Femoral Plaque Echogenicity and Cardiovascular Risk in Claudicants

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OBJECTIVES The present study was designed to verify whether the evaluation of femoral plaque echogenicity might be a useful tool for cardiovascular risk assessment in patients affected by lower extremity peripheral arterial disease.

BACKGROUND Lower extremity peripheral arterial disease is a common manifestation of atherosclerosis and is associated with a high risk of developing major cardiovascular events. Vulnerable atherosclerotic plaque plays a central role in the occurrence of acute ischemic events in different vascular territories. Furthermore, atherosclerosis is a systemic disease, and the presence of an unstable atherosclerotic plaque in a certain vascular district, characterized by low echogenicity at B-mode ultrasound, is associated to a greater prevalence of unstable plaques in other vascular beds.

METHODS Femoral plaque echogenicity of 246 claudicants with ankle/brachial index ≤0.90 was evaluated at B-mode ultrasound by visual analysis and by calculating the grayscale median (GSM) value. In these patients, the occurrence of myocardial infarction and stroke was prospectively assessed.

RESULTS Femoral GSM values and plaque types assessed by visual analysis were highly correlated by Spearman analysis (rho = 0.905, $p < 0.001$). During a median follow-up of 30 months, 32 patients (13%) had a major cardiovascular event. Compared with patients without events, those who experienced an event during the follow-up had a lower femoral plaque GSM value (42.9 ± 26.2 vs. 58.8 ± 19.3, $p = 0.002$) and a higher prevalence of hypoechoic femoral plaque at visual analysis (68.8% vs. 19.6%, $p < 0.001$). At Cox analysis, femoral GSM showed an inverse relationship with cardiovascular risk, even after adjustment for possible confounders (hazard ratio: 0.96, 95% confidence interval [CI]: 0.95 to 0.98, $p < 0.001$). Furthermore, patients with hypoechoic femoral plaques at visual analysis had a 7.24-fold increased cardiovascular risk compared with patients with hyperechoic plaques after adjustment for possible confounders (95% CI: 3.23 to 16.22, $p < 0.001$).

CONCLUSIONS This study demonstrates that the presence of hypoechoic atherosclerotic femoral plaques is associated with higher cardiovascular risk in lower extremity peripheral arterial disease patients. (J Am Coll Cardiol Img 2012;5:348–57) © 2012 by the American College of Cardiology Foundation

Vulnerable atherosclerotic plaque plays a central role in the occurrence of acute ischemic events in different vascular districts (1–4). Culprit lesions responsible for acute coronary syndromes most commonly result from disruption of vulnerable plaques that are angiographically modest in severity (5–8). These rupture-prone plaques have certain characteristics, such as: a thin cap; a large lipid core composed of cholesterol, lipids, and dead foam cells; and increased inflammatory activity (1,3,4,8,9). Similarly, histopathological studies demonstrated that certain morphological features of carotid atheroma, such as a large lipid core separated from the lumen by a thin or ruptured fibrous cap and the presence of intraplaque hemorrhage, are associated with increased stroke risk (2). To date, little is known about the pathophysiological implication and the impact on clinical outcome of unstable plaques in the lower limbs of patients affected by lower extremity peripheral arterial disease (LE-PAD), a common manifestation of atherosclerosis characterized by a high risk of developing ischemic vascular events and death (10,11).

Plaque echogenicity, evaluated by B-mode ultrasound imaging, has been found to be a simple and reliable tool in predicting plaque composition. Plaques that appear hypoechoic present an inflammatory infiltration and are lipid-rich (12,13), whereas those with high echogenicity have more fibrous tissue and calcific components, which make them more stable (12,13). It is noteworthy that atherosclerosis is a systemic disease that commonly involves several vascular territories, and recent studies indicate that plaque instability may not be confined to a single vascular bed and may involve different arterial districts simultaneously (14,15). Thus, the early noninvasive visualization of "sensitive" hypoechoic spots in the arterial tree might have important clinical implications, potentially identifying a subgroup of patients exposed to a higher cardiovascular risk. Indeed, patients with unstable femoral or carotid plaques are more likely to have unstable plaques in the contralateral artery (14,15). In LE-PAD patients, the presence of hypoechoic femoral plaques is associated with a greater prevalence of hypoechoic carotid plaques (16). Furthermore, subjects with history of cerebrovascular disease and hypoechoic carotid plaques are at increased risk not only of developing an acute cerebrovascular event, but also an acute coronary syndrome (17,18). However, to date, little is known about the prognostic implications of a hypoechoic plaque in the lower limbs on cardiovascular events. This is particularly unfortunate because LE-PAD has a widespread impact on health beyond the lower extremity circulation (10,11). Accordingly, we conducted a prospective study aimed at evaluating the prognostic impact of femoral plaque echogenicity on the incidence of myocardial infarction and stroke in a homogeneous cohort of LE-PAD patients.

