JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 75, NO. 14, 2020

ORIGINAL INVESTIGATIONS

Short-Term Progression of Multiterritorial Subclinical Atherosclerosis

Beatriz López-Melgar, MD, PhD,[a,b] Leticia Fernández-Friera, MD, PhD,[a,b,c] Belén Oliva, MSc,[a] José Manuel García-Ruiz, MD,[a,c,d] Fátima Sánchez-Cabo, PhD,[a] Héctor Bueno, MD, PhD,[a,c,e] José María Mendiguren, MD,[f] Enrique Lara-Pezzi, PhD,[a,c] Vicente Andrés, PhD,[a,c] Borja Ibáñez, MD, PhD,[a,c,g] Antonio Fernández-Ortiz, MD, PhD,[a,c,h] Javier Sanz, MD,[a,i] Valentín Fuster, MD, PhD[a,i]

ABSTRACT

BACKGROUND Atherosclerosis progression predicts cardiovascular events; however, progression of multiterritorial subclinical atherosclerosis is incompletely understood.

OBJECTIVES This study sought to study short-term progression of atherosclerosis using different noninvasive imaging techniques and their relationship with cardiovascular risk.

METHODS The study included 3,514 PESA (Progression of Early Subclinical Atherosclerosis) study participants (45.7 ± 4.2 years of age; 63% men). Participants underwent 2-dimensional vascular ultrasound (2DVUS) of abdominal aorta, carotid, iliac, and femoral territories to determine a plaque number score; 3DVUS to quantify carotid and femoral plaque volume; and coronary artery calcium score (CACS) at baseline and 2.8 years later. The authors calculated the rate of new disease incidence and changes in disease extent. Logistic regression models were used to evaluate associations of progression rates with baseline cardiovascular risk factors and estimated 10-year risk.

RESULTS Imaging detected short-term (3-year) atherosclerosis progression in 41.5% of participants (26.4% by 2DVUS, 21.3% by 3DVUS, and 11.5% by CACS), particularly in peripheral territories examined by vascular ultrasound. New atherosclerosis onset accounted for approximately one-third of total progression, also more frequently by 2DVUS and 3DVUS (29.1% and 16.6%, respectively), than by CACS (2.9%). Participants with baseline disease by all 3 modalities (n = 432) also showed significant atherosclerosis progression (median: 1 plaque [interquartile range (IQR): −1 to 3 plaques] by 2DVUS; 7.6 mm^3 [IQR: −32.2 to 57.6 mm^3] by 3DVUS; and 21.6 Agatston units [IQR: 4.8 to 62.6 Agatston units] by CACS). Age, sex, dyslipidemia, hypertension, smoking, and family history of premature cardiovascular disease contributed to progression, with dyslipidemia the strongest modifiable risk factor. Although disease progression correlated with cardiovascular risk, progression was detected in 36.5% of participants categorized as low risk.

CONCLUSIONS With this multimodal and multiterritorial approach, the authors detected short-term progression of early subclinical atherosclerosis in a substantial proportion (41.5%) of apparently healthy middle-aged men and women, more frequently by peripheral 2D/3DVUS than by CACS. Disease progression, as defined in this study, correlated with almost all cardiovascular risk factors and estimated risk. (Progression of Early Subclinical Atherosclerosis [PESA]; NCT01410318) (J Am Coll Cardiol 2020;75:1617–27) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Listen to this manuscript's audio summary by Editor-in-Chief Dr. Valentin Fuster on JACC.org.

From the [a]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; [b]HM Hospitales-Centro Integral de Enfermedades Cardiovasculares HM CIEC, Madrid, Spain; [c]CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain; [d]Instituto de Investigación Sanitaria del Principado de Asturias, Hospital Universitario de Cabueñes, Gijón, Spain; [e]Hospital Universitario 12 de Octubre, Madrid, Spain; [f]Banco de Santander, Madrid, Spain; [g]IIS-Fundación Jiménez Díaz Hospital, Madrid, Spain; [h]Hospital Clínico San Carlos IdISSC, Madrid, Spain; and [i]The Zena and Michael A. Wiener Cardiovascular Institute/Marie-Josée and Henry R. Kravis Center for Cardiovascular Health, Mount Sinai School of Medicine, New York, New York. The PESA-CNIC-Santander study is funded by the Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain, and Banco

ISSN 0735-1097 https://doi.org/10.1016/j.jacc.2020.02.026

ABBREVIATIONS AND ACRONYMS

2DVUS = 2-dimensional vascular ultrasound

3DVUS = 3-dimensional vascular ultrasound

ASCVD = atherosclerotic cardiovascular disease

CACS = coronary artery calcium score

CI = confidence interval

CVRF = cardiovascular risk factor

CT = computed tomography

GPV = global plaque volume

IMT = intima-media thickness

MDD = minimum detectable difference

VUS = vascular ultrasound

Atherosclerosis is a systemic process, and its progression is linked to incident cardiovascular events (1-3). The most widely used biomarker for studying subclinical coronary disease in asymptomatic individuals is the coronary artery calcium score (CACS) (4,5); however, this technique only identifies the advanced epiphenomenon of calcification. Another biomarker is intima-media thickness (IMT) (6-12), but this does not necessarily reflect atherosclerosis (13) and is a weaker predictor of clinical events (14,15). Only a few studies have evaluated plaque progression noninvasively with carotid 2-dimensional vascular ultrasound (2DVUS) (6,16), and there is, therefore, a lack of information about varying progression in different arterial beds (7). A more comprehensive picture of the atherosclerotic process would be provided through combined assessment of multiple vascular sites and different atherosclerotic manifestations, namely peripheral plaque formation and growth by vascular ultrasound (VUS) and coronary calcification by CACS. Moreover, this would help to optimize the allocation of available screening tests and the design of strategies for timely detection of early progression of subclinical disease.

SEE PAGE 1628

We therefore used a range of noninvasive imaging modalities (2D and 3D vascular ultrasound [3DVUS], and CACS) to study the progression of subclinical atherosclerosis burden over a short time period (3-year follow-up) in the PESA-CNIC-Santander (Progression of Early Subclinical Atherosclerosis) study cohort. We used statistical modeling of measurement errors for 2D and 3DVUS and previous data for CACS to define progression by each imaging method. Moreover, we proposed a definition of individual atherosclerosis progression on the basis of the combination of information obtained by the 3 imaging techniques, and attempted to substantiate it by determining its relation to cardiovascular risk factors (CVRFs) and estimated risk.

