Prevalence, Vascular Distribution and Multi-territorial Extent of Subclinical Atherosclerosis in a Middle-Aged Population: The PESA (Progression of Early Subclinical Atherosclerosis) Study

Fernandez-Friera et al: Multi-territorial extent of early atherosclerosis

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ABSTRACT

Background: Data are limited regarding the presence, distribution and extent of subclinical atherosclerosis in middle-aged populations.

Methods and Results: The PESA (Progression of Early Subclinical Atherosclerosis) study prospectively enrolled 4184 asymptomatic participants aged 40-54 years (mean age 45.8 years, 63.3% male) to evaluate the systemic extent of atherosclerosis in the carotid, abdominal aortic and ilio-femoral territories by 2D/3D ultrasound and coronary artery calcification (CAC) by computed tomography. The extent of subclinical atherosclerosis, defined as presence of plaque or CAC≥1, was classified as focal (one site affected), intermediate (2-3 sites) or generalized atherosclerosis (4-6 sites) after exploring each vascular site (right/left carotids, aorta, right/left ilio-femorals and coronary arteries).

Subclinical atherosclerosis was present in 62.5% of participants (70.7% of men; 48.4% of women). Intermediate and generalized atherosclerosis was identified in 41.2%. Plaques were most common in the ilio-femorals (43.7%), followed by carotids (31.3%) and aorta (24.5%), while CAC was present in 17.7%. Among participants with low Framingham Heart Study (FHS) 10-year risk, subclinical disease was detected in 58.2%, with intermediate or generalized disease in 35.7%. When assessing longer-term risk (30-year Framingham Heart Study), 82.5% of participants at high-risk had atherosclerosis, with 65.8% classified as intermediate or generalized.

Conclusions: Subclinical atherosclerosis was highly prevalent in this middle-aged population, with almost 50% of participants classified as having intermediate or generalized disease. Most participants at high FHS risk had subclinical disease; nonetheless, extensive atherosclerosis was also present in a substantial number of low-risk individuals, suggesting added value of imaging for diagnosis and prevention.

Clinical Trial Registration: ClinicalTrials.gov. NCT01410318

KEYWORDS

Atherosclerosis, imaging, population, epidemiology, risk factors

ABBREVIATIONS

ABI: Ankle-brachial index

CAC: Coronary artery calcification

CACS: Coronary artery calcification score

CT: Computed tomography

CV: Cardiovascular

FHS: Framingham Heart Study

IMT: Intima-media thickness

The natural history of atherosclerosis involves a protracted subclinical phase, with disease often detected only at an advanced symptomatic stage or following a cardiovascular (CV) event. This is of particular importance as CV events are often fatal, and many deaths attributable to coronary artery disease are sudden[1]. There is thus a clear need to identify disease at an early stage, and as a result primary prevention forms the cornerstone of management. Currently, risk stratification scores for prevention rely on the presence of identifiable risk factors and levels of biochemical markers. However, conventional risk assessment has well-recognized limitations, notably in lower-risk groups such as women and younger people[2, 3]. Detection of atherosclerosis in its subclinical stage may help identify strategies to arrest disease development. The clinical significance of early detection was recently highlighted in the US High Risk Plaque Study, which has demonstrated an association between the presence of subclinical disease and CV events[4].

The introduction of noninvasive imaging techniques has unlocked the potential to evaluate atherosclerosis in asymptomatic populations. Specific imaging modalities include vascular ultrasound, computed tomography (CT), and magnetic resonance imaging[5, 6]. Many imaging studies evaluated individual vascular territories, but given the systemic nature of atherosclerosis a multi-territory analysis has the potential to provide a more comprehensive overview of the distribution and burden of atherosclerosis.

The PESA (Progression of Early Subclinical Atherosclerosis) study evaluates atherosclerosis in the carotid, aortic, coronary and ilio-femoral territories using accessible noninvasive imaging techniques in an asymptomatic middle-aged

population[7]. Through the evaluation of multiple vascular beds in relatively young individuals we aim to improve understanding of the origin and progression of atherosclerosis. Here, we present the prevalence, vascular distribution and extent of subclinical atherosclerosis in the PESA cohort and their relation to CV risk algorithms.

METHODS

Study population

The rationale and design of the PESA study has been previously described[7]. In brief, PESA-CNIC Santander is a prospective cohort study of asymptomatic employees of the Santander Bank in Madrid, aged 40 to 54 years and consecutively recruited between June 2010 and February 2014. Participants with prior CV disease and any condition reducing life expectancy or affecting study adherence were not included. Participants were examined at baseline by ankle-brachial index (ABI), vascular ultrasound and non-contrast CT, and will be followed up at 3- and 6-years. In addition, each visit includes clinical interviews, physical examination, fasting blood draw, urine sample, and a 12-lead electrocardiogram. The study protocol has been approved by the *Instituto de Salud Carlos III* Ethics Committee and all eligible participants have provided written informed consent.

CV risk factors were determined from blood samples and interviews, as follows: 1) diabetes: fasting plasma glucose ≥126 mg/dL, or treatment with insulin or oral hypoglycemic medication[8]; 2) arterial hypertension: systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or use of antihypertensive medication[8]; 3) dyslipidemia: total cholesterol ≥240 mg/dL, LDL-cholesterol ≥160 mg/dL, HDL-cholesterol <40 mg/dL, or use of lipid-lowering drugs[9]; 4) smoking: current smoking

status, or a lifetime consumption of >100 cigarettes[5, 10]; 5) family history of CV disease: first degree-relative diagnosed with atherosclerosis before the age of 55 years in men and 65 in women[11]. CV risk was assessed by the 10-year risk of coronary heart disease and the 30-year risk of CV disease from the Framingham Heart Study (FHS)[10, 12], and additionally by the European Society of Cardiology's SCORE (Systematic COronary Risk Evaluation), which calculates 10-year risk of fatal CV disease[11]. Low, moderate and high-risk cut-off values were <10%, ≥10 to 20% and >20% for the FHS and <1%, ≥1 to <5%, ≥5 for the European SCORE.

Vascular ultrasound imaging

The 2D/3D vascular ultrasound protocol has been described[13]. Presence of atherosclerotic plaques was assessed by cross-sectional sweep of carotids, infra-renal abdominal aorta and ilio-femoral arteries. Plaque was defined as a focal protrusion into the arterial lumen of thickness >0.5 mm or >50% of the surrounding intima-media thickness (IMT), or a diffuse thickness >1.5 mm measured between the media-adventitia and intima-lumen interfaces[13, 14]. Semi-automated detection of IMT was also assessed at the level of the posterior wall of the common carotid and femoral arteries in longitudinal views. Values >0.9 were considered abnormal, as established in previous studies[14] and guidelines[15]. Ultrasound studies were analyzed with QLab9 (Philips Healthcare, Bothel, Seattle) at the CNIC Core Imaging Laboratory, as reported[13]. Good reproducibility was found for presence of plaque in all territories (kappa = 0.75 for carotids, 0.89 for aorta, 0.88 for ilio-femorals). Similarly, excellent intraclass correlation coefficient (ICC>95%) was found for both carotid and femoral IMT measurements. Imaging quality was evaluated as optimal, suboptimal or non-interpretable, and inclusion of studies was determined by consensus. The ABI was

calculated as the ratio of the systolic blood pressure in the posterior tibial artery to the systolic blood pressure in the brachial artery using Doppler ultrasound and a standard sphygmomanometer. Values of ABI<0.9[16] were considered abnormal.

Coronary artery calcification by computed tomography

Coronary artery calcification (CAC) was detected with a 16-slice CT scanner (Philips Brilliance, Philips Healthcare, Andover) using non-contrast prospective electrocardiography-gated acquisition. Estimated absorbed radiation was 0.6-1.2 mSv. CAC score (CACS) was calculated by the Agatston method and graded as 1-99, 100-399 and ≥400[17]. CACS was quantified by three trained technicians blinded to other imaging results and supervised by experienced physicians.