METHODS

Patients. A total of 410 consecutive subjects who were referred to our vascular laboratory for suspected intermittent claudication were screened for enrollment in the study. LE-PAD was diagnosed on the basis of an ankle-brachial index (ABI) ≤0.90 associated with 1 or more stenoses of lower extremity arteries at B-mode ultrasound. According to these criteria, 43 subjects were classified as not having LE-PAD. In addition, 14 claudicants with ABI >1.4 were excluded because these values may be falsely elevated due to severe arterial calcification, and 11 patients were excluded because they were affected by critical limb ischemia. Thus, 342 patients affected by intermittent claudication were initially selected. Of these, 24 were excluded for 1 of the following exclusion criteria: acute coronary syndromes, cerebrovascular events, or revascularization procedures during the previous 6 months; abnormal myocardial ischemia stress test at enrollment; significant renal, hepatic, or inflammatory disease; decompensated heart failure; or malignant neoplasia. Therefore, 318 patients affected by LE-PAD at stage II of Fontaine classification were potentially suitable to be enrolled in the study. To be included, claudicants should have at least an atherosclerotic plaque in 1 or both femoral arterial districts within 4 cm proximal and 1 cm distal to the femoral bifurcation. Thus, 72 patients were excluded for 1 of the following reasons: absence of femoral plaque (n = 3); presence of plaque in femoral arteries <1.3 mm because such plaque could not be clearly separated from diffused thickened intima-media complex (n = 4) (19); presence of calcified femoral plaque with an acoustic shadow because it was technically not reliable to determine its echogenicity (n = 34); previous femoral revascularization procedure (n = 20), under the assumption that the revascularized artery might be that at higher risk; occlusion of femoral arteries in the tract

examined (n = 11). Therefore, the study was finally conducted in 246 LE-PAD patients affected by intermittent claudication. All the women were post-menopausal, and none was receiving hormone replacement therapy. Participants gave written informed consent to the study, which was approved by our institutional ethics committee.

In each patient, clinical history and risk factors were assessed. Smokers included current and former smokers. Hypertension was diagnosed if systolic arterial pressure exceeded 140 mm Hg and/or diastolic arterial pressure exceeded 90 mm Hg on repeated measurements, or if the patient used anti-hypertensive drugs because of a history of arterial hypertension. Hypercholesterolemia was diagnosed if fasting plasma low-density lipoprotein cholesterol exceeded 130 mg/dl, or if the patient used lipid-lowering drugs because of a history of hypercholesterolemia (20). Diabetes mellitus was diagnosed if plasma fasting glucose exceeded 126 mg/dl or if the patient used hypoglycemic agents. Body mass index was calculated as body mass (kg) divided by height (m) squared.

All patients were treated according to the most recent guidelines (11), and response to therapy (including side effects) was evaluated at regular clinical evaluations during follow-up and, when necessary, therapeutic adjustments were performed.

ABI was measured after participants rested supine for 5 min. The systolic blood pressure in both brachial arteries and the ankle systolic blood pressure in the right and left posterior tibial and dorsalis pedis arteries were measured using a Doppler probe. The ABI for each leg was then determined using the higher of the 2 readings from either the posterior tibial or dorsalis pedis arteries and the higher of the 2 brachial readings. The lower ABI of the 2 legs was used for diagnostic purposes.

Definition of femoral plaque type by visual analysis. All patients underwent ultrasound examinations of the lower limbs with an ImagePoint Hx Ultrasound System (Hewlett-Packard, Andover, Massachusetts) with a 7.5-MHz linear array transducer. Several variables on the scanner were standardized to optimize image quality and to avoid variability in B-mode image acquisition between subjects. The aim was to allow acquisition of maximal data with the least image modification by the scanner or operator. Briefly, dynamic range was set at 55 dB, persistence at 2, gain at 55, and depth gain compensation was maintained as flat. B-mode-images of femoral arteries were kept in longitudinal and transverse sections in order to obtain full visualiza-

tion of the femoral district and extent of the lesions. The best longitudinal section with full display of the plaque and adventitia was stored and used for visual and computer-assisted analysis. In case both femoral districts presented an atherosclerotic plaque or there was more than a plaque in the same femoral district, only the 1 with lower echogenicity was considered for the analyses. Femoral plaques were classified according to a modified version of the classification proposed by Gray-Weale et al. (21,22) and graded as: type 1 = echolucent; type 2 = predominantly echolucent; type 3 = predominantly echogenic; or type 4 = echogenic. The study population was then categorized into 2 groups according to the femoral plaque echogenicity. Patients with types 1 or 2 plaque were considered to have hypoechoic plaque, whereas patients with types 3 or 4 plaque were considered to have hyperechoic plaque. A single well-trained investigator (V.S.), unaware of the patient's clinical status, performed all the ultrasound examinations, stored the best longitudinal images of the femoral plaque, and evaluated plaque echogenicity at visual analysis.