METHODS

STUDY POPULATION. The PESA-CNIC-Santander study (NCT01410318) rationale and design have been reported (17). Briefly, between June 2010 and February 2014, the PESA study enrolled 4,184 volunteers age 40 to 54 years (employees at the Santander Bank headquarters in Madrid, Spain) with no history of cardiovascular disease. Participants are examined at baseline and at 3- and 6-year follow-up by 2DVUS and 3DVUS to evaluate peripheral atherosclerosis at multiple arterial sites and by noncontrast cardiac computed tomography (CT) to quantify CACS. Enrollees also complete clinical interviews with standardized questionnaires and undergo a physical examination on all visits. The present analysis included participants who had completed visits 1 (baseline) and 2 (3 years). CVRF definitions were as reported (18), and the American College of Cardiology/American Heart Association atherosclerotic cardiovascular disease (ASCVD) risk score was quantified and categorized for 10-year risk as low (<5%), borderline (5% to 7.4%), or intermediate-high (≥7.5%) (19,20). The study protocol was approved by the ethics committee of the Carlos III Institute of Health (Madrid, Spain), and all participants provided written informed consent.

IMAGE ACQUISITION PROTOCOL AND ANALYSIS. The imaging methodologies used in the PESA study have been reported (17,18,21,22). Briefly, noncontrast CT studies were performed using a 16-slice Philips Brilliance scanner (Philips Healthcare, Andover, Massachusetts) and standard methodology. Peripheral 2DVUS and 3DVUS images were acquired with a Philips iU22 ultrasound system equipped with L9-12 linear and VL13-5 3D volume-linear array transducers. Manual 2DVUS cross-sectional sweeps and selected longitudinal views were obtained in the bilateral carotid arteries, the infrarenal aorta, and the bilateral iliac and bilateral femoral arteries (7 sites). 3DVUS images were acquired of the bilateral carotid and femoral arteries (4 sites) with an automatic axial 30° sweep (≈6-cm long) centered at each bifurcation.

Santander, Madrid, Spain. The study also receives funding from the Instituto de Salud Carlos III (ISC III) (PI15/02019) and the European Regional Development Fund (ERDF) "Una manera de hacer Europa." The CNIC is supported by the Ministerio de Ciencia e Innovación, the ISC III and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence (SEV-2015-0505). Dr. Bueno has received research funding from the Instituto de Salud Carlos III, Spain (PIE16/00021 & PI17/01799); has received research funding from AstraZeneca, Bristol-Myers Squibb, and Novartis; has received consulting fees from AstraZeneca, Bayer, Bristol-Myers Squibb-Pfizer, and Novartis; and has received speaker fees or support for attending scientific meetings from Amgen, AstraZeneca, Bayer, Bristol-Myers Squibb-Pfizer, Novartis, and MEDSCAPE-the heart.org. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. P.K. Shah, MD, served as Guest Editor-in-Chief for this paper.

Manuscript received November 16, 2019; revised manuscript received December 27, 2019, accepted February 4, 2020.

All images were analyzed off-line at the CNIC Imaging Core Laboratory using dedicated analysis tools within QLABv10.2 (Philips Healthcare). Analysis was performed by technicians blinded to clinical information and previous imaging.

In 2DVUS and 3DVUS assessments, plaque was defined according to the Mannheim criteria as a focal protrusion into the arterial lumen measuring >0.5 mm or >50% of the adjacent IMT, or as IMT >1.5 mm (23). For 2DVUS, we recorded the number of plaques present in each arterial site, categorized as 0 = no plaques; 1 = 1 plaque; 2 = 2 plaques; and 3 = 3 or more plaques. Scores from the 7 explored sites were combined to produce a per-individual 2D-Plaque Score (2D-Score) from 0 to 21. For 3DVUS, plaque volumes in each of the 4 explored sites were summed to obtain the global plaque volume (3D-GPV in mm^3) per participant. CACS was quantified by the Agatston method (24).

For each imaging modality, we quantified the prevalence and extent of disease at visits 1 and 2, as well as the 3-year incidence of new disease, the extent of new disease, and the 3-year change in disease extent. These parameters were measured for the whole population and separately for the subcohorts without disease at baseline (absence of atherosclerosis in all territories by all modalities) and with any disease at baseline (presence of atherosclerosis in 1 or more territory by 1 or more imaging modality), because these may represent different pathophysiological processes (new disease onset versus progression of existing disease) (5,16,25).

TABLE 1 PESA Cohort Baseline Characteristics (Visit 1)

	Total Cohort (N = 3,514)	Without Disease at Baseline (n = 1,342)	With Any Disease at Baseline (n = 2,172)	p Value
Age, yrs	45.7 ± 4.2	44.3 ± 3.7	46.5 ± 4.3	<0.001
Male	2,212 (63.0)	661 (49.2)	1,551 (71.4)	<0.001
CV risk factors				
Dyslipidemia	1,438 (40.9)	397 (29.6)	1,041 (47.9)	<0.001
Diabetes	56 (1.6)	8 (0.6)	48 (2.2)	<0.001
Hypertension	381 (10.8)	88 (6.6)	293 (13.5)	<0.001
Smoking	700 (19.9)	180 (13.4)	520 (23.9)	<0.001
Obesity	466 (13.3)	142 (10.6)	324 (14.9)	<0.001
Family history of CV disease	539 (15.3)	193 (14.4)	346 (15.9)	0.216
CV risk factors therapy				
Lipid-lowering	231 (6.6)	39 (2.9)	192 (8.8)	<0.001
Antihypertensive	248 (7.1)	49 (3.7)	199 (9.2)	<0.001
Antidiabetic	44 (1.3)	8 (0.6)	36 (1.7)	0.006
Number of CV risk factors				
0	1,580 (45.0)	785 (58.5)	795 (36.6)	<0.001
1	1,367 (38.9)	444 (33.1)	923 (42.5)	<0.001
2	498 (14.1)	110 (8.2)	388 (17.9)	<0.001
>2	69 (2.0)	3 (0.2)	66 (3.0)	<0.001
Conventional risk scales				
10-yr ASCVD risk*	1.95 (0.87–3.93)	1.23 (0.57–2.60)	2.56 (1.26–4.86)	<0.001

Values are mean ± SD, n (%), or median (interquartile range). *Log-transformed for the analysis (n = 3,051).
ASCVD = atherosclerosis cardiovascular disease risk; CV = cardiovascular; PESA = Progression of Early Subclinical Atherosclerosis study.

DEFINITIONS OF ATHEROSCLEROSIS PROGRESSION. Definitions for each imaging technique. There are no established criteria for determining progression by 2DVUS and 3DVUS. In the PESA cohort, the high prevalence of zeros (participants identified as disease-free by imaging modalities) leads to a severely right-skewed distribution of the imaging dataset and precludes normalization of continuous data for a reliable analysis. Therefore, we opted for a binary approach using cutoff values to define significant changes. For 2D-Score and 3D-GPV, changes were assessed by estimating the minimum detectable difference (MDD) from the interobserver variability for each technique. This approach not only avoids assumptions about progression or stability rates (i.e., based on tertiles of change from baseline) but also, account for technique-specific measurement errors (26). We derived MDD from Bland-Altman limits of agreement of log-transformed values for avoiding plaque size effect on the measurement of change, and then back-transformed for meaningful interpretation as a percentage of change (details in the Supplemental Appendix). For CACS change, we used the square-root method described by Budoff et al. (27), which shows an association with all-cause mortality (27). For all 3 modalities, disease progression in individuals without baseline disease was defined as a score >0 in visit 2. Cutoffs used to define progression in individuals with disease at baseline were a ≥2 point increase in the 2D-Score, a ≥100% increase in 3D-GPV, and/or a >2.5 change in the square-root method for CACS (27). With 3DVUS, we could only confidently detect changes in 3D-GPV of 100% (i.e., at least doubling), contrasting a previous study reporting a MDD between 12% and 35% (28). The need for larger relative volume changes in our study is likely due to the smaller plaque size in our population (median volume 32 mm^3 in PESA vs. 276 mm^3 in that previous study), since measurement variability is known to increase with decreasing plaque size (28,29). In addition, the complete dataset from visit 1 (20,768 imaging and clinical variables) was analyzed with machine-learning models to independently validate the estimated cutoff values for 2DVUS and 3DVUS (Supplemental Appendix).

