients with steatosis versus 59% of patients without steatosis had a positive CAC score (CAC > 0; $P = 0.03$) and 31% versus 24%, respectively, had a severe CAC score (>100; $P < 0.001$). CAC gradually increased across FLI tertiles (98 ± 273, 140 ± 300, and 184 ± 434; $P < 0.001$). FLI predicted C-IMT and CAC independent of age, type 2 diabetes, high blood pressure, and tobacco use (beta = 0.060, $P = 0.001$; and beta = 0.083, $P < 0.001$, respectively) (Table 2). In the same model, among individual components of FLI, only waist circumference was associated with both C-IMT and CAC (beta = 0.105, $P = 0.001$; and beta = 0.206, $P < 0.001$, respectively) (Supporting Information Table S1). Steatosis was associated with CAC independent of the presence of CP and FP (Supporting Information Table S1, Model 2). Adjusting for age, steatosis and the number of traditional CVRFs were independently associated with C-IMT (beta = 0.063, $P = 0.001$; and beta = 0.119, $P < 0.001$) and CAC (beta = 0.054, $P = 0.006$; and beta = 0.153, $P < 0.001$).

TABLE 2. Independent Predictors of C-IMT and CAC

	C-IMT		CAC	
	Beta	*P*	Beta	*P*
Age	0.445	< 0.001	0.226	< 0.001
Type 2 diabetes	0.034	0.066	0.039	0.053
High blood pressure	0.105	< 0.001	0.048	0.023
Tobacco	0.052	0.004	0.008	0.690
hsCRP	−0.018	0.329	−0.045	0.023
Steatosis*	0.065	0.001	0.091	< 0.001

*FLI as a continuous variable.
Abbreviation: hsCRP, highly sensitive quantification of C-reactive protein.

Femoral Atherosclerosis

The prevalence of femoral plaques was not different in patients with and without steatosis (53% versus 50%, $P = 0.099$). Steatosis correlated with the presence of femoral plaques in univariate analysis (beta = 0.060, $P = 0.003$). However, in multivariate models, steatosis was no longer an independent predictor of femoral atherosclerosis. Instead, age, male sex, active tobacco consumption, and the number of classical CVRFs were independent predictors for the presence of femoral plaques (Table 3). In the same model, among the individual components of FLI, GGT positively correlated with FP, whereas BMI showed a negative correlation (Supporting Information Table S2). Almost two-thirds of patients with FP had concomitant coronary atherosclerosis and more than one-third of them had a CAC score greater than 100 Agatston units.

RELATIONSHIP BETWEEN STEATOSIS AND MULTIPLE-SITE ATHEROSCLEROSIS

At least 1 atherosclerotic lesion was present in 78% of patients: 1 site in 25%, 2 sites in 25%, and 3 sites in 28%. The proportion of patients with steatosis

TABLE 3. Independent Predictors of Femoral Plaques

	Femoral Plaques			
	Model 1		Model 2	
	OR	*P*	OR	*P*
Age	1.081 (1.07-1.09)	< 0.001	1.058 (1.04-1.06)	< 0.001
Male sex	2.107 (1.754-2.53)	< 0.001	1.972 (1.64-2.36)	< 0.001
Type 2 diabetes	0.904 (0.70-1.16)	0.437	-	-
High blood pressure	1.109 (0.92-1.33)	0.279	-	-
Current smoking	2.737 (2.15-3.47)	< 0.001	-	-
Steatosis*	1 (0.99-1.003)	0.856	0.994 (0.99-1.00)	0.054
Number of CVRFs†	-	-	1.420 (1.28-1.56)	< 0.001

*FLI as a continuous variable.
†CVRFs tested were age, type 2 diabetes, high blood pressure, dyslipidemia, and current smoking.