Femoral grayscale median analysis. The grayscale median (GSM) analysis was performed by a single investigator (G.S.), unaware of the visual analysis, who analyzed echogenicity in all the plaque images with the Image J software (National Institutes of Health, Bethesda, Maryland). GSM of the frequency distribution of gray values of the pixels within the plaque served as a measure of echogenicity. Standardization was obtained as previously reported (23). Subsequently, the plaque was outlined and its overall brightness evaluated by means of GSM grayscale range of 0 (black) to 255 (white). The GSM value of the plaque was adjusted linearly so that the median value of blood was 0 and that of adventitia was 190.

Measurement of femoral plaque thickness and stenosis. The maximum thickness and the percentage stenosis of the femoral plaque considered for visual and GSM analyses were measured from a longitudinal view of the femoral district by the investigator who performed the visual analysis (V.S.). Femoral plaque thickness was considered as the maximum distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface. To evaluate the femoral percentage stenosis, the reference (normal) lumen diameter of the artery and the residual lumen diameter at the point of maximum stenosis were obtained using the cursors on the ultrasound image. The percentage stenosis was obtained comparing

the residual with the reference lumen diameter. In obtaining these measurements, the operator took care to optimize the view of the artery and the stenosis and used the outline of the color area and the background B-mode image to determine where best to place the cursors for diameter measurements. Figure 1 reports representative ultrasound images of 2 femoral atherosclerotic plaques obtained in our population.

Assessment of carotid intima-media thickness. Carotid ultrasound examination was performed at baseline in all patients enrolled in the study using the ultrasound system previously described. According to the Mannheim Consensus (24), carotid intima-media thickness (IMT) was evaluated in a 10-mm long segment just proximal to the carotid bulb using scans of the common carotid artery. IMT was measured as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall (24). Maximum carotid IMT, defined as the greatest axial thickness obtained in both carotid arteries, was considered for each patient.

Assessment of cardiovascular events. Patients were contacted for follow-up at 3-month intervals. The occurrence of fatal and nonfatal acute myocardial infarction and ischemic stroke was prospectively assessed. The minimum follow-up period was 6 months. Medical records and death certificates of all patients who had an event were obtained and validated by a physician unaware of the echogenicity of femoral plaques (G.G.). For patients who had more than 1 event, only the first event was considered for the analysis.

Statistical analysis. Statistical analyses were performed using SPSS version 12.0 (SPSS, Inc., Chicago, Illinois) and Stata version 10.0 (Stata Corp., College Station, Texas). Variables were expressed as absolute number and percentage, mean $\pm$ SD, or median (interquartile range [IQR]). Comparisons were made by chi-square test, *t* test for unpaired samples, or Mann-Whitney *U* test, as appropriate.

To assess the intraoperator reproducibility, 40 plaques were re-evaluated by the same operator blinded to the previous results. Intraoperator agreement was assessed by kappa statistic for visual analysis and by intraclass correlation coefficient (ICC) with 95% confidence interval (CI) for GSM analysis. The ICC chosen was of single measures and absolute agreement with random effect. The agreement between 2 repeated evaluations of the plaques was excellent for both visual (kappa = 0.85) and GSM analyses (ICC = 0.91, 95% CI: 0.84 to 0.95).