Definition of subclinical atherosclerosis

Subclinical atherosclerosis was defined as the presence of atherosclerotic plaques in the carotid, aortic or ilio-femoral territories or CACS≥1. The multi-territorial extent of subclinical atherosclerosis was defined according to the number of vascular sites affected (right carotid, left carotid, abdominal aorta, right ilio-femoral, left ilio-femoral and coronary arteries). Participants were classified as disease-free (0 vascular sites affected) or having focal (1 site), intermediate (2-3 sites) or generalized atherosclerosis (4-6 sites).

Statistical analysis

Baseline characteristics were calculated using mean and standard deviation for continuous variables and count and proportions for categorical variables. Age and gender-adjusted associations between risk factors and vascular disease in each territory

were examined using logistic regression models. The reproducibility of ultrasound measurements was studied by replicating the analysis of a random sample of 60 studies 3 months after the initial assessment. Cohen's Kappa and intraclass correlation coefficients were used to quantity agreement between measurements. Statistical analyses were conducted using Stata 12 (StataCorp, College Station, TX, USA).

RESULTS

The PESA cohort comprised 4184 participants (78% of the eligible population). At the time of publication, 34 individuals (0.8%) had discontinued the study and 84 (2.0%) were either excluded for missing data or were pending evaluation, resulting in 4066 participants eligible for analysis. After excluding non-interpretable images (64 participants, 1.5%), the population available for imaging analysis was 4002 (98% of the cohort). Only 1 case of abnormal ABI was detected in the first 2536 participants, and therefore the protocol was amended to discontinue measurement of ABI in subsequent examinations. Baseline demographic characteristics and traditional risk factors are summarized in Table 1. The average age of participants was 45.8 years, 63.3% were male and 99.9% Caucasian. The most prevalent risk factor was dyslipidemia (41.6%), followed by smoking (20.5%), family history (15.9%), hypertension (11.8%), and diabetes (2.0%). Overall, the presence of risk factors was higher in men, with the exception of smoking (23.4% women and 18.9% men) and family history (16.6% women and 15.5% men). Most participants (62.1%) had at least one risk factor, 18.4% had two and 5.2% had three or more. Aside from family history, the prevalence of risk factors increased with age except for smoking and diabetes in women (Table S1). There were no significant differences on the distribution of the group not included in the imaging analysis (1.5%), thus excluding the introduction of systematic bias.

Prevalence, vascular distribution and extent of subclinical atherosclerosis

Overall prevalence of subclinical atherosclerosis (presence of plaque or CACS≥1) was 62.5%. Plaques were detected by ultrasound in 60.2% of participants (31.3% in the carotids, 24.5% in the aorta and 43.7% in the ilio-femoral arteries) and 17.7% had CAC (CACS 1-99 in 14.4%, 100-399 in 2.6% and ≥400 in 0.7%). Of those with CACS≥1, 87.4% had plaques present at other vascular sites, whereas 54.4% of participants with CACS=0 had plaques in other territories. Classification of participants according to the extent of atherosclerosis showed that disease was focal in 21.2%, intermediate in 27.8% and generalized in 13.4%.

Subclinical atherosclerosis was more prevalent in men (70.7% vs. 48.4% in women) across all vascular territories, with differences most pronounced in the ilio-femoral and coronary arteries (Figure 1). Of the 23 participants with CACS≥400, only one was female (Table S1). Atherosclerosis prevalence increased with age for both genders and across all vascular territories, and only the presence of aortic disease was found to be independent of gender (Figure 2). The prevalence of atherosclerosis affecting multiple vascular sites was greater in men, increased with age, and was related to the presence of CV risk factors (Figure S1, Table S2). Notably, the extent of subclinical atherosclerosis in men aged 40-44 years, was similar to that in women 5 to 10 years older (Figure S1).

Associations between risk factors and subclinical atherosclerosis in different territories are shown in Table 2. Age and male gender were significantly associated with the presence of plaque in any territory, and particularly with CAC. All risk factors were associated with atherosclerosis across all territories, although results did not reach

significance for the association of diabetes or hypertension with aortic disease and of family history with carotid disease. Diabetes was most strongly associated with carotid disease and CAC, whereas smoking had a stronger association with aortic and ilio-femoral disease and family history had a stronger association with CAC. The presence of ilio-femoral disease was more strongly correlated with aortic disease and CAC than with carotid disease. Furthermore, having disease in the ilio-femorals corresponds to a 69.7% probability of finding disease in any other territory explored. Conversely, the absence of plaque in the ilio-femorals confers a 66.7% probability of being disease-free in the other vascular territories. In participants with carotid disease, the odds ratio for co-existing plaque was slightly higher in the aortic and ilio-femoral territories compared with CAC, and the probability of subclinical atherosclerosis in any other territory was 71.7%, with a negative predictive value of 54.6%.

Intima-media thickness and subclinical atherosclerosis

Mean IMT was 0.59 mm in carotid arteries and 0.61 mm in femoral arteries. Abnormal IMT (>0.9 mm)[14] was detected in 8.7% of participants, and was more frequent in femoral than in carotid arteries (8.4% vs. 0.8%). Prevalence of abnormal IMT was higher in men and increased with age (Table S1). The sensitivity, specificity, and positive and negative predictive values for IMT to detect subclinical atherosclerosis were 13.8%, 99.8%, 99.1% and 41.0%, respectively.

CV risk scales and subclinical atherosclerosis

The FHS 10-year score was 5.9%, and most participants (85.0%) were at low-risk, compared with 13.6% at moderate risk and 1.4% at high risk. Similarly, most individuals (84.5%) were at low-risk by the European SCORE compared with 15.4% at

moderate and 0.07% at high risk. To assess longer-term risk, mean FHS 30-year score was calculated, yielding a mean value of 17.8% and higher proportions of participants at moderate and high-risk (30.3% and 35.0%, respectively).

The relationship between risk categories according to CV risk scales and subclinical atherosclerosis is shown in Figures 3 and 4. Among participants at low 10-year FHS risk, 58.2% had subclinical atherosclerosis, including 35.7% with intermediate or generalized disease. Most FHS high-risk participants (94.6%) had atherosclerosis, with intermediate or generalized disease in 85.5%. Similarly, among participants with a low European SCORE, 57.5% had subclinical atherosclerosis, including 34.7% with intermediate or generalized disease, and all high-risk participants had intermediate or generalized disease. Given the low numbers of individuals at high-risk using the 10-year FHS scale (55 participants) or the European SCORE (3 participants), we also examined the relationship between atherosclerosis and 30-year FHS score. Among individuals at high 30-year FHS risk, 82.5% had subclinical atherosclerosis, with intermediate or generalized disease in 65.8%, whereas of low-risk individuals, 43.1% had atherosclerosis and 21.8% had intermediate or generalized disease. Such associations were also observed for each vascular territory analyzed separately (Figures S3, S4 and S5).

Participants classified at low-risk but who had ≥2 vascular sites affected were further analyzed to investigate the involvement of specific arterial sites. In individuals with intermediate or generalized atherosclerosis and a low 10-year FHS risk (n= 1215), prevalence of ilio-femoral disease was 38.4%, carotid disease 28% and aortic disease 22.1%, and CACS≥1 was 13.7%. Using the European SCORE, disease prevalence in

low-risk individuals with intermediate or generalized disease (n= 1174) was 43.4% for ilio-femoral arteries, 31.0% for carotids, 24.3% for aorta and 17.4% for CACS≥1.