TABLE 2 Disease Prevalence and Extent by Imaging Modality at Baseline and Follow-Up in the Complete PESA Cohort (N = 3,514)

	2DVUS (2D-Score)	3DVUS (GPV)	CACS (Agatston Units)	Any (2DVUS and/or 3DVUS and/or CACS)
Baseline				
Participants with presence of disease	2,040 (58.1)	1,520 (43.3)	614 (17.5)	2,172 (61.8)
Disease extent in participants with disease	2 (1 to 4)	50.3 (19.3 to 118.6)	18.9 (4.8 to 62.4)	N/A
Disease extent in the total cohort	1 (0 to 3)	0 (0 to 36.6)	0 (0 to 0)	N/A
3-yr follow-up				
Participants with presence of disease	2,248 (64.0)	1,840 (52.4)	783 (22.3)	2,358 (67.1)
Disease extent in participants with disease	2 (1 to 5)	46.8 (17.7 to 109.1)	22.9 (6.6 to 87.3)	N/A
Disease extent in the total cohort	1 (0 to 3)	4.6 (0 to 50.1)	0 (0 to 0)	N/A
Baseline-to-3-yr change				
Incidence of new disease	208 (5.9)*	320 (9.1)*	169 (4.8)*	186 (5.3)*
Change in disease extent, total cohort	0 (0 to 1)*	0 (0 to 11.5)*	0 (0 to 0)*	N/A
Change in disease extent, participants with any disease	1 (−1 to 2)*	7.8 (−12.4 to 35.5)*	9.8 (2.2 to 35.8)*	N/A
Change in disease extent, participants without disease	0 (0 to 1)*	0 (0 to 0)*	0 (0 to 0)*	N/A

Values are n (%) or median (interquartile range). *p < 0.001 for the significance of the 3-year change.
2DVUS = 2-dimensional vascular ultrasound; 3DVUS = 3-dimensional vascular ultrasound; CACS = coronary artery calcium score; GPV = global plaque volume; N/A = not applicable.

Definitions for individuals. Participants were defined as progressors or nonprogressors by combining the definitions given in the previous section for each imaging modality as follows.

- Progressor: Individual fulfilling the progression criteria in at least 1 imaging modality.
- Nonprogressor: Individual with an absence of progression criteria in all 3 imaging modalities.

SUBANALYSIS OF PROGRESSION USING CONTINUOUS VARIABLES. To evaluate progression as a continuous standardized metric and enable more direct comparison among imaging techniques, we analyzed the small subset of participants with disease detected with all 3 imaging modalities (thus excluding participants free of disease by 1 or more imaging techniques). This analysis also served as a validity test for the proposed dichotomous thresholds.

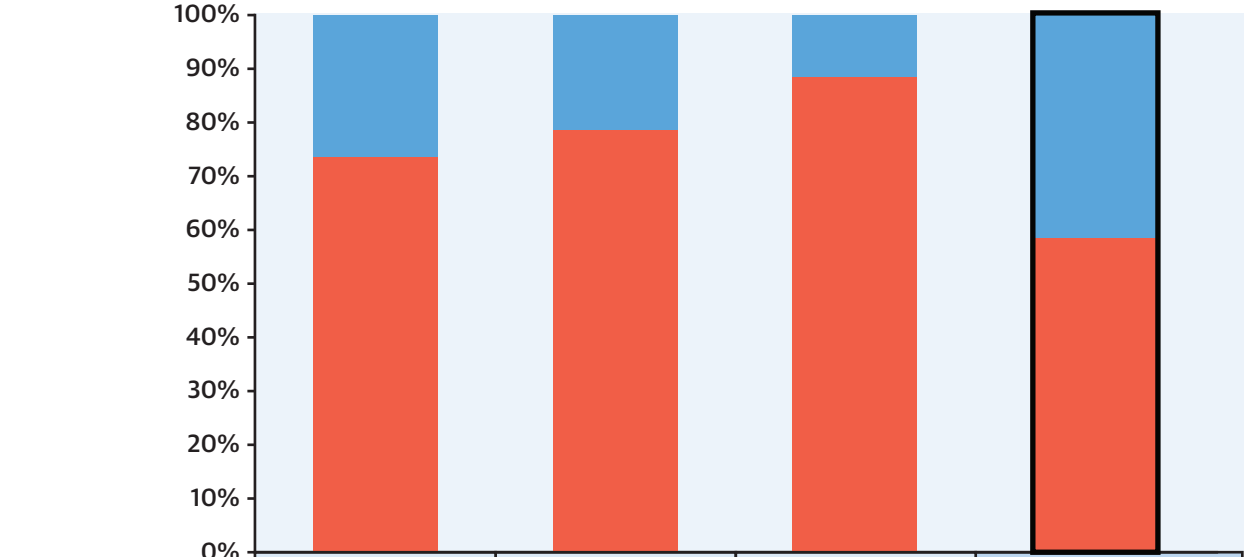
TABLE 3 Disease Extent by Imaging Modality in the PESA Subcohort With Disease Detected by All Imaging Techniques at Baseline and Follow-Up (n = 432)

	2DVUS (2D-Score)	3DVUS (GPV)	CACS (Agatston Units)	All (2DVUS, 3DVUS, and CACS)
Visit 1				
Disease extent in the total cohort	4.5 (2 to 8)	104.0 (42.7 to 232.7)	26.2 (6.8 to 83.4)	N/A
Visit 2				
Disease extent in the total cohort	5 (3 to 9.5)	106.4 (49.1 to 245.7)	54.1 (17.5 to 137.1)	N/A
Baseline to 3-year change				
Change in disease extent	1 (−1 to 3)*	7.6 (−32.2 to 57.6)*	21.6 (4.8 to 62.6)*	N/A

Values are median (interquartile range). *p < 0.001 for the significance of the 3-year change.
Abbreviations as in Table 2.