The relationship between plaque type evaluated by visual analysis and GSM value was assessed by
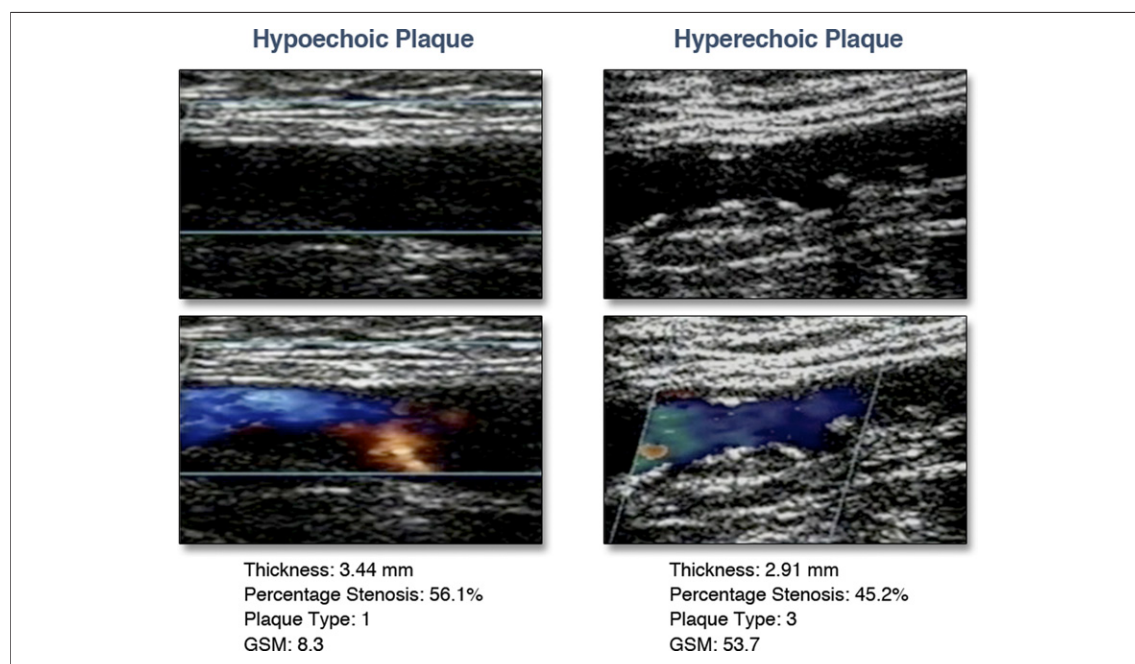

Figure 1. Femoral Plaques

Representative ultrasound images of 2 atherosclerotic femoral plaques obtained in the study population with their respective characteristics. GSM = grayscale median.

Spearman analysis. Cox proportional hazard analyses were performed to verify if femoral GSM (continuous variable) and the presence of hypoechoic femoral plaques at visual analysis (categorical variable) were associated with the occurrence of future myocardial infarction or stroke. The following covariates, potential contributors of cardiovascular risk in LE-PAD patients, were included in the adjusted model: age; sex; previous myocardial infarction or stroke; ABI; maximum carotid IMT; and femoral plaque thickness.

To identify the GSM threshold level that provided the best cutoff for outcome prediction, we chose the value in which the sum of specificity and sensitivity was the highest. This value was obtained by receiver-operating characteristic (ROC) curve analysis (25). To test the robustness of the results, we used the bootstrap approach (26). After randomly sampling the study population, drawn with a replacement 200 times, the mean and the 95% CI of the 200 bootstrapped cutoff estimates were calculated. This mean value was used as the best cutoff. Cumulative event rates in patients with GSM ≤ cutoff obtained by bootstrap approach versus patients with higher GSM values were estimated by Kaplan-Meier curves and probability values by log-rank test. Similarly, cumulative event rates were calculated in patients with hypoechoic versus hyperechoic femoral plaques at visual analysis.

The C statistic was used to assess the ability to classify risk (27). Comparison between C statistics was made by the pairwise comparison of ROC curves.

All statistical tests were 2-sided. For all tests, a p value <0.05 was considered statistically significant.

RESULTS

Patient characteristics. Table 1 shows the baseline characteristics of the study population. Importantly, the LE-PAD population enrolled in this study exhibited a high prevalence of classic cardiovascular risk factors and a high cardiovascular comorbidity.

Table 2 reports baseline characteristics of the study population according to the presence of hypoechoic or hyperechoic femoral plaques at visual analysis. Notably, patients with hypoechoic plaques (n = 64) showed a higher prevalence of previous myocardial infarction and tended to be younger than subjects with hyperechoic plaques (n = 182). Conversely, no differences between the 2 groups were observed with respect to ABI, prevalence of other classic cardiovascular risk factors, cardiovas-

cular medications, maximum carotid IMT, and femoral plaque severity.

Table 3 shows the use of cardiovascular medications after 6 months of follow-up in all the study population and after categorization in those with hypoechoic versus hyperechoic femoral plaques. Importantly, no difference between the groups could be observed at this time point.