In light of recent US guidelines for statin therapy[18], a further analysis was performed using the 10-year ASCVD risk algorithm, an atherosclerotic CV risk calculator based on Pooled Cohort Equations[6, 18], to compare individuals at ≥7.5% and <7.5% risk. The ≥7.5% 10-year ASCVD risk population (9.9%) had a significantly higher prevalence of subclinical atherosclerosis (91.5% vs 59.6%, p<0.001) and of intermediate and generalized disease (35.8% and 27.1% vs 42.5% and 10.3%; p<0.001). Comparison by vascular territory revealed carotid disease in 57.0% vs 28.6%, aortic disease in 42.3% vs 22.7%, CACS≥1 in 45.1% vs 14.8%, and ilio-femoral disease in 79.4% vs 40.1% for the ≥7.5% vs <7.5% risk groups, respectively (p<0.001 for all comparisons).

DISCUSSION

The main findings from the PESA cohort are as follows: 1) Subclinical atherosclerosis is highly prevalent in this middle-aged asymptomatic population; 2) The ilio-femoral territory is the most frequently affected vascular site in the early stages of atherosclerosis; 3) Most individuals classified at high-risk by traditional risk scales (FHS and European SCORE), had subclinical atherosclerosis, but atherosclerosis is also present in nearly 60% of participants classified at low risk, with intermediate or generalized disease in one-third. Ongoing long-term PESA follow-up over at least 6 years will enable the study of associations between subclinical disease evaluated at baseline and subsequent occurrence of CV events.

Subclinical atherosclerosis is prevalent in a middle-aged population

Few population studies have investigated the prevalence and extent of subclinical atherosclerosis across multiple vascular sites in a middle-aged population, despite the fact that atherosclerosis is a systemic disease with a long latent subclinical phase. In the MESA and CARDIA studies, evaluation of atherosclerosis was limited to carotid IMT and CAC[3, 5]. The ARIC study focused only on carotid and popliteal territories assessed by ultrasound[19]. The Heinz Nixdorf Recall, High Risk Plaque, and Rotterdam studies recruited older populations with prior CV disease or a high-risk profile[1, 20, 21]. Using an innovative multi-territory evaluation, the PESA study detects a high prevalence of subclinical disease, with almost 50% of participants classified as having intermediate or generalized disease despite being predominantly at low-risk according to traditional risk scoring systems. This finding is probably due to the examination of several territories, including vascular areas more susceptible to disease such as the ilio-femoral arteries, which were not explored in earlier studies. Other studies that did investigate multiple vascular sites included only men with at least one risk factor[22], examined participants at higher risk[23], utilized basic imaging techniques (for example, aortic calcification by CT), or explored fewer territories[24].

The added value of a multi-territory vascular evaluation of CV risk is supported by a study of 10,000 participants, showing that scanning only carotids or only femorals predicts on average 15% and 13% fewer events than examining both territories[25]. This finding supports the view that the wider sampling that comes from exploring several territories overcomes the problem of not detecting a lesion when examining only one territory. The predictive value of multi-territory imaging will be assessed in detail with the appearance of events during PESA follow-up. The prognostic relevance of subclinical atherosclerosis is supported by the US High Risk Plaque Study, which has

shown strong associations between CV events and presence of subclinical carotid and coronary disease[4]. Also, the Northern Manhattan Study demonstrated that subclinical carotid plaque is a precursor of CV events[26]. In the CAFES-CAVE study, the evaluation of subclinical disease in the carotids and also in the femoral arteries was associated with CV events in a 10-year follow-up[25]. These studies highlight the potential value of evaluating subclinical atherosclerosis in multiple territories for CV event prediction.

The association of increased prevalence of subclinical atherosclerosis with male gender and increased age is consistent with previous reports[27], and may be related to the natural history of the disease. Interestingly, the risk of atherosclerosis in men is similar to that in women aged five to ten years older in this cohort. This discovery may assist in determining the most appropriate time window for atherosclerosis screening and intensification of primary CV prevention. We also found that individuals at ≥7.5% 10-year ASCVD risk had substantial atherosclerosis compared with lower-risk individuals, especially regarding CAC, and had a 4-fold higher prevalence of generalized disease. This interesting finding is in line with the proposed intensification of statin treatment in the most recent guidelines on the treatment of blood cholesterol[6].

Consistent with previous studies[28-30], IMT measurements did not correlate with the presence of subclinical atherosclerosis (presence of plaque or CACS≥1). Prevalence of abnormal IMT was low (8.7%), especially in the carotid arteries (0.8%), despite the overall high prevalence of subclinical disease (62.5%). The increasing rates of abnormal IMT with age suggest a confounding influence of factors not directly related to atherosclerosis. In addition, almost all participants with abnormal IMT also had disease (99.1%), indicating that IMT provides little information that cannot be derived by

directly determining the presence of plaques. Further evidence of the limited utility of IMT for assessing subclinical atherosclerosis is provided by its low sensitivity in our population (13.8%), the lack of standard cut-off values for normal age reference intervals[31], and its reported poor predictive value for CV disease compared with plaque-based markers[29]. In this regard, the most recent American College of Cardiology/American Heart Association (ACC/AHA) Guide on the assessment of CV risk no longer recommends carotid IMT for routine clinical assessment of the risk of a first atherosclerotic CV event[6].

The ilio-femoral territory is the most frequently affected vascular site

The clear predominance of disease in the ilio-femoral arteries is possibly related to specific patterns of shear stress and disturbed flow caused by the vessel curvature[32]. Early detection of ilio-femoral disease is important since advanced stages of peripheral artery disease are associated with a 4-fold higher risk of myocardial infarction and a 2- to 3-fold greater risk of stroke[33, 34]. The ilio-femoral territory has not been examined as extensively as the carotids and CAC; however, compared with carotid disease, peripheral artery disease has been shown to have comparable predictive power for CV events[25]. Moreover, compared with coronary artery disease, peripheral disease carries a greater risk of all-cause of mortality, CV death, stroke, myocardial infarction and hospitalization for atherotrombotic events (21.1% vs 15.2%)[35]. This high morbimortality translates into a high economic burden, with costs for treatment and 1-year follow-up of peripheral artery disease ~5% more than for coronary artery disease[36].

In PESA participants, the presence of ilio-femoral disease increases the risk of concurrent CAC and is predictive of disease elsewhere. Moreover, the absence of ilio-

femoral disease is strongly associated with the absence of atherosclerosis at other vascular sites. Thus, imaging of peripheral arteries may be a useful population-wide screening tool for detecting atherosclerosis in its early stages. Indeed, evaluation of the ilio-femoral arteries appears to be more valuable than CAC for detecting subclinical atherosclerosis, given the high prevalence (82.3%) of a zero CACS in this low-risk middle-aged population. It is also interesting that most participants with CAC also had plaques at other vascular sites, while half of participants without CAC were not disease-free, indicating that CAC represents a more advanced stage of disease. ABI measurement for early stages of atherosclerosis is likely to be of limited value given the low prevalence of abnormal ABI in this young population (40-54 years), contrasting with the association detected between ABI and subclinical disease in older participants[37].

Subclinical atherosclerosis and traditional CV risk scales

Current risk stratification strategies have successfully identified individuals at risk of CV events. Traditional risk scales include the widely used FHS and SCORE, an adaptation of FHS that avoids risk overestimation in European populations with less coronary heart disease[11, 38]. However, the impact of these scales in younger, low-risk populations is limited, with many individuals still suffering CV events and little success in promoting lifestyle changes[39]. In a cohort of 122,458 patients with coronary disease, 9%-13% of those aged <55 years had no conventional risk factors[40], indicating disparity between traditional risk factors and the presence of disease in younger populations.