STATISTICAL METHODS. Categorical data are presented as n (%) and continuous variables as mean ± SD if normally distributed, or otherwise as median (interquartile range [IQR]). Variables with non-normal distribution were log-transformed before comparison. Differences between visits 1 and 2 were assessed by paired Student's *t*-test or Wilcoxon signed rank test as appropriate. Comparisons between participants without and with any baseline disease were made with unpaired Student's *t*-test and chi-square test for continuous and categorical variables, respectively. After power transformation of both the baseline and follow-up data, we used the Cohen's Dav method to obtain a standardized continuous measure of progression, thus allowing comparison in the participant subcohort with disease identified by all 3 imaging techniques. We used a logistic regression model adjusted by sex, age, smoking, dyslipidemia, diabetes, hypertension, obesity, and family history of cardiovascular disease to assess the association between baseline CVRFs and overall atherosclerosis progression using our proposed definition. To compare the magnitude of the effects of different CVRFs on plaque progression, we calculated the "adequacy" index, which is the proportion of the full model log-likelihood explainable by each predictor (21,30). In addition, we used again logistic regression models to explore the association between overall atherosclerosis progression rates and ASCVD risk score. Statistical analyses were conducted with Stata 12 software (StataCorp, College Station, Texas).

CENTRAL ILLUSTRATION **3-Year Progression Rates of Subclinical Atherosclerosis Evaluated by 3 Imaging Techniques, Separately and in Combination**



	2-Dimensional Vascular Ultrasound	3-Dimensional Vascular Ultrasound	Coronary Artery Calcium Score	COMBINING IMAGING MODALITIES
Progressors	26.4%	21.3%	11.5%	41.5%
Nonprogressors	73.6%	78.7%	88.5%	58.5%

López-Melgar, B. et al. J Am Coll Cardiol. 2020;75(14):1617-27.

RESULTS

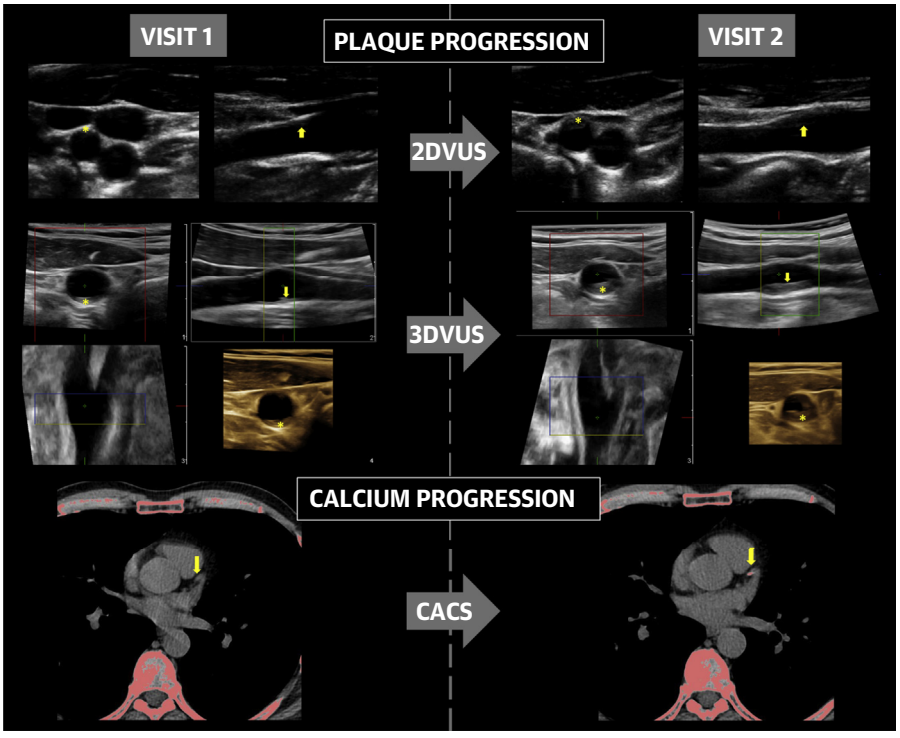
SHORT-TERM (3-YEAR) CHANGES IN SUBCLINICAL ATHEROSCLEROSIS. The study cohort comprised 3,514 individuals (excluding 137 participants who missed visit 2, 383 with incomplete and 117 with uninterpretable imaging studies, and 33 without clinical data) (Supplemental Figure 1). Baseline mean age was 45.7 ± 4.2 years, 37% were women, and the overall risk profile was low (median 10-year ASCVD risk ~2%) (**Table 1**). The median between-visit interval was 2.8 years (IQR: 2.6 to 3.0 years).

Subclinical atherosclerosis at baseline was detected in 2,172 participants (61.8%): 2,040 (58.1%) by 2DVUS, 1,520 (43.3%) by 3DVUS, and 614 (17.5%) by CACS. The overall 3-year change in disease prevalence was 5.3% (5.9%, 9.1%, and 4.8% by 2DVUS, 3DVUS, and CACS, respectively) (**Table 2**, Supplemental Figure 2). Participants with disease at baseline and/or follow-up had a median change of 1 plaque (IQR: −1 to 2 plaques) in the 2D-Score, 7.8 mm^3 (IQR: −12.4 to 35.5 mm^3) in 3D-GPV, and 9.8 Agatston units (IQR: 2.2 to 35.8 Agatston units) by CACS (all p values <0.001) (**Table 2**).

Among the 1,342 participants without baseline disease, 419 (31.2%) developed new atherosclerosis by 3-year follow-up (Supplemental Table 1), with disease onset more frequently found in the peripheral territories by VUS (2DVUS 29.1% [95% confidence interval (CI): 26.6% to 31.6%]; 3DVUS 16.6% [95% CI: 14.7% to 18.7%]) than in the coronary territory by CACS (2.9% [95% CI: 2.1% to 4.0%]). Among participants with any disease at baseline (Supplemental Table 2), 2DVUS detected an absolute reduction in disease prevalence but a statistically significant increase in disease extent, whereas 3DVUS and CACS identified significant increases in both disease prevalence and extent. In addition, the 3-year rate of conversion to CACS >0 in participants with any extracoronary disease at baseline was twice that of participants without (5.9% vs. 2.9%; p < 0.001).

Evidence of disease by all 3 imaging modalities was observed in only 432 participants (12.3%). As expected, the participants in this subcohort were older (age 48.8 ± 4.0 years; p < 0.001), predominantly men (n = 405; 93.7%), and had a higher burden of CVRFs and estimated 10-year ASCVD risk (5.10 [IQR: 2.91 to 7.71]; p < 0.001). Three-year change in disease extent

FIGURE 1 Examples of Atherosclerosis Progression Using Different Imaging Modalities



Intimal irregularities in the carotid bulb **(arrow)** were detected on visit 1 by 2DVUS. By visit 2, this had progressed to a hypoechogenic atherosclerotic plaque **(arrow)** extending into the external carotid artery **(asterisk)**. Analysis by 3DVUS detected and quantified significant growth of a plaque already present in the carotid bulb on visit 1 **(arrow and asterisk)**. Computed tomography images show new onset of a calcified lesion in the left anterior descending coronary artery on visit 2 **(arrow)**. 2DVUS = 2-dimensional vascular ultrasound; 3DVUS = 3-dimensional vascular ultrasound; CACS = coronary artery calcium score.

was also significant in this subcohort with disease detected by all imaging techniques, with an observed median change of 1 plaque (IQR: −1 to 3 plaques) by 2DVUS, 7.6 mm^3 (IQR: −32.2 to 57.6 mm^3) by 3DVUS, and 21.6 Agatston units (IQR: 4.8 to 62.6 Agatston units) by CACS **(Table 3)**.