Femoral plaque characteristics and outcome. During a median follow-up of 30.0 (IQR: 13.0 to 41.0) months, 32 of the 246 patients (13%) had an event:

Table 1. Baseline Characteristics of the Study Population (n = 246)

	n	Mean ± SD, % or Median (IQR)
Age, yrs		65.5 ± 9.2
Men	206	83.7%
Risk factors		
Smoking	224	91.1%
Hypertension	198	80.5%
SBP, mm Hg		137.3 ± 25.0
DBP, mm Hg		76.6 ± 14.7
Hypercholesterolemia	194	78.9%
Total cholesterol, mg/dl		193.6 ± 43.7
LDL, mg/dl		117.1 ± 40.3
HDL, mg/dl		49.9 ± 12.8
Diabetes mellitus	98	39.8%
Blood glucose, mg/dl		116.3 ± 42.7
BMI, kg/m²		26.5 ± 3.5
Comorbidity		
CAD	168	68.3%
Previous MI	118	48.0%
Previous stroke	12	4.9%
Medications		
Antiplatelets	222	90.2%
Beta-blockers	76	30.9%
ACE-inhibitors	160	65.0%
Statins	154	62.6%
Carotid IMT		
Maximum IMT, mm		1.23 ± 0.16
LE-PAD severity		
Fontaine stage IIA	136	55.3%
Fontaine stage IIB	110	44.7%
ABI		0.67 ± 0.19
Femoral plaque features		
Thickness, mm		2.91 ± 0.83
Percentage stenosis		43.5 ± 13.1
Hypoechoic plaque	64	26.0%
GSM		61.4 (42.0–74.9)

ABI = ankle-brachial index; ACE = angiotensin-converting enzyme; BMI = body mass index; CAD = coronary artery disease; DBP = diastolic blood pressure; GSM = grayscale median; HDL = high-density lipoprotein; IMT = intima-media thickness; IQR = interquartile range; LDL = low-density lipoprotein; LE-PAD = lower extremity-peripheral arterial disease; MI = myocardial infarction; SBP = systolic blood pressure.

26 had a myocardial infarction and 6 had a stroke. Compared with patients without events, those who experienced an event during the follow-up had a lower femoral plaque GSM value (42.9 ± 26.2 vs. 58.8 ± 19.3, p = 0.002), and a higher prevalence of hypoechoic femoral plaque at visual analysis (68.8% vs. 19.6%, p < 0.001).

Considered as a continuous variable, femoral GSM was inversely associated with cardiovascular risk (Table 4). This association remained statistically significant after adjustment for potential confounders (Table 4).

Spearman analysis showed that femoral GSM value and plaque type assessed by visual analysis were highly correlated (rho = 0.905, p < 0.001) (Fig. 2). Consistently, Cox analyses revealed that the presence of hypoechoic femoral plaques at visual analysis was associated with an increased risk of developing myocardial infarction or stroke, even after adjustment for possible confounding factors (Table 4).

Figure 3 displays the ROC curve for GSM in relation to the occurrence of myocardial infarction or stroke. The GSM cutoff value that provided the maximum sum of the specificity and sensitivity in predicting the outcome was 30.2. The C statistic (area under the curve) for this GSM value was 0.68 (95% CI: 0.63 to 0.73, p < 0.001). The bootstrapped cutoff value estimate for GSM was 31.4 (95% CI: 25.2 to 35.7).

Kaplan-Meier survival curves showed that the incidence of myocardial infarction or stroke during follow-up was significantly higher in patients with femoral GSM value ≤31.4 versus those with greater GSM value (Fig. 4). Similar results were observed when patients were categorized according to visual analysis (Fig. 4). Notably, at Cox analysis adjusted for potential confounders, patients with GSM ≤31.4 presented a 5.74-fold increased risk of developing a cardiovascular event compared with patients with higher GSM value (Table 4).

The C statistic for the risk prediction model incorporating age, sex, smoking, hypertension, hypercholesterolemia, diabetes, body mass index, previous myocardial infarction or stroke, ABI, carotid IMT, and femoral plaque thickness and percentage stenosis was 0.71 (95% CI: 0.65 to 0.76), similar to that of GSM alone (0.68, 95% CI: 0.63 to 0.73, p = 0.624). Notably, it significantly increased to 0.78 (95% CI: 0.72 to 0.83, p = 0.048) when the model incorporated also GSM, suggesting a significant contribution of femoral plaque echogenicity evaluation on cardiovascular risk assessment.

Table 2. Characteristics of LE-PAD Patients According to the Presence of Hypoechoic or Hyperechoic Femoral Plaque at Visual Analysis