Although the FHS and the European SCORE scales were designed to assess risk of CV events derived from atherosclerosis, and not the presence of subclinical atherosclerosis,

we aimed to complement these predictive models by comparing the presence and extent of subclinical disease across different risk categories. Interestingly, most individuals classified at high-risk by both algorithms have subclinical atherosclerosis, with a high proportion having intermediate or generalized disease. Moreover, subclinical atherosclerosis is present in nearly 60% of PESA participants at low risk, with one third having at least two sites affected. In this regard, a sub-study of the MESA and CARDIA population detected higher carotid IMT and higher CAC in individuals with low 10-year but high lifetime risk compared with individuals with low 10-year and low lifetime risk[3]. Together these results strongly suggest an association of atherosclerosis with characteristics different from the classic CV risk factors not considered in standard risk scales that will be the basis for extensive further investigation in PESA (exercise, sleeping disorders, etc.). Multi-territory vascular imaging appears to have the potential to help identify new factors and thus complement traditional risk scales, helping to achieve the goal of individualized risk assessment.

Limitations

This study presents a cross-sectional analysis of the PESA cohort at baseline, and therefore cannot yet evaluate clinical events, precluding the possibility of establishing causality; these current findings will be complemented by long-term follow up monitoring of atherosclerosis progression. The PESA population consists of middle-aged, predominantly male white-collar workers, which may limit the generalizability of the results. Detection of plaques in the iliac arteries may be limited by the penetration of the vascular probe used and the presence of air (22.9% of iliac studies were suboptimal, compared with 2.3% for carotids, 10.2% for aorta, and 5.8% for femorals). However, a further evaluation of variability in the iliac arteries showed good results (kappa = 0.84),

and only 1.2% of iliac studies were non-interpretable. When classifying the extent of subclinical atherosclerosis, aortic and coronary sites were each considered as single territories, and greater weight was therefore given to carotid and ilio-femoral territories; however, the multi-territorial extent of disease includes the concept of laterality and introduces a novel evaluation of atherosclerosis. Although CAC scan is a well-established test for the evaluation of subclinical coronary disease, it is not suitable for non-calcified plaques. In the interest of clarity and ease of clinical application, atherosclerotic plaques and CAC were considered as dichotomized variables (presence or absence) to evaluate the extent of subclinical atherosclerosis.

In conclusion, subclinical atherosclerosis is highly prevalent in a middle-aged asymptomatic population, with almost half the participants presenting with intermediate or generalized disease. Prevalence is higher in men and in the ilio-femoral arteries, highlighting the value of screening this territory for early detection of atherosclerosis. As a substantial proportion of low-risk participants had subclinical atherosclerosis, imaging of early atherosclerosis may be particularly valuable in this setting. Long-term follow up will determine whether early detection of subclinical atherosclerosis has any impact on predicting and preventing CV events.

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DISCLOSURES

None.

REFERENCES

1. Erbel R, Delaney JA, Lehmann N, McClelland RL, Mohlenkamp S, Kronmal RA, Schmermund A, Moebus S, Dragano N, Stang A, Jockel KH, Budoff MJ. Signs of subclinical coronary atherosclerosis in relation to risk factor distribution in the Multi-Ethnic Study of Atherosclerosis (MESA) and the Heinz Nixdorf Recall Study (HNR). *Eur Heart J.* Nov 2008;29(22):2782-2791.

2. Sibley C, Blumenthal RS, Merz CN, Mosca L. Limitations of current cardiovascular disease risk assessment strategies in women. *J Womens Health (Larchmt).* Jan-Feb 2006;15(1):54-56.

3. Berry JD, Liu K, Folsom AR, Lewis CE, Carr JJ, Polak JF, Shea S, Sidney S, O'Leary DH, Chan C, Lloyd-Jones DM. 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.* Jan 27 2009;119(3):382-389.

4. Baber U SS, Schoos M, Sillesen H, Muntendam P, Garcia M, Gregson J, Pocock S, Falk E, Fuster V. Prevalence, Impact and Predictive Value of Detecting Subclinical Coronary and Carotid Atherosclerosis In Asymptomatic Adults: Insights from the BioImage Study. *J Am Coll Cardiol.* 2014;63(12 S):998.

5. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR, Jr., Kronmal R, Liu K, Nelson JC, O'Leary D, Saad MF, Shea S, Szklo M, Tracy RP. Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol.* Nov 1 2002;156(9):871-881.

6. Goff DC, Jr., Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, Greenland P, Lackland DT, Levy D, O'Donnell CJ, Robinson JG, Schwartz

JS, Shero ST, Smith SC, Jr., Sorlie P, Stone NJ, Wilson PW, Jordan HS, Nevo L, Wnek J, Anderson JL, Halperin JL, Albert NM, Bozkurt B, Brindis RG, Curtis LH, DeMets D, Hochman JS, Kovacs RJ, Ohman EM, Pressler SJ, Sellke FW, Shen WK, Smith SC, Jr., Tomaselli GF. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation.* 2014;129(25 Suppl 2):S49-73.

7. Fernandez-Ortiz A, Jimenez-Borreguero LJ, Penalvo JL, Ordovas JM, Mocoroa A, Fernandez-Friera L, Laclaustra M, Garcia L, Molina J, Mendiguren JM, Lopez-Melgar B, de Vega VM, Alonso-Farto JC, Guallar E, Sillesen H, Rudd JH, Fayad ZA, Ibanez B, Sanz G, Fuster V. The Progression and Early detection of Subclinical Atherosclerosis (PESA) study: rationale and design. *Am Heart J.* Dec 2013;166(6):990-998.

8. Pearson T PL, Artinian N, Carnethon M, Criqui M, Daniels S, Fonarow G, Fortmann S, Franklin B, Galloway J, Goff D, Heath G, Holland Frank A, Kris-Etherton P, Labarthe D, Murabito J, Sacco R, Sasson C, Turner M. American Heart Association Guide for Improving Cardiovascular Health at the Community Level, 2013, Update. A scientific Statement for Public Health Practinioners, Healthcare Providers and Health Policy Makers *Circulation.* 2013;127:1730-1753.

9. Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA.* May 16 2001;285(19):2486-2497.

10. Ford ES, Giles WH, Mokdad AH. The distribution of 10-Year risk for coronary heart disease among US adults: findings from the National Health and Nutrition Examination Survey III. *J Am Coll Cardiol.* May 19 2004;43(10):1791-1796.

11. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syvanne M, Scholte op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). *Eur Heart J.* Jul 2012;33(13):1635-1701.

12. Pencina MJ, D'Agostino RB, Sr., Larson MG, Massaro JM, Vasan RS. Predicting the 30-year risk of cardiovascular disease: the framingham heart study. *Circulation.* Jun 23 2009;119(24):3078-3084.

13. Sillesen H, Muntendam P, Adourian A, Entrekin R, Garcia M, Falk E, Fuster V. Carotid plaque burden as a measure of subclinical atherosclerosis: comparison with other tests for subclinical arterial disease in the High Risk Plaque BioImage study. *JACC Cardiovasc Imaging.* Jul 2012;5(7):681-689.

14. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Desvarieux M, Ebrahim S, Fatar M, Hernandez Hernandez R, Kownator S, Prati P, Rundek T, Taylor A, Bornstein N, Csiba L, Vicaut E, Woo KS, Zannad F, Advisory Board of the 3rd Watching the Risk Symposium tESC. Mannheim intima-media thickness consensus. *Cerebrovasc Dis.* 2004;18(4):346-349.

15. Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, Dallongeville J, De Backer G, Ebrahim S, Gjelsvik B, Herrmann-Lingen C, Hoes A, Humphries S, Knapton M, Perk J, Priori SG, Pyorala K, Reiner Z, Ruilope L, Sans-Menendez S, Op Reimer WS, Weissberg P, Wood D, Yarnell J, Zamorano JL, Walma E, Fitzgerald T, Cooney MT, Dudina A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Funck-Brentano C, Filippatos G, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Altiner A, Bonora E, Durrington PN, Fagard R, Giampaoli S, Hemingway H, Hakansson J, Kjeldsen SE, Larsen m L, Mancia G, Manolis AJ, Orth-Gomer K, Pedersen T, Rayner M, Ryden L, Sammut M, Schneiderman N, Stalenhoef AF, Tokgozoglu L, Wiklund O, Zampelas A. European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). *Eur J Cardiovasc Prev Rehabil.* Sep 2007;14 Suppl 2:S1-113.