TABLE 4. Independent Predictors of ≥ 2-Site Atherosclerosis

	Model 1		Model 2		Model 3	
	OR	*P*	OR	*P*	OR	*P*
Age	1.092 (1.08-1.10)	< 0.001	1.09 (1.08-1.10)	< 0.001	1.08 (1.07-1.09)	< 0.001
Tobacco	1.737 (1.37-2.20)	< 0.001	1.75 (1.37-2.21)	< 0.001	-	-
Type 2 diabetes	0.976 (0.75-1.26)	0.850	1.06 (0.82-1.37)	0.619	-	-
High blood pressure	1.346 (1.11-1.62)	0.002	-	-	-	-
Dyslipidemia	-	-	2.11 (1.65-2.69)	< 0.001	-	-
Clustering of CVRFs (≥ 4)	-	-	-	-	2.38 (1.77-3.20)	< 0.001
Presence of steatosis*	1.240 (1.03-1.49)	0.023	1.22 (1.01-1.46)	0.034	1.21 (1.01-1.45)	0.035

*FLI ≥ 60.

gradually increased with the number of atherosclerosis sites involved: 34% for 1-site, 38% for 2-site, and 40% for 3-site atherosclerosis (*P* = 0.025). Conversely, the proportion of patients with multiple-site atherosclerosis increased across FLI tertiles (46%, 55% and 56%; *P* < 0.001). In univariate analysis, the age-adjusted odds ratio (OR) of steatosis for multiple-site atherosclerosis was 1.32, 95% confidence interval (CI) 1.11-1.58. Among traditional CVRFs, dyslipidemia, tobacco, and clustering of CVRFs (≥ 4 CVRFs) had the greatest age-adjusted OR for multiple-site atherosclerosis: OR = 2.15 (1.69-2.74), OR = 1.73 (1.36-2.19) and OR = 2.50 (1.86-3.35), respectively. In multivariate analysis, steatosis predicted multiple-site atherosclerosis independent of age, individual, or clustering of CVRFs (Table 4).

RELATIONSHIP AMONG STEATOSIS, ATHEROSCLEROSIS SITES, AND 10-YEAR FRS

Forty percent of patients had 3 or more CVRFs. The proportion of patients with multiple CVRFs gradually increased across FLI tertiles: 25%, 42% and 54%; *P* < 0.001. Forty-one percent of patients (n = 1040) had an intermediate/high FRS (≥10%); among them, 58% had steatosis. Patients with steatosis had significantly higher FRS then those without (14% ± 8% versus 8% ± 6%; *P* < 0.001); FRS gradually increased across FLI tertiles (6% ± 5%, 10% ± 6%, and 14% ± 8%; *P* < 0.001). After adjusting for age and sex, steatosis was associated with FRS beyond the traditional CVRFs (beta = 0.320, *P* < 0.001) or individual atherosclerosis sites among CP, FP, and CAC (beta = 0.048, *P* = 0.001; beta = 0.062, *P* < 0.001; and beta = 0.032, *P* = 0.024) (Supporting Information Table S3).

TABLE 5. AUCs of CVRFs, Combined Atherosclerosis Sites, and Steatosis (FLI) for Intermediate/High Framingham Score

	AUC	95% CI	*P**
CVRFs†	0.768	0.750-0.786	Ref
FLI	0.761	0.742-0.780	0.616
CP + FP + CAC	0.686	0.666-0.707	< 0.001
Model 1: FLI + CP	0.777	0.758-0.795	< 0.562
Model 2: FLI + FP	0.788	0.771-0.806	< 0.124
Model 3: FLI + CAC	0.786	0.768-0.804	< 0.189
Model 4: FLI + CP + FP + CAC	0.803	0.786-0.820	0.009
Model 5: FLI + CVRF	0.848	0.833-0.862	< 0.001

*Versus CVRF (Ref).
†CVRFs tested were age, type 2 diabetes, high blood pressure, dyslipidemia, and current smoking.