	Hypoechoic Plaque (n = 64)	Hyperechoic Plaque (n = 182)	p Value
Age, yrs	63.6 ± 9.2	66.1 ± 9.1	0.054
Men	54 (84.4)	152 (83.5)	0.873
Risk factors			
Smoking	58 (90.6)	176 (91.2)	0.888
Hypertension	50 (78.1)	148 (81.3)	0.579
SBP, mm Hg	134.7 ± 27.9	138.2 ± 23.9	0.334
DBP, mm Hg	74.0 ± 11.1	77.5 ± 15.7	0.104
Hypercholesterolemia	52 (81.3)	142 (78.0)	0.586
Total cholesterol, mg/dl	189.8 ± 46.7	194.8 ± 42.7	0.460
LDL, mg/dl	112.2 ± 44.6	118.6 ± 39.0	0.416
HDL, mg/dl	47.6 ± 11.4	50.6 ± 13.2	0.203
Diabetes mellitus	24 (37.5)	74 (40.7)	0.657
Blood glucose, mg/dl	119.7 ± 51.8	115.0 ± 39.0	0.203
BMI, kg/m²	26.3 ± 3.7	26.5 ± 3.4	0.650
Comorbidity			
CAD	46 (71.9)	122 (67.0)	0.474
Previous MI	38 (59.4)	80 (44.0)	0.034
Previous stroke	4 (6.2)	8 (4.4)	0.554
Medications			
Antiplatelets	56 (87.5)	166 (91.2)	0.390
Beta-blockers	20 (31.3)	56 (30.8)	0.943
ACE-inhibitors	44 (68.8)	116 (63.7)	0.469
Statins	37 (57.8)	117 (64.3)	0.357
Carotid IMT			
Maximum IMT, mm	1.25 ± 0.15	1.22 ± 0.16	0.108
LE-PAD severity			
ABI	0.67 ± 0.17	0.67 ± 0.20	0.989
Femoral plaque severity			
Thickness, mm	2.98 ± 0.81	2.87 ± 0.83	0.348
Percentage stenosis	44.7 ± 14.9	43.0 ± 12.4	0.365

Values are mean ± SD or n (%).
Abbreviations as in Table 1.

DISCUSSION

Our prospective study demonstrates that LE-PAD patients with a hypoechoic plaque in the femoral arteries are exposed to a significantly higher risk of developing myocardial infarction or stroke com-

Table 3. Cardiovascular Medications at 6 Months Follow-Up

	All Patients (n = 246)	Hypoechoic Plaque (n = 64)	Hyperechoic Plaque (n = 182)	p Value*
Antiplatelets	242 (98.4)	64 (100.0)	178 (97.8)	0.232
Beta-blockers	86 (35.0)	24 (37.5)	62 (34.1)	0.620
ACE inhibitors	172 (69.9)	44 (68.8)	128 (70.3)	0.813
Statins	183 (74.4)	48 (75.0)	135 (74.2)	0.897

Values are n (%). *p value for difference between patients with hypoechoic versus hyperechoic femoral plaque.
Abbreviation as in Table 1.

Table 4. Crude and Adjusted HR of Developing MI or Stroke in LE-PAD Patients (Cox Analysis)

	Femoral GSM*			Hypoechoic Femoral Plaque			Femoral GSM ≤31.4		
	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value
Univariate	0.96	0.95–0.98	0.001	7.15	3.38–15.11	0.001	7.10	3.53–14.29	0.001
Adjusted model†	0.96	0.95–0.98	0.001	7.24	3.23–16.22	0.001	5.74	2.64–12.47	0.001

*For continuous variable, HR is shown for an increase of 1 U. †Multivariate Cox analyses adjusted for age, sex, previous MI or stroke, ABI, maximum carotid IMT, and femoral plaque thickness.
CI = confidence interval; HR = hazard ratio; other abbreviations as in Table 1.

pared with those with a hyperechoic femoral plaque. Similar findings were obtained with both computer-assisted and visual analyses of plaque echogenicity. Notably, these results remained unaltered after accounting for factors known to have important impacts on LE-PAD patients' outcome, including age, sex, previous cardiovascular events, and ABI.

Acute ischemic events are more closely related to the histopathological characteristics of atherosclerotic plaques than to the number of plaques or the degree of vascular stenosis (2,5–8,28,29). In this regard, the PROSPECT (Providing Regional Observations to Study Predictors of Events in the Coronary Tree) trial (8) has recently shown that lesions responsible for recurrent cardiac ischemic events are frequently angiographically mild, most are thin-cap fibroatheromas, or are characterized by a large plaque burden, a small luminal area, or some combination of these characteristics, as determined by grayscale and radiofrequency intravascular ultrasonography. Although histology is the gold standard to determine plaque structures, it requires invasive arterial explants. In contrast, high-resolution B-mode ultrasound is a simple, low cost, and reliable noninvasive method for the evaluation of vascular stenoses and plaque echogenicity, which is

known to be related to the histological characteristics of the atherosclerotic lesion (13,21). Indeed, plaques that appear hypoechoic on B-mode ultrasound have a pronounced inflammatory infiltration, a high lipid content, and are more prone to rupture (12,30,31), whereas hyperechoic plaques consist mainly of fibrous tissue, collagen, and calcium, which make them more stable (12,13).