16. Heald CL, Fowkes FG, Murray GD, Price JF, Ankle Brachial Index C. Risk of mortality and cardiovascular disease associated with the ankle-brachial index: Systematic review. *Atherosclerosis.* Nov 2006;189(1):61-69.

17. 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.* Mar 15 1990;15(4):827-832.

18. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC, Jr., Watson K, Wilson PW. 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.* Jul 1 2014;63(25 Pt B):2889-2934.

19. Investigators TA. The Atherosclerosis Risk in Communities Study: design and objectives. *Am J Epidemiol.* 2002;156:871-881.

20. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, Kuipers EJ, Nijsten TE, Stricker BH, Tiemeier H, Uitterlinden AG, Vernooij MW, Witteman JC. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol.* Aug;26(8):657-686.

21. Falk E, Sillesen H, Muntendam P, Fuster V. The high-risk plaque initiative: primary prevention of atherothrombotic events in the asymptomatic population. *Curr Atheroscler Rep.* Oct 2011;13(5):359-366.

22. Simon A, Giral P, Levenson J. Extracoronary atherosclerotic plaque at multiple sites and total coronary calcification deposit in asymptomatic men. Association with coronary risk profile. *Circulation.* Sep 15 1995;92(6):1414-1421.

23. Megnien JL, Sene V, Jeannin S, Hernigou A, Plainfosse MC, Merli I, Atger V, Moatti N, Levenson J, Simon A. Coronary calcification and its relation to extracoronary atherosclerosis in asymptomatic hypercholesterolemic men. The PCV METRA Group. *Circulation.* May 1992;85(5):1799-1807.

24. Karim R, Hodis HN, Detrano R, Liu CR, Liu CH, Mack WJ. Relation of Framingham risk score to subclinical atherosclerosis evaluated across three arterial sites. *Am J Cardiol.* Oct 1 2008;102(7):825-830.

25. Belcaro G, Nicolaides AN, Ramaswami G, Cesarone MR, De Sanctis M, Incandela L, Ferrari P, Geroulakos G, Barsotti A, Griffin M, Dhanjil S, Sabetai

M, Bucci M, Martines G. Carotid and femoral ultrasound morphology screening and cardiovascular events in low risk subjects: a 10-year follow-up study (the CAFES-CAVE study(1)). *Atherosclerosis.* Jun 2001;156(2):379-387.

26. Rundek T, Arif H, Boden-Albala B, Elkind MS, Paik MC, Sacco RL. Carotid plaque, a subclinical precursor of vascular events: the Northern Manhattan Study. *Neurology.* Apr 1 2008;70(14):1200-1207.

27. Jain A, McClelland RL, Polak JF, Shea S, Burke GL, Bild DE, Watson KE, Budoff MJ, Liu K, Post WS, Folsom AR, Lima JA, Bluemke DA. Cardiovascular imaging for assessing cardiovascular risk in asymptomatic men versus women: the multi-ethnic study of atherosclerosis (MESA). *Circ Cardiovasc Imaging.* Jan 2011;4(1):8-15.

28. 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.* Jan 2012;220(1):128-133.

29. Yerly P, Rodondi N, Viswanathan B, Riesen W, Vogt P, Bovet P. Association between conventional risk factors and different ultrasound-based markers of atherosclerosis at carotid and femoral levels in a middle-aged population. *Int J Cardiovasc Imaging.* Mar 2013;29(3):589-599.

30. Lorenz MW, Schaefer C, Steinmetz H, Sitzer M. Is carotid intima media thickness useful for individual prediction of cardiovascular risk? Ten-year results from the Carotid Atherosclerosis Progression Study (CAPS). *Eur Heart J.* Aug 2010;31(16):2041-2048.

31. Engelen L, Ferreira I, Stehouwer CD, Boutouyrie P, Laurent S, Reference Values for Arterial Measurements C. Reference intervals for common carotid

intima-media thickness measured with echotracking: relation with risk factors. *Eur Heart J.* Aug 2013;34(30):2368-2380.

32. Gallino A, Aboyans V, Diehm C, Cosentino F, Stricker H, Falk E, Schouten O, Lekakis J, Amann-Vesti B, Siclari F, Poredos P, Novo S, Brodmann M, Schulte KL, Vlachopoulos C, De Caterina R, Libby P, Baumgartner I. Non-coronary atherosclerosis. *Eur Heart J.* Mar 3 2014;35(17):112-9.

33. Faxon DP, Creager MA, Smith SC, Jr., Pasternak RC, Olin JW, Bettmann MA, Criqui MH, Milani RV, Loscalzo J, Kaufman JA, Jones DW, Pearce WH. Atherosclerotic Vascular Disease Conference: Executive summary: Atherosclerotic Vascular Disease Conference proceeding for healthcare professionals from a special writing group of the American Heart Association. *Circulation.* Jun 1 2004;109(21):2595-2604.

34. Aronow H, Hiatt WR. The burden of peripheral artery disease and the role of antiplatelet therapy. *Postgrad Med.* Jul 2009;121(4):123-135.

35. Steg PG, Bhatt DL, Wilson PW, D'Agostino R, Sr., Ohman EM, Rother J, Liau CS, Hirsch AT, Mas JL, Ikeda Y, Pencina MJ, Goto S, Investigators RR. One-year cardiovascular event rates in outpatients with atherothrombosis. *JAMA.* Mar 21 2007;297(11):1197-1206.

36. MR J. Peripheral artery disease costlier to treat than coronary artery disease. *International Simposium on Endovascular Therapy, Hollywood, FL.* 2008.

37. O'Hare AM, Katz R, Shlipak MG, Cushman M, Newman AB. Mortality and cardiovascular risk across the ankle-arm index spectrum: results from the Cardiovascular Health Study. *Circulation.* Jan 24 2006;113(3):388-393.

38. Hense HW, Schulte H, Lowel H, Assmann G, Keil U. Framingham risk function overestimates risk of coronary heart disease in men and women from Germany--

results from the MONICA Augsburg and the PROCAM cohorts. *Eur Heart J.* May 2003;24(10):937-945.

39. Wister A, Loewen N, Kennedy-Symonds H, McGowan B, McCoy B, Singer J. One-year follow-up of a therapeutic lifestyle intervention targeting cardiovascular disease risk. *Cmaj.* Oct 9 2007;177(8):859-865.

40. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, Ellis SG, Lincoff AM, Topol EJ. Prevalence of conventional risk factors in patients with coronary heart disease. *Jama.* Aug 20 2003;290(7):898-904.

FIGURE LEGENDS

Figure 1. Prevalence of subclinical atherosclerosis by vascular territories in PESA.

Figure 2. Prevalence of subclinical atherosclerosis by age and gender in each vascular territory.

Figure 3. Distribution of subclinical atherosclerosis detected by noninvasive imaging according to Framingham Heart Study risk categories. Vascular sites examined were the right and left carotids, abdominal aorta and the right and left ilio-femoral arteries (presence of plaque), and the coronary vessels (CAC). FHS scores were classified as low (<10%), moderate (≥10 to 20%) or high risk (>20%)[10, 12].

Figure 4. Distribution of subclinical atherosclerosis detected by noninvasive imaging according to European SCORE categories. European SCORE was classified as low (<1%), moderate (≥1 to <5%), or high risk (≥5 to <10%)[11].