The AUROC for the prediction of FRS ≥ 10%, considering a model that included age, dyslipidemia, type 2 diabetes, high blood pressure and current smoking, increased from 0.768 (95% CI 0.750-0.786) to 0.848 (95% CI 0.833-0.862) when steatosis was added to the model (*P* < 0.001). A model adding steatosis to individual-site atherosclerosis (CP, FP, and CAC) predicted FRS ≥ 10% better than a combination of individual-site atherosclerosis (AUC 0.803 [95% CI 0.786-0.820] versus AUC 0.686 [95% CI 0.666-0.707], respectively; *P* < 0.001) (Table 5 and Fig. 1). Adding steatosis to CVRFs correctly classified 86% of patients versus 78% with CVRFs alone. Adding steatosis to individual atherosclerosis sites correctly classified 82% of patients versus 71% with individual atherosclerosis sites alone. Thus, between 8% and 11% of patients shifted FRS risk categories when their steatosis status, as measured by FLI, was known.

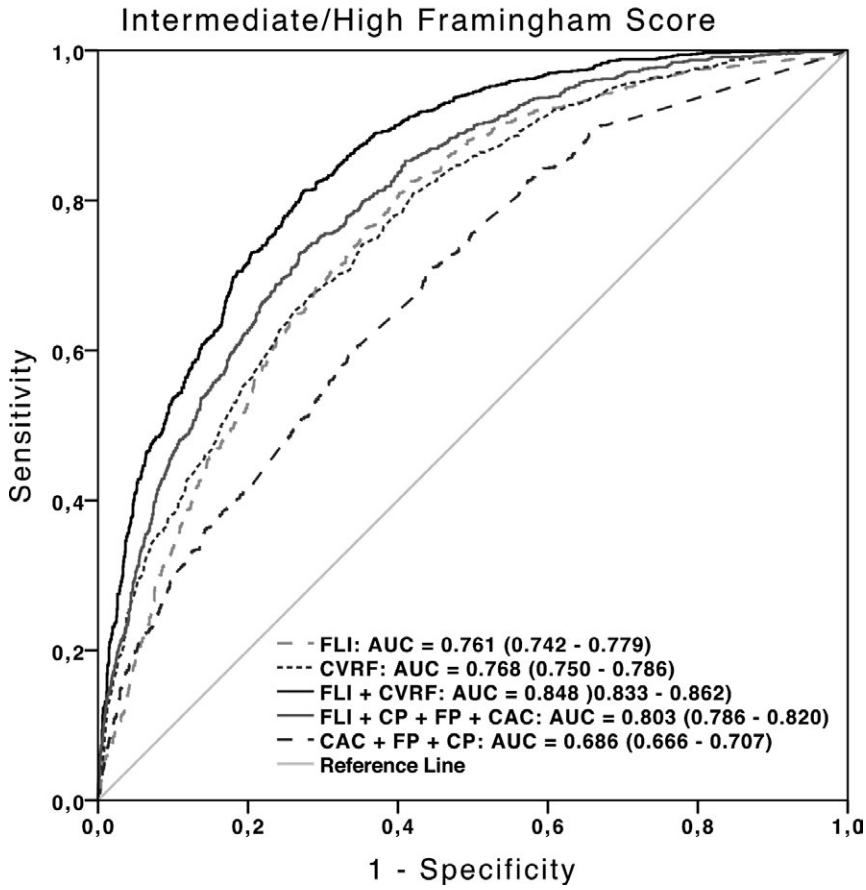

FIG. 1. Area under the receiver operating curve (AUC) for an intermediate/high Framingham Score (≥10%) as an outcome and different models including steatosis (FLI), traditional cardiovascular risk factors (CVFRF) and atherosclerotic sites (carotid and femoral plaques, CP and FP, respectively) as input variables.

Discussion

In this study we investigated the site-specific relationship between steatosis and subclinical atherosclerosis assessed in the carotid, coronary, and femoral arteries territory. Although steatosis predicted both carotid and coronary atherosclerosis beyond the traditional CVRFs, which is in agreement with previous studies,[21-23] there was no significant association between steatosis and FP. Steatosis was associated with FP only in men and this relationship disappeared after controlling for CVRFs. Instead, male sex, active tobacco consumption, and clustering of CVRFs were the strongest predictors for the presence of FP. Therefore, our results suggest that steatosis differentially affects the co-existence of early atherosclerosis at different arterial sites. However, because this is a cross-sectional, retrospective study, further insight into this relationship can only be gained by longitudinal studies testing incident early atherosclerotic lesions at different vascular sites in patients with or without baseline steatosis.