Notably, plaque instability has been considered a multivessel phenomenon, and thus, vulnerable plaques may occur simultaneously in different parts of the arterial tree in the same individual. Indeed, patients with an acute coronary syndrome show the concomitant presence of multiple unstable plaques in the coronary tree and in the carotid district (32–34). Furthermore, although it is known that patients with history of cerebrovascular events and hypoechoic, probably unstable, carotid plaques are at increased risk of future ischemic events in the cerebral and coronary districts (17,18,29), little is known about the predictive role of femoral plaques echogenicity in LE-PAD.

Although LE-PAD is a common manifestation of atherosclerosis and is associated to a high incidence of major cardiovascular events (10,11), this increased risk appears to be poorly related to classic risk factors (10,35). In this regard, other parameters have been described as independent risk predictors in LE-PAD, such as the disease severity evaluated by ABI, a worse endothelial function, and a more pronounced inflammatory status (11,36–42). The present study suggests that the presence of hypoechoic femoral plaques might be considered a new marker of cardiovascular risk in LE-PAD. Indeed, the identification of a vulnerable site (hypoechoic spot) in femoral arteries might identify patients at increased risk of "remote" coronary or carotid adverse events, suggesting that the presence of an unstable plaque in a certain artery might be associated with the presence of unstable plaques in other districts. At this regard, autoptic and echographic studies show that atherosclerosis develops slowly in the femoral artery compared with coronary and carotid arteries (43,44), suggesting that the
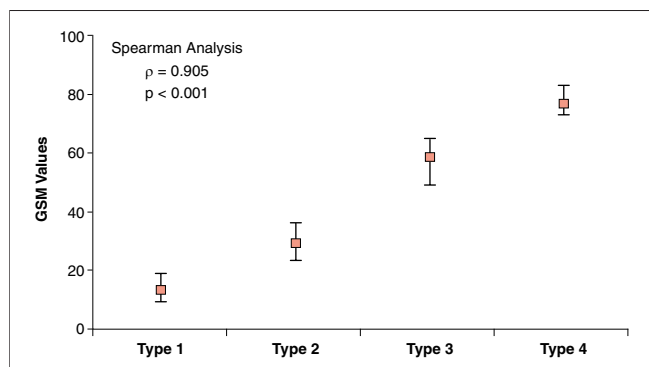
Figure 2. Correlation Between Visual and Computer-Assisted Echogenicity Analyses

Correlation between visual classification of plaque echogenicity (4 types) and GSM value (presented as median and interquartile range). Abbreviation as in Figure 1.
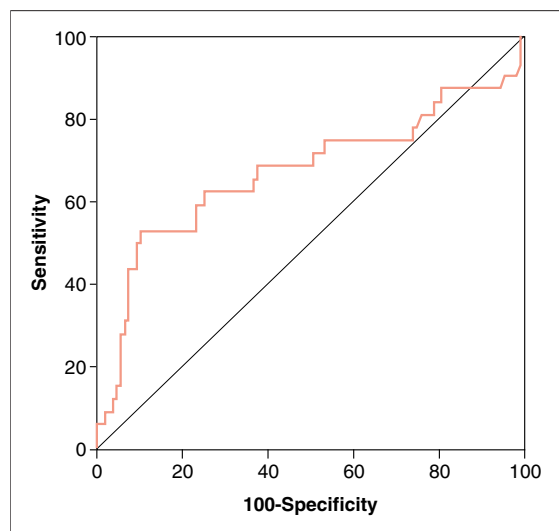
Figure 3. GSM ROC Curve

Receiver-operating characteristic (ROC) curve illustrating the sensitivity and specificity of GSM in predicting the occurrence of myocardial infarction or stroke. Abbreviation as in Figure 1.

sound during the routine diagnostic evaluation. Although the echogenicity evaluation of the major number of plaques might represent the optimal strategy to estimate arterial health, systematic evaluation of all arterial plaques of the lower limbs might be extremely difficult and time-consuming. In contrast, the femoral district next to the bifurcation is the most commonly involved in lower limbs arterial atherosclerosis, and it can be easily and reliably visualized in the vast majority of patients undergoing lower limbs ultrasound evaluation. Indeed, in the present study, femoral plaque echogenicity evaluation was applicable in almost 80% of LE-PAD at stage II of Fontaine classification.