RATES OF SHORT-TERM ATHEROSCLEROSIS PROGRESSION. Over 3 years, in the total cohort and combining findings from all 3 imaging techniques, atherosclerosis progressed significantly in 41.5% of participants (**Central Illustration**). Evaluating each imaging modality separately, atherosclerosis progression was detected in 26.4% of participants by 2D-Score, in 21.3% by 3D-GPV, and in 11.5% by CACS. Nonprogressors were assessed by applying the inverse of the progression criteria: a decrease of ≥2 points in the 2D-Score, a 100% reduction in 3D-GPV, and a change >−2.5 in the square-root method for CACS. According to these criteria, reductions in disease at 3-year follow-up occurred in 9.1% of participants by 2DVUS, 2.7% by 3DVUS, and 0.3% by CACS. Progression rates were also higher for atherosclerotic plaques detected in peripheral arteries by VUS than for coronary calcification detected by CACS in the participant subcohorts without and with any baseline disease (Supplemental Table 3). Interestingly, CACS progressed approximately 5 times more frequently among participants with any baseline disease than in those without (16.8% vs. 3.1%; $p < 0.001$). However, in the subcohort with disease detected by all 3 imaging modalities, progression by CACS was highest, followed by 2DVUS and then 3DVUS, both when the measure used was continuous (Cohen's Dav coefficient = 0.42 by CACS, 0.27 by 2DVUS, and 0.09 by 3DVUS) or dichotomous (progression = 40.1% by CACS, 38.2% by 2DVUS, and 21.2% by 3DVUS).

Plaque progression by 2D/3DVUS was significantly higher among participants with CACS progression than in those without (52.8% vs. 33.9%, respectively; $p < 0.001$) (Supplemental Table 4). In addition, the CACS progression was twice as high in those

TABLE 4 Univariate and Multivariate Predictors of Overall Atherosclerosis Progression

	OR (95% CI)	AI	p Value
Univariate OR*			
Sex, male	2.20 (1.91–2.55)		<0.001
Age, ×5 yrs	1.50 (1.38–1.64)		<0.001
Smoking	1.30 (1.10–1.54)		0.002
Dyslipidemia	1.98 (1.72–2.27)		<0.001
Diabetes	1.90 (1.11–3.23)		0.019
Hypertension	2.06 (1.66–2.56)		<0.001
Family history of CV disease	1.30 (1.08–1.56)		0.006
Obesity, BMI ≥30 kg/m^2	1.62 (1.33–1.97)		<0.001
10-yr ASCVD risk	1.16 (1.13–1.19)		<0.001
Multivariate OR†			
Sex, male	1.78 (1.52–2.08)	0.47	<0.001
Age, ×5 yrs	1.35 (1.24–1.48)	0.39	<0.001
Smoking	1.32 (1.11–1.57)	0.04	0.002
Dyslipidemia	1.47 (1.27–1.71)	0.38	<0.001
Diabetes	1.07 (0.62–1.87)		0.805
Hypertension	1.37 (1.09–1.72)	0.17	0.008
Family history of CV disease	1.30 (1.07–1.57)	0.03	0.008
Obesity, BMI ≥30 kg/m^2	1.17 (0.95–1.44)		0.148

*Unadjusted odds ratios for conventional cardiovascular risk factors (CVRFs) and ASCVD risk. †Adjusted model by sex, age, and CVRF (smoking, dyslipidemia, diabetes, hypertension, family history of CV disease, and obesity).

AI = adequacy index; ASCVD = atherosclerosis cardiovascular disease; BMI = body mass index; CV = cardiovascular; OR = odds ratio.

participants with 2D/3DVUS progression than in those without (16.9% vs. 8.5%, respectively; $p < 0.001$). The 3 imaging modalities showed strong agreement for absence of progression in 2,054 participants (58.5%), whereas progression was detected by all 3 methods in only 52 individuals (1.5%) (Supplemental Table 5). Examples of disease progression are shown in Figure 1.

ASSOCIATION OF ATHEROSCLEROSIS PROGRESSION WITH CVRF AND ESTIMATED ASCVD RISK. Overall atherosclerosis progression showed an independent association with age, male sex, and all other CVRFs except obesity and diabetes (Table 4). The strongest associations were for age and male sex, and the strongest modifiable predictor of short-term atherosclerosis progression was dyslipidemia. A sensitivity analysis with the 670 excluded participants showed no significant differences (data not shown). Comparison of participant subgroups without and with any baseline disease revealed that new atherosclerosis onset on visit 2 in disease-free participants was significantly associated with age, male sex, and dyslipidemia, whereas disease progression on visit 2 in already diseased individuals was linked to age, male sex, dyslipidemia, smoking, and family history. However, differences in odds ratios between these subgroups were not statistically significant (Figure 2).

Atherosclerosis progression increased significantly across cardiovascular risk categories, regardless of the imaging modality used (all $p < 0.001$) (Figure 3). Atherosclerosis detected by any technique progressed in 36.5% of participants categorized as low risk (23.8%, 19.9%, and 7.9% by 2DVUS, 3DVUS, and CACS, respectively) and in 63.1% of intermediate-high-risk participants (36.5%, 25.7%, and 31.1%, respectively).