Other studies have shown that CVRFs do not have the same impact at different atherosclerosis sites. In the Pathological Determination of Atherosclerosis in Youth study, smoking selectively increased 3-fold the risk of atherosclerotic lesions of the abdominal aorta, while not influencing the risk of coronary lesions. Glycated hemoglobin was strongly related to coronary atherosclerosis but not with abdominal aorta atherosclerosis.[24] In a large cohort from the Offspring Framingham Heart Study,[25] Mellinger et al. demonstrated that steatosis was an independent predictor of coronary but not abdominal aortic calcium. It is

currently believed that many factors including genetic background, sex, immune status, oxidative stress, chronic low-grade inflammation, and blood flow parameters interact in different and complex ways to generate an atherosclerotic lesion at a particular site.[26] Understanding the determinants of atherosclerosis at different anatomical sites is of clinical relevance, as this will translate into different clinical event rates. For example, carotid atherosclerosis is responsible for 25% of strokes, with an annual risk in asymptomatic patients increasing from 1% to 3% according to the severity of stenosis. Peripheral artery atherosclerosis, although often underdiagnosed, is associated with the highest risk of cardiovascular death and cardiovascular events dues to atherothrombosis.[27] In asymptomatic subjects from the Aragon Worker's Health Study, the prediction of severe coronary atherosclerosis by traditional CVRFs was significantly improved when taking into account the presence of FP.[11]

Another finding of this study is that patients with steatosis had a higher prevalence of diffuse atherosclerotic disease, involving multiple sites among carotid, coronary, and femoral atherosclerosis. The proportion of patients with multiple-site atherosclerosis in our study gradually increased across FLI tertiles, suggesting a quantitative relationship, with the amount of steatosis playing a major role in the development of diffuse atherosclerosis. Multiple-site atherosclerosis is a major determinant of clinical events: the 1-year clinical event rate (cardiovascular death, stroke, myocardial infarction) significantly increased with the number of symptomatic arterial disease location, ranging from 2.2% in patients with one territory involved to 9.2% in patients with three territories involved.[27] More recently, the Registry Reduction in Atherothrombosis for Continued Health Registry (REACH) also highlighted that patients with multiple-site atherosclerosis have a higher rate of cardiovascular fatal and nonfatal events than patients with just one territory affected.[28] Interestingly, retrospective long-term follow-up studies with repeat cardiovascular screening for early atherosclerosis (either carotid or coronary)[4,6] suggest that steatosis predates the occurrence of early atherosclerosis. If this is further confirmed by prospective studies and if a quantitative relationship does indeed exist, then it would be important to determine whether steatosis reversal early in the disease process can prevent the occurrence of early atherosclerosis.

Finally, we have shown that steatosis is associated with cardiovascular risk beyond the traditional CVRFs. In fact, adding steatosis to traditional CVRFs significantly improves cardiovascular risk prediction particularly for patients with high FRS. This was not the case in the Multi-ethnic Study of Atherosclerosis (MESA). In that study, patients with NAFLD had a higher risk of nonfatal cardiovascular events and overall mortality; however, adding NAFLD to traditional CVRFs did not improve the cardiovascular risk prediction when adjusted for age, sex, ethnicity, and traditional CVRFs.[29] These discordant results may arise from differences in the study populations. In the current study, patients were at least 6 years younger than in the MESA study, had at least 1 CVRF with a high proportion of patients with dyslipidemia, and had a higher mean FRS. The different methods used for the diagnosis of steatosis (CT scan in the MESA study, FLI in our study) as well as the different design of the two studies may also account for the discordant results.