Table 1. Demographic characteristics and traditional cardiovascular risk factors

	Total (n=4066)	Men (n=2573)	Women (n=1493)	P value
Baseline characteristics				
Age (years)	45.8±4.3	46.3±4.4	45±3.9	<0.001
BMI (kg/m^2)	26.2±3.8	27.4±3.4	24.1±3.6	<0.001
SBP (mmHg)	116±12.5	121±11.1	109±11	<0.001
DBP (mmHg)	72.5±9.4	74.7±9.1	68.7±8.7	<0.001
Total cholesterol (mg/dL)	201±33.3	203±34.2	196±31.2	<0.001
LDLc (mg/dL)	132±29.8	136±30.3	125±27.5	<0.001
HDLc (mg/dL)	49±12.2	44.8±10.2	56.3±11.9	<0.001
Triglycerides (mg/dL)	95±57.2	109±64	70.6±29.9	<0.001
Fasting glucose (mg/dL)	90.6±13.8	93.4±15	85.7±9.7	<0.001
HbA1c (%)	5.44±0.5	5.49±0.5	5.36±0.4	<0.001
Lipid-lowering therapy	287(7.1)	242(9.4)	45(3)	<0.001
Antihypertensive therapy	309(7.6)	266(10.3)	43(2.9)	<0.001
Antidiabetic therapy	64(1.6)	56(2.2)	8(0.5)	<0.001
CV risk factors				
Dyslipidemia	1691(41.6)	1374(53.4)	317(21.2)	<0.001
Total cholesterol≥240 mg/dL	475(11.7)	345(13.4)	130(8.7)	<0.001
LDLc≥160 mg/dL	688(16.9)	522(20.3)	166(11.1)	<0.001
HDLc<40mg/dL	983(24.2)	880(34.2)	103(6.9)	<0.001
Current smoking	835(20.5)	486(18.9)	349(23.4)	<0.001
Hypertension	481(11.8)	409(15.9)	72(4.8)	<0.001
Diabetes	81(2.0)	72(2.8)	9(0.6)	<0.001
Family history of CV disease	646(15.9)	398(15.5)	248(16.6)	0.337
Number of CV risk factors				
0	1535(37.7)	777(30.2)	758(50.8)	<0.001
1	1572(38.7)	1053(40.9)	519(34.8)	<0.001
2	746(18.4)	569(22.1)	177(11.8)	<0.001
>2	213(5.2)	174(6.8)	39(2.6)	<0.001

Data are expressed as mean ± SD or n (%). BMI: Body mass index. CV: Cardiovascular; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; LDLc: Low-density lipoprotein cholesterol; HDLc: High-density lipoprotein cholesterol.

Table 2. Association between the presence of atherosclerosis in individual vascular territories and age, gender, risk factors and vascular distribution of disease

	Carotid disease	Coronary calcification	Aortic disease	Ilio-femoral disease
Age and gender				
40-44 years	1 (ref)	1 (ref)	1 (ref)	1 (ref)
45-49 years	1.45(1.23-1.70) <0.001	1.88(1.52-2.33) <0.001	1.72(1.44-2.06) <0.001	2.06(1.78-2.39) <0.001
50-54 years	2.54(2.15-3.01) <0.001	5.09(4.13-6.28) <0.001	3.20(2.67-3.84) <0.001	4.22(3.56-5.00) <0.001
Female	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Male	1.83(1.58-2.11) <0.001	7.04(5.43-9.13) <0.001	1.25(1.08-1.46) 0.004	2.76(2.41-3.17) <0.001
Cardiovascular risk factors				
Hypertension	1.36(1.11-1.68) 0.003	1.42(1.13-1.80) 0.003	1.10(0.88-1.37) 0.422	1.33(1.07-1.64) 0.009
Diabetes	1.89(1.17-3.04) 0.009	2.18(1.33-3.60) 0.002	1.39(0.86-2.27) 0.183	2.55(1.44-4.52) 0.001
Smoking	1.75(1.48-2.06) <0.001	1.57(1.27-1.93) <0.001	2.30(1.94-2.72) <0.001	2.57(2.17-3.05) <0.001
Dyslipidemia	1.43(1.23-1.65) <0.001	1.65(1.38-1.98) <0.001	1.39(1.19-1.63) <0.001	1.70(1.48-1.96) <0.001
Family history	1.16(0.97-1.40) 0.111	1.74(1.39-2.18) <0.001	1.35(1.12-1.64) 0.002	1.23(1.03-1.48) <0.025
Vascular territories				
Carotid disease	(-)	2.06(1.73-2.47) <0.001	2.80(2.40-3.27) <0.001	2.13(1.84-2.46) <0.001
Coronary calcification	2.06(1.73-2.47) <0.001	(-)	2.48(2.06-2.99) <0.001	3.16(2.60-3.84) <0.001

Aortic disease	2.80(2.40-3.27) <0.001	2.48(2.06-2.99) <0.001	(-)	4.85(4.09-5.75) <0.001
Ilio-femoral disease	2.13(1.84-2.46) <0.001	3.16(2.60-3.84) <0.001	4.85(4.09-5.75) <0.001	(-)

Data are expressed as odds ratio (95% confidence interval) and p value and is adjusted by age and gender for cardiovascular risk factors and for vascular territories.

Figure 1.

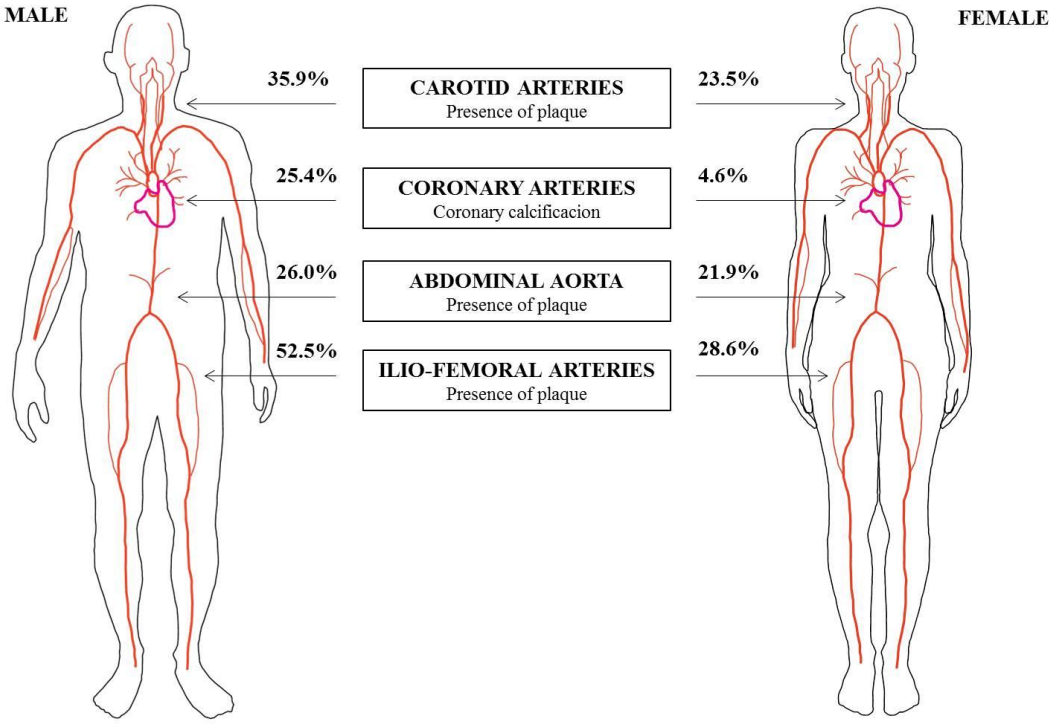

MALE
FEMALE
35.9%
CAROTID ARTERIES
Presence of plaque
23.5%
25.4%
CORONARY ARTERIES
Coronary calcificacion
4.6%
26.0%
ABDOMINAL AORTA
Presence of plaque
21.9%
52.5%
ILIO-FEMORAL ARTERIES
Presence of plaque
28.6%

Figure 2.