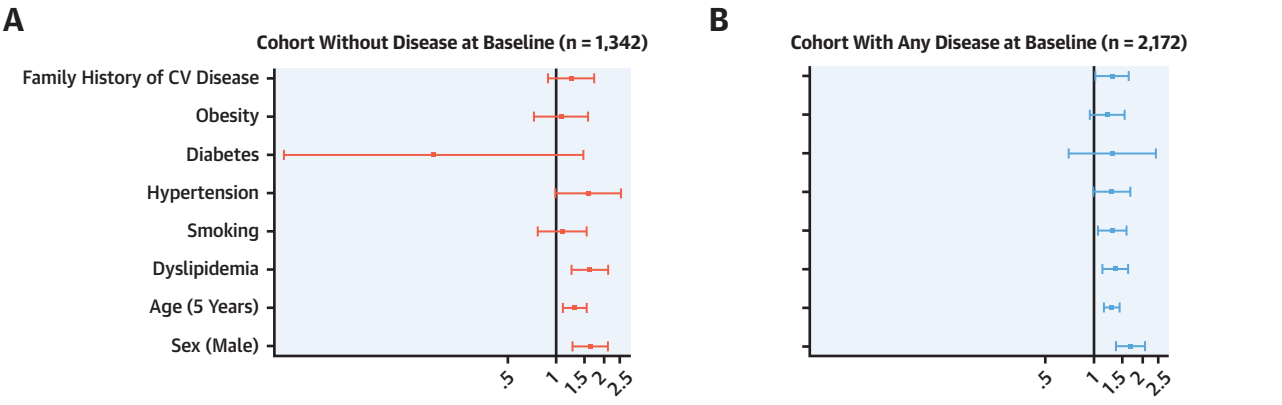
DISCUSSION

To our knowledge, this is the first large prospective study reporting the short-term progression of subclinical atherosclerosis across coronary and multiple noncoronary territories as detected by noninvasive imaging modalities (VUS and CACS). Atherosclerosis progression, as defined here, can be detected in 41.5% of middle-aged individuals over a relatively short period (3 years). New disease onset and disease progression was detected much earlier in noncoronary vessels assessed by 2D and 3DVUS than in coronary arteries assessed by CACS, and CACS progression was more common in individuals with detectable baseline atherosclerosis. Atherosclerosis progression using our definition was associated with most conventional CVRFs, in particular age, male sex, and dyslipidemia. Although progression rates increased in parallel with estimated 10-year ASCVD risk, progression was detected in a substantial proportion (36.5%) of individuals categorized as low risk.

SHORT-TERM PROGRESSION OF SUBCLINICAL ATHEROSCLEROSIS. We propose a new definition of disease progression that combines the information obtained with different imaging techniques to determine an individual's status as a "progressor." According to the criteria defined here, atherosclerosis progressed in 41.5% of PESA participants over ~3 years. Progression by all 3 modalities was present in only 1.5% of participants, which may reflect heterogeneity in disease progression (anatomic or methodological) or the identification of different disease stages with different techniques (atherogenesis vs. calcification). Evaluation of the potential clinical impact of the proposed criteria will require assessment of their association with future events in ongoing follow-up (31); however, we believe that the criteria are indirectly validated by: 1) use of previously validated criteria (CACS) or criteria developed from reproducibility measurements (VUS); 2) good general agreement among modalities for the absence of progression; 3) agreement between findings from machine-learning analyses; 4) agreement between continuous-based and cutoff-based measures of

FIGURE 2 Relationship Between CVRF and Atherosclerosis Development

Multivariate Analysis



	OR (95% CI)	AI	p Value	OR (95% CI)	AI	p Value	*p Interaction*
Family History of CV Disease	1.24 (0.89 to 1.73)		0.208	1.30 (1.02 to 1.64)	0.04	**0.031**	*0.815*
Obesity	1.07 (0.73 to 1.58)		0.720	1.21 (0.94 to 1.55)		0.139	*0.580*
Diabetes	0.17 (0.02 to 1.48)		0.110	1.30 (0.70 to 2.41)		0.398	*0.073*
Hypertension	1.59 (0.99 to 2.55)		0.051	1.29 (0.99 to 1.68)		0.061	*0.503*
Smoking	1.09 (0.77 to 1.55)		0.614	1.30 (1.06 to 1.59)	0.04	**0.012**	*0.392*
Dyslipidemia	1.62 (1.25 to 2.11)	0.43	**<0.001**	1.35 (1.13 to 1.62)	0.34	**0.001**	*0.250*
Age (5 Years)	1.30 (1.10 to 1.55)	0.26	**0.003**	1.29 (1.15 to 1.44)	0.38	**<0.001**	*0.907*
Sex (Male)	1.63 (1.27 to 2.10)	0.46	**<0.001**	1.68 (1.37 to 2.06)	0.44	**<0.001**	*0.884*

The relationship between cardiovascular risk factor (CVRF) and atherosclerosis development was analyzed separately in the subgroups without **(A)** and with **(B)** any disease at baseline. Adjusted models by sex, age, CVRF (smoking, dyslipidemia, diabetes, hypertension, family history of CV disease, and obesity). AI = adequacy index; CI = confidence interval; CV = cardiovascular; OR = odds ratio.

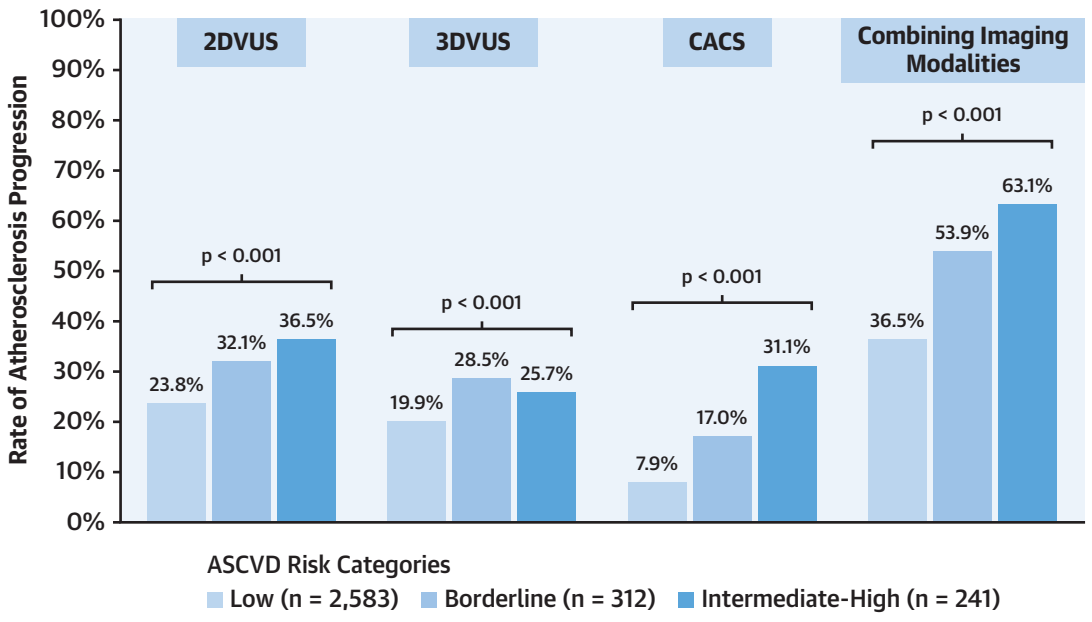
progression; and 5) significant associations with CVRFs and estimated risk.

Among nonprogressors, we observed possible disease regression, particularly as determined by 2DVUS. The Tromsø study reported regression in total carotid plaque area in 14% of men and 11% of women (6), whereas others have considered regression as methodological error (7,32). An important role for technical factors is supported by our detection of possible regression predominantly by 1 modality, but the existence of genuine regression cannot be excluded. Confirmation will require careful image review on a case-by-case basis to exclude simple measurement variability.

Among the imaging modalities used here, data on CACS progression has been more widely explored, with the middle-aged MESA (Multi-Ethnic Study of Atherosclerosis) and CARDIA (Coronary Artery Risk Development in Young Adults) cohorts (33) generating results comparable to those observed in the PESA study. Our vascular ultrasound results expand the limited information available on atherosclerosis progression at multiple extracoronary territories and include unique 3DVUS data.