Additionally, we have shown that adding steatosis to multiple-site atherosclerosis predicted FRS better than classical CVRFs. This strengthens observations from the global REACH Registry that patients without established atherothrombosis but with risk factors only had a lower risk of cardiovascular events or death when compared with patients with established atherothrombosis with or without prior clinical cardiovascular events.[30] The mechanisms whereby steatosis increases cardiovascular risk and cardiovascular mortality by itself (i.e., in addition to traditional CVRFs) is under investigation. Chronic low-grade inflammation and insulin resistance not only play a pivotal role in the occurrence, progression, and complications of atherosclerosis plaques, but also in the progression of liver damage, and is therefore a shared pathophysiological link between NAFLD and atherosclerosis. Inflammatory gene expression in adipose tissue strongly correlates with the progression of liver damage.[31] The concomitant presence of NAFLD and systemic inflammation (as assessed by highly sensitive quantification of C-reactive protein) increased the risk of CAC development over 4 years among 1,500 healthy Korean patients.[32] Activation of hepatic nuclear factor kappa B and c-Jun kinase pathways was responsible for an increased production of pro-inflammatory cytokines, which further aggravated insulin resistance and favored the progression of liver disease

in addition to promoting accelerated atherosclerosis. Interestingly, despite similar C-IMT, patients with steatosis appeared to have a higher inflammation of the arterial wall compared with patients without steatosis.[33] This is of interest because the composition and inflammation of atherosclerosis plaques are better correlated with the risk of future cardiovascular events than the degree of stenosis itself.[34]

The strength of the current study is the large sample size, with more than 2,500 patients included and multiple-site atherosclerosis evaluation. A limitation of this study is the use of FLI as a surrogate marker for hepatic steatosis instead of the assessment of steatosis by imaging or histology. This is important because at least three components of the FLI were shown to be associated with cardiovascular disease (BMI, waist circumference, and serum triglycerides), and as such, these variables could account for the associations seen in this report. Moreover, despite the numerical range of the FLI index, its ability to quantify is certainly inferior to that of imaging methods that measure directly the hepatic fat signal, such as MRI-based proton density fat fraction.[35] However, the FLI has been validated extensively both in the general population[17] and in tertiary care referral centers with a good accuracy for discriminating presence from absence of steatosis defined histologically or by ultrasound.[36] Moreover, there are data demonstrating that FLI predicts steatosis on liver biopsy better its components.[36] Ideally, this study should have incorporated an independent assessment of steatosis; unfortunately, this cohort was not initially designed for the study of liver outcomes. Interestingly, all serum-based biomarker panels proposed so far and used in patients seen in clinics are based on one or several biochemical variables in relation to the metabolic syndrome.[17,36-38] Therefore, these results should be interpreted with caution as they are suggestive but fall short of a definitive demonstration of the association between steatosis and early atherosclerotic lesions.

It remains to be determined whether there is clinical relevance to the incremental diagnostic gain of adding a surrogate marker of hepatic steatosis to traditional CVRFs for the diagnosis of early atherosclerotic lesions. The improvement in the AUROC reported here is not trivial given the insensitivity of the AUROC statistic to improved prediction by new markers[39,40] and the fact that a 0.85 value is in the range of accepted diagnostic methods with good discriminative value.[41] However, our results need to be tested in independent data sets. Ultimately, prospective studies should confirm an increase in predictive value, and cost-efficacy should be demonstrated before the assessment of steatosis can be used for prediction in clinical practice.[42]

In conclusion, we have shown that steatosis differently affects early atherosclerosis development depending on the arterial site involved, and found a quantitative relationship between the amount of steatosis and the severity of early atherosclerosis. Steatosis status reinforces the prediction of cardiovascular mortality over traditional CVRFs and over imaging-based measurements of atherosclerotic lesions. The detection of steatosis has important prognostic implications in patients with CVRFs. Conversely, in patients seen primarily for hepatic steatosis, a thorough cardiovascular evaluation is necessary, considering the increased cardiovascular and atherosclerotic risk conferred upon by NAFLD.

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