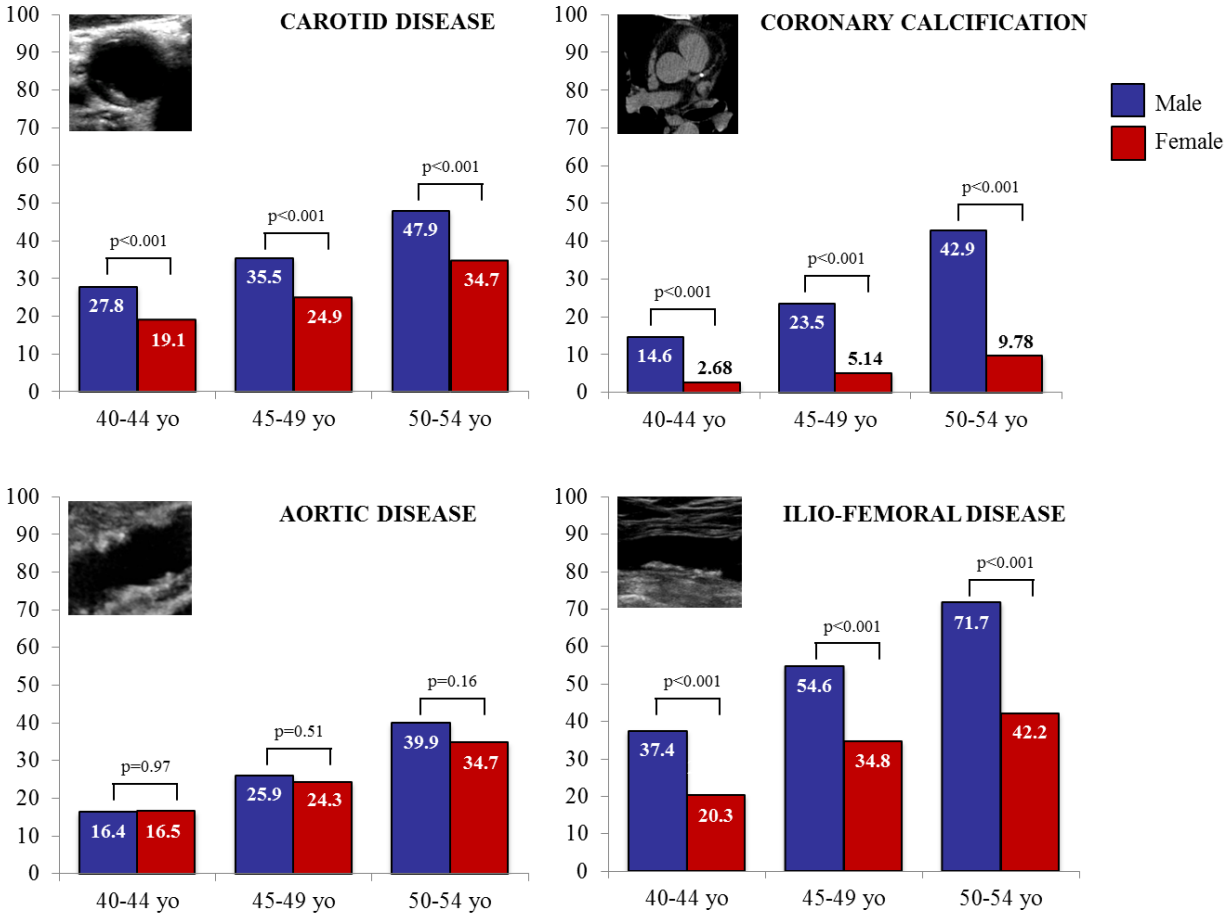

CAROTID DISEASE
CORONARY CALCIFICATION
AORTIC DISEASE
ILIO-FEMORAL DISEASE
Male
Female
100
90
80
70
60
50
40
30
20
10
0
p<0.001
p=0.97
p=0.51
p=0.16
27.8
19.1
35.5
24.9
47.9
34.7
14.6
2.68
23.5
5.14
42.9
9.78
16.4
16.5
25.9
24.3
39.9
34.7
37.4
20.3
54.6
34.8
71.7
42.2
40-44 yo
45-49 yo
50-54 yo

Figure 3.

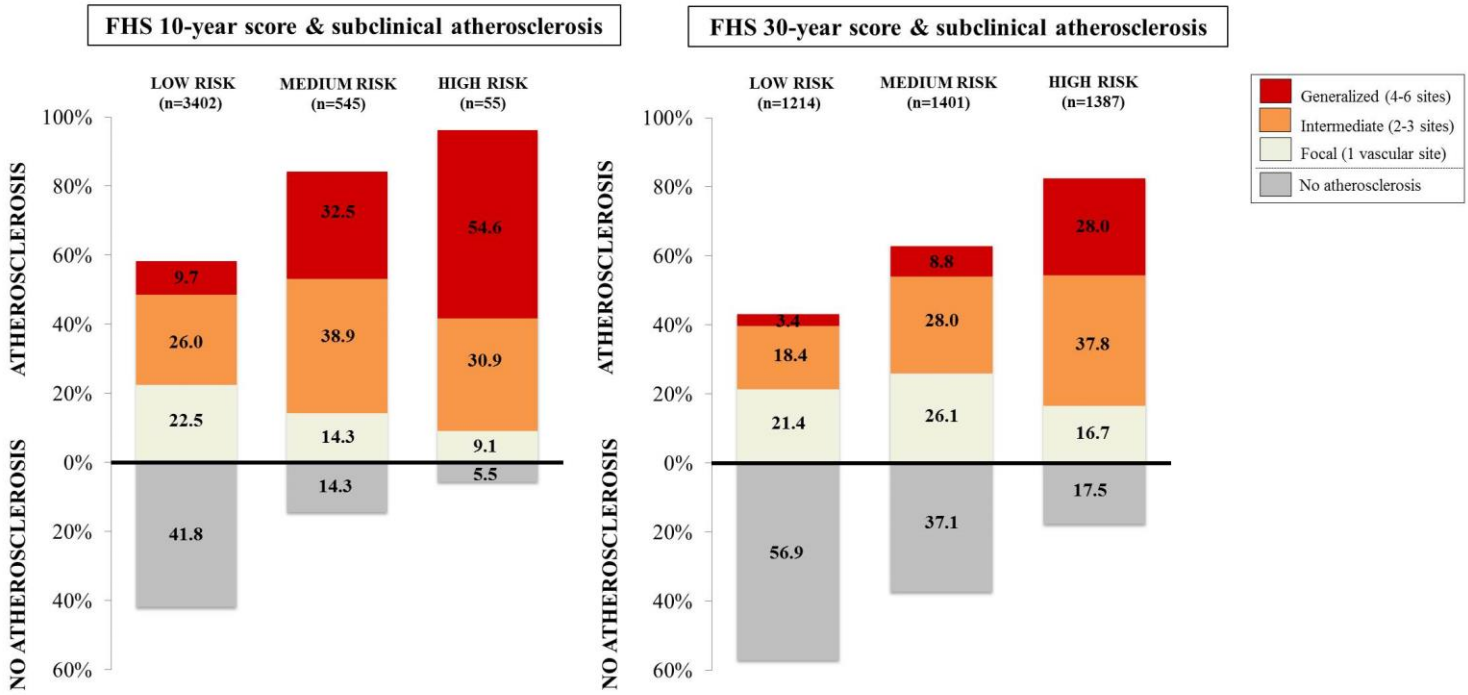
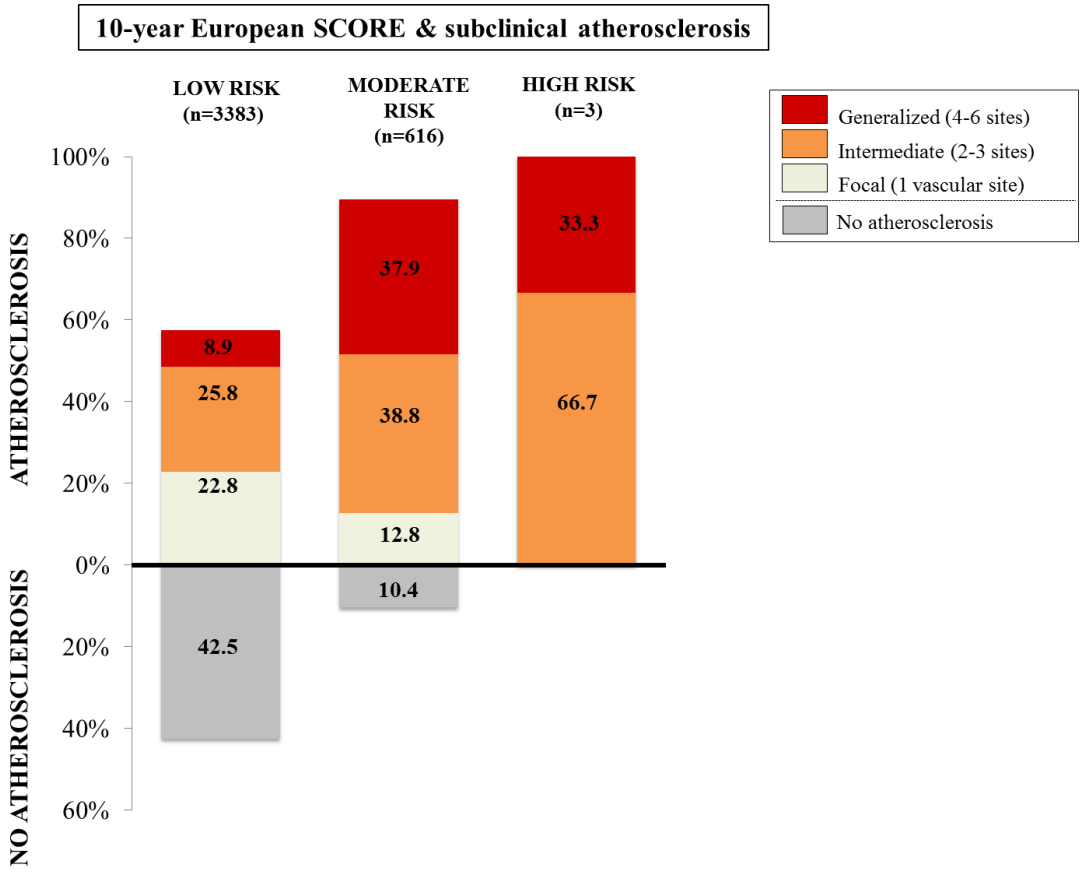