PERIPHERAL ATHEROSCLEROSIS PROGRESSION COMPARED WITH CACS PROGRESSION. Atherosclerosis progression rates in noncoronary territories (26% by 2DVUS and 21% by 3DVUS) were more than double those in the coronary arteries (11% by CACS). Moreover, new atherosclerosis onset in individuals without baseline disease was 10 and 5 times more frequent in extracoronary arteries by VUS (29.1% by 2DVUS and 16.6% by 3DVUS, than 2.9% by CACS, respectively). This large difference does not necessarily indicate that disease develops earlier in peripheral arteries than in the coronary circulation because atheroma formation and calcification represent different stages of atherogenesis (CACS does not

FIGURE 3 Relationship Between Early Atherosclerosis Progression and Estimated Cardiovascular Risk



Distribution of disease among ASCVD risk categories stratified by each imaging modality separately and in combination (N = 3,136 participants in which 10-year ASCVD risk score could be calculated at visit 1). ASCVD = atherosclerotic cardiovascular disease; other abbreviations as in Figure 1.

detect early noncalcified atherosclerosis). Nonetheless, this difference may have important implications for scheduling and/or allocating screening for subclinical atherosclerosis in middle-aged individuals, because most participants were considered "stable" according to CACS despite frequent disease progression in peripheral territories (up to one-third). Furthermore, participants without peripheral plaque progression rarely developed or showed progression in coronary artery calcification, with most participants "stable" by 2D/3DVUS also being "stable" by CACS.

Notwithstanding these observations, in the subgroup of individuals with disease detected by all modalities, CACS progressed faster than VUS-based peripheral atherosclerosis. This may indicate that once atherosclerosis is extensive or advanced, coronary calcification evaluated by CACS progresses faster than calcific and noncalcific atheroma features evaluated by VUS. Another explanation for the faster CACS progression in these participants could be the "densification" of calcific deposits as part of the natural process of plaque healing. This phenomenon would lead to overestimated disease progression by the Agatston method (34). Nevertheless, this subgroup is not representative of the whole PESA cohort, and these findings should thus be interpreted as hypotheses generating.

SUBCLINICAL ATHEROSCLEROSIS PROGRESSION AND RISK FACTORS. In line with previous studies evaluating carotid plaques (6,7), no individual CVRF seems to preferentially determine progression, particularly the progression of established atherosclerosis. Only age, male sex, and dyslipidemia were independently associated with new-onset atherosclerosis in previously disease-free individuals. These findings are similar to those of the REFINE (Risk Evaluation for Infarct Estimates) study (16) and highlight the crucial role of cholesterol in plaque development among the modifiable determinants (35). Moreover, these differences suggest that different CVRFs might have differential effects on atherosclerosis onset versus progression of existing plaques, although further research is needed to confirm this trend.

Although progression increased in step with risk, considerable atherosclerosis progression was detected in all ASCVD risk categories, even the low-risk stratum, confirming the well-known mismatch between cardiovascular risk profile determined by risk scales and the presence of subclinical atherosclerosis

(18,33). Continued clinical follow-up of the PESA cohort will monitor incident cardiovascular events and help to determine whether plaque progression has additive prognostic value over conventional risk stratification.

STUDY LIMITATIONS. PESA enrolled a middle-aged, Caucasian, overall low-risk, and relatively homogeneous cohort, and our findings might not be automatically extrapolated to other populations. Although indirectly validated, the definitions of progression and stability (or regression) are not yet standardized or may not be optimal, and other definitions would likely yield different progression rates. Ultimately, the validation of the proposed criteria will come from the association, or lack thereof, with future incident events. The intermodality comparison of progression in a continuous fashion was limited to only 432 individuals with disease detected in all 3 imaging tests due to the high skewness of the data for the total cohort. Ultrasound detected any plaque in more than 1 territory, whereas CACS is an integral measure of calcified plaques on the coronary vessels, which limits the comparison of actual progression of systemic atherosclerosis, but allows the comparison of the performance of available screening tests. Also, we studied a single form of coronary atherosclerosis (calcification), and therefore, development and/or progression of noncalcified coronary plaques could not be assessed.

CONCLUSIONS

Atherosclerosis progressed significantly in 41.5% PESA participants over a 3-year follow-up period, with progression, as defined in the current study, detected more frequently in peripheral arteries by 2D and 3DVUS than in the coronary arteries by CACS. Development or progression of coronary calcification appears to be rare in individuals without manifest peripheral atherosclerosis disease. The definition of overall atherosclerosis progression used in the PESA study is associated with conventional CVRFs and estimated cardiovascular risk, and allows detection of substantial disease progression even in individuals categorized as low risk.

ACKNOWLEDGMENTS The authors acknowledge the remarkable work on the acquisition and supervision of the imaging studies by the PESA technical staff: the team of imaging technicians (Alberto Ávila, Aurora del Barrio, Iris García, Marta Gavilán, Ángel Macías, Rosario Pérez, and Braulio Pérez), the research nurses and sonographers (Virginia Mass and Inés Gutierrez), radiologists (Ana Álvarez, Chawar Hayoun, Susana Linares, Vicente Martínez de Vega, Diana Mollinedo and Verónica Muñoz,), and cardiologists (Inés García-Lunar, Sandra Gómez-Talavera, and Andrea Moreno). The authors also thank the epidemiology technicians, the IT team (Sergio Cárdenas and Jesús Molina), the CNIC Bioinformatics Unit (Fernando Benito), the CNIC administrative team (Marta García and Marta Fernández), Javier Sanchez-González (Philips engineer and scientist), Ángel Ciprés (PESA study manager), Alberto Sanz (CNIC general manager), and the medical and technical team at Banco Santander Health Services for their contribution to keeping the study up to the high standards of quality and innovation. The authors also thank Simon Bartlett (CNIC editing services) for English revision. Finally, the authors are particularly grateful to all the PESA study participants.

ADDRESS FOR CORRESPONDENCE: Dr. Valentín Fuster, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Melchor Fernandez Almagro, 3, 28029, Madrid, Spain. E-mail: vfuster@cnic.es or valentin.fuster@mountsinai.org. OR Dr. Javier Sanz, Centro Nacional de Investigaciones Cardiovasculares (CNIC). Melchor Fernández Almagro, 3, 28029, Madrid, Spain. E-mail: javier.sanz@cnic.es or javier.sanz@mountsinai.org.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Early subclinical atherosclerosis progresses over 3 years in ~40% of apparently healthy middle-aged men and women. Progression is more often detected by 2D/3D ultrasound evaluation of peripheral arterial plaques than by CT imaging of coronary calcification, and is associated with cardiovascular risk factors but also occurs in low-risk individuals.

TRANSLATIONAL OUTLOOK: Further research is needed to clarify how these imaging modalities complement conventional risk stratification and confirm the association of progression of early disease with cardiovascular events during long-term follow-up.