Our results might inspire future studies aimed at verifying the current hypothesis that plaque vulnerability is a systemic phenomenon. Indeed, a large body of data suggest that plaque vulnerability is only partly determined by local factors, because systemic factors such as infection, inflammation, autoimmunity, or specific genes may also be involved (45–48). In particular, high systemic levels of inflammatory markers are associated with the presence of vulnerable plaques in multiple vascular districts (32,49). In this regard, in LE-PAD patients, the vascular bed of lower limbs provides a large surface that, in the presence of vulnerable plaques, may represent a release's source of inflam-
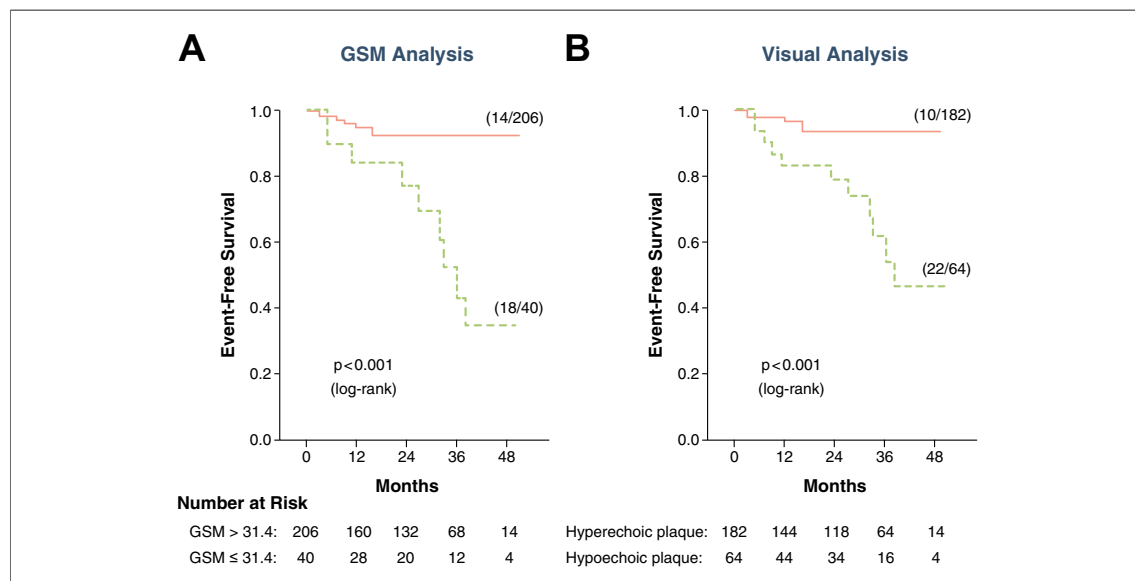
presence of femoral plaques might indicate a generalized susceptibility to atherosclerosis and vulnerability to coronary events.

These findings are clinically relevant, because nearly all LE-PAD patients present in the femoral bifurcation an atherosclerotic plaque, whose echogenicity can be easily evaluated by B-mode ultra-

Figure 4. Patients' Outcome According to Femoral Plaque Echogenicity

Kaplan-Meier analysis of event-free survival curves in patients with hypoechoic **(green line)** and hyperechoic **(red line)** femoral plaques. **(A)** Analysis considering patients with GSM ≤31.4 (hypoechoic plaque) versus patients with higher GSM value (hyperechoic plaque). **(B)** Analysis considering patients with hypoechoic (types 1 + 2) versus patients with hyperechoic (types 3 + 4) plaques at visual analysis. Number of events/number of patients in each subgroup is reported in parentheses. Abbreviation as in Figure 1

matory molecules that might contribute to vascular impairment at distant sites.

Study limitations. Our results refer to a restricted category of patients with significant and severe atherosclerotic disease of the lower limbs, ABI ≤0.90, and high prevalence of cardiovascular risk factors and cardiovascular comorbidity. In particular, we enrolled only patients with LE-PAD at stage II of Fontaine classification (intermittent claudication). Therefore, our results cannot be extended to the general population or to patients affected by critical limb ischemia (stages III and IV of Fontaine classification), and, especially, to patients with asymptomatic disease (stage I of Fontaine classification), who represent the majority of the LE-PAD population and whose cardiovascular risk is not much lower than that of claudicants (50). Furthermore, it is important to emphasize that about 22% of the LE-PAD patients at stage II of Fontaine classification who were potentially suitable to be enrolled were excluded from the study due to limitations regarding femoral plaque echogenicity evaluation.

CONCLUSIONS

The presence of hypoechoic atherosclerotic plaques in the femoral arteries entails an increased risk of future myocardial infarction and ischemic stroke in LE-PAD. This information may be obtained by computer-assisted or visual analyses. These findings are clinically relevant, because the simple, inexpensive, and noninvasive visual evaluation of femoral plaque echogenicity by B-mode ultrasound might help to identify vulnerable LE-PAD patients potentially exposed to higher cardiovascular risk.

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Key Words: cardiovascular risk ■ femoral plaque echogenicity ■ peripheral arterial disease ■ vulnerable plaque.