REFERENCES

1. Polak JF, Pencina MJ, O'Leary DH, D'Agostino RB. Common carotid artery intima-media thickness progression as a predictor of stroke in Multi-Ethnic Study of Atherosclerosis. Stroke 2011;42:3017–21.

2. Azen SP, Mack WJ, Cashin-Hemphill L, et al. Progression of coronary artery disease predicts clinical coronary events. Long-term follow-up from the Cholesterol Lowering Atherosclerosis Study. Circulation 1996;93:34–41.

3. Lehmann N, Erbel R, Mahabadi AA, et al. Value of progression of coronary artery calcification for risk prediction of coronary and cardiovascular events: result of the HNR study (Heinz Nixdorf Recall). Circulation 2018;137:665–79.

4. McEvoy JW, Blaha MJ, Defilippis AP, et al. Coronary artery calcium progression: an important clinical measurement? A review of published reports. J Am Coll Cardiol 2010;56:1613–22.

5. Kronmal RA, McClelland RL, Detrano R, et al. Risk factors for the progression of coronary artery calcification in asymptomatic subjects: results from the Multi-Ethnic Study of Atherosclerosis (MESA). Circulation 2007;115:2722–30.

6. Herder M, Johnsen SH, Arntzen KA, Mathiesen EB. Risk factors for progression of carotid intima-media thickness and total plaque area: a 13-year follow-up study: the Tromso study. Stroke 2012;43:1818–23.

7. van der Meer IM, Iglesias del Sol A, Hak AE, Bots ML, Hofman A, Witteman JC. Risk factors for progression of atherosclerosis measured at multiple sites in the arterial tree: the Rotterdam Study. Stroke 2003;34:2374–9.

8. Chambless LE, Folsom AR, Davis V, et al. Risk factors for progression of common carotid atherosclerosis: the Atherosclerosis Risk in Communities Study, 1987-1998. Am J Epidemiol 2002;155:38–47.

9. Lorenz MW, Polak JF, Kavousi M, et al. Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data. Lancet (London, England) 2012;379:2053–62.

10. Rosvall M, Persson M, Ostling G, et al. Risk factors for the progression of carotid intima-media thickness over a 16-year follow-up period: the Malmo Diet and Cancer Study. Atherosclerosis 2015;239:615–21.

11. Johnson HM, Douglas PS, Srinivasan SR, et al. Predictors of carotid intima-media thickness progression in young adults: the Bogalusa Heart Study. Stroke 2007;38:900–5.

12. Salonen R, Salonen JT. Progression of carotid atherosclerosis and its determinants: a population-based ultrasonography study. Atherosclerosis 1990;81:33–40.

13. Finn AV, Kolodgie FD, Virmani R. Correlation between carotid intimal/medial thickness and atherosclerosis: a point of view from pathology. Arterioscler Thromb Vasc Biol 2010;30:177–81.

14. Inaba Y, Chen JA, Bergmann SR. Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis. Atherosclerosis 2012;220:128–33.

15. Peters SA, den Ruijter HM, Bots ML, Moons KG. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. Heart (British Cardiac Society) 2012;98:177–84.

16. Sturlaugsdottir R, Aspelund T, Bjornsdottir G, et al. Predictors of carotid plaque progression over a 4-year follow-up in the Reykjavik REFINE-study. Atherosclerosis 2018;269:57–62.

17. Fernandez-Ortiz A, Jimenez-Borreguero LJ, Penalvo JL, et al. The Progression and Early detection of Subclinical Atherosclerosis (PESA) study: rationale and design. Am Heart J 2013;166:990–8.

18. Fernandez-Friera L, Penalvo JL, Fernandez-Ortiz A, et al. Prevalence, vascular distribution, and multiterritorial extent of subclinical atherosclerosis in a middle-aged cohort: the PESA (Progression of Early Subclinical Atherosclerosis) study. Circulation 2015;131:2104–13.

19. Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2014;63:2889–934.

20. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol 2018;73:e285–350.

21. Lopez-Melgar B, Fernandez-Friera L, Sanchez-Gonzalez J, et al. Accurate quantification of atherosclerotic plaque volume by 3D vascular ultrasound using the volumetric linear array method. Atherosclerosis 2016;248:230–7.

22. Lopez-Melgar B, Fernandez-Friera L, Oliva B, et al. Subclinical atherosclerosis burden by 3D ultrasound in mid-life: the PESA study. J Am Coll Cardiol 2017;70:301–13.

23. Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim intima-media thickness consensus. Cerebrovasc Dis 2004;18:346–9.

24. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990;15:827–32.

25. Lehmann N, Mohlenkamp S, Mahabadi AA, et al. Effect of smoking and other traditional risk factors on the onset of coronary artery calcification: results of the Heinz Nixdorf recall study. Atherosclerosis 2014;232:339–45.

26. Wannarong T, Parraga G, Buchanan D, et al. Progression of carotid plaque volume predicts cardiovascular events. Stroke 2013;44:1859–65.

27. Budoff MJ, Hokanson JE, Nasir K, et al. Progression of coronary artery calcium predicts all-cause mortality. J Am Coll Cardiol Img 2010;3:1229–36.

28. Landry A, Spence JD, Fenster A. Measurement of carotid plaque volume by 3-dimensional ultrasound. Stroke 2004;35:864–9.

29. Landry A, Spence JD, Fenster A. Quantification of carotid plaque volume measurements using 3D ultrasound imaging. Ultrasound Med Biol 2005;31:751–62.

30. Califf RM, Phillips HR 3rd., Hindman MC, et al. Prognostic value of a coronary artery jeopardy score. J Am Coll Cardiol 1985;5:1055–63.

31. Budoff MJ, Young R, Lopez VA, et al. Progression of coronary calcium and incident coronary heart disease events: MESA (Multi-Ethnic Study of Atherosclerosis). J Am Coll Cardiol 2013;61:1231–9.

32. Henein MY, Koulaouzidis G, Granasen G, Wiklund U, Guerci A, Schmermund A. The natural history of coronary calcification: a meta-analysis from St Francis and EBEAT trials. Int J Cardiol 2013;168:3944–8.

33. Berry JD, Liu K, Folsom AR, et al. Prevalence and progression of subclinical atherosclerosis in younger adults with low short-term but high lifetime estimated risk for cardiovascular disease: the Coronary Artery Risk Development in Young Adults study and Multi-Ethnic Study of Atherosclerosis. Circulation 2009;119:382–9.

34. Criqui MH, Denenberg JO, Ix JH, et al. Calcium density of coronary artery plaque and risk of incident cardiovascular events. JAMA 2014;311:271–8.

35. Fernandez-Friera L, Fuster V, Lopez-Melgar B, et al. Normal LDL-cholesterol levels are associated with subclinical atherosclerosis in the absence of risk factors. J Am Coll Cardiol 2017;70:2979–91.

KEY WORDS cardiovascular risk, cardiovascular risk factors, coronary and noncoronary atherosclerosis progression, multiterritory noninvasive imaging, short-term atherosclerosis progression

APPENDIX For an expanded Methods section as well as supplemental figures and tables, please see the online version of this paper.