FHS 10-year score & subclinical atherosclerosis
FHS 30-year score & subclinical atherosclerosis
LOW RISK (n=3402)
MEDIUM RISK (n=545)
HIGH RISK (n=55)
LOW RISK (n=1214)
MEDIUM RISK (n=1401)
HIGH RISK (n=1387)
ATHEROSCLEROSIS
NO ATHEROSCLEROSIS
100%
80%
60%
40%
20%
0%
20%
40%
60%
9.7
26.0
22.5
41.8
32.5
38.9
14.3
14.3
54.6
30.9
9.1
5.5
3.4
18.4
21.4
56.9
8.8
28.0
26.1
37.1
28.0
37.8
16.7
17.5
Generalized (4-6 sites)
Intermediate (2-3 sites)
Focal (1 vascular site)
No atherosclerosis

Figure 4.


10-year European SCORE & subclinical atherosclerosis
LOW RISK (n=3383)
MODERATE RISK (n=616)
HIGH RISK (n=3)
Generalized (4-6 sites)
Intermediate (2-3 sites)
Focal (1 vascular site)
No atherosclerosis
ATHEROSCLEROSIS
NO ATHEROSCLEROSIS
100%
80%
60%
40%
20%
0%
20%
40%
60%
8.9
25.8
22.8
42.5
37.9
38.8
12.8
10.4
33.3
66.7

SUPPLEMENTAL MATERIAL

Supplemental Tables

Table S1. Prevalence of risk factors, risk scales, IMT and CACS by age and gender

	MEN			WOMEN		
	40-44 yo (n=1000)	**45-49 yo** (n=822)	**50-54 yo** (n=702)	**40-44 yo** (n=747)	**45-49 yo** (n=506)	**50-54 yo** (n=225)
CV risk factors						
Dyslipidemia	483 (48.3)	449 (54.6)	406 (57.8)	113 (15.1)	121 (23.9)	81 (36)
Smoking	170 (17)	159 (19.3)	146 (20.8)	163 (21.8)	131 (25.9)	50 (22.2)^{NS}
Hypertension	84 (8.4)	124 (15.1)	179 (25.5)	13 (1.74)	34 (6.72)	25 (11.1)
Diabetes	12 (1.2)	18 (2.19)	37 (5.27)	3 (0.4)	2 (0.4)	2 (0.89)^{NS}
Family history	148 (14.8)	136 (16.5)	104 (14.8) ^{NS}	123 (16.5)	91 (18.0)	32 (14.2) ^{NS}
Number of risk factors						
0	365 (36.5)	234 (28.5)	173 (24.6)	419 (56.1)	238 (47.1)	96 (42.7)
1	415 (41.5)	353 (42.9)	267 (38.1)^{NS}	249 (33.3)	180 (35.6)	81 (36.0) ^{NS}
2	181 (18.1)	181 (22.0)	193 (27.5)	71 (9.5)	67 (13.2)	38 (16.9)
>2	39 (3.9)	54 (6.6)	69 (9.8)	8 (1.1)	21 (4.1)	10 (4.4)
CV risk scales (%)						
10y SCORE	0.39 ± 0.23	0.77 ± 0.37	1.43 ± 0.73	0.06 ± 0.05	0.17 ± 0.09	0.42 ± 0.25
10y FHS	5.84 ± 0.10	7.78 ± 0.13	10.3 ± 0.19	1.81 ± 0.04	3.3 ± 0.09	4.9 ± 0.18
30y FHS	17.3 ± 0.27	23.4 ± 0.35	30.4 ± 0.45	6.68 ± 0.13	10.5 ± 0.27	14.4 ± 0.54
CACS						
CACS (Agatston units)	6.73 ± 0.98	18.6 ± 3.53	45.4 ± 5.72	0.36 ± 0.01	1.52 ± 0.48	4.37 ± 2.06
CACS 0	854 (85.4)	629 (76.5)	401 (57.1)	727 (97.3)	480 (94.9)	203 (90.2)
CACS 1-99	126 (12.6)	159 (19.3)	227 (32.3)	20 (2.7)	24 (4.7)	20 (8.9)
CACS 100-399	19 (1.9)	26 (3.2)	57 (8.1)	0 (0)	2 (0.4)	1 (0.4)
CACS ≥400	1 (0.1)	8 (1)	17 (2.4)	0 (0)	0 (0)	1 (0.4)
IMT (mm)						
Carotid IMT	0.59 ± 0.003	0.61 ± 0.003	0.64± 0.004	0.53 ± 0.002	0.56 ± 0.003	0.60 ± 0.006
Femoral IMT	0.60 ± 0.007	0.66 ± 0.009	0.73± 0.012	0.50 ± 0.004	0.55 ± 0.007	0.57 ± 0.011
Carotid IMT > 0.9	7 (0.7)	4 (0.5)	18 (2.6)	0 (0)	0 (0)	2 (0.9)
Femoral IMT > 0.9	67 (6.7)	99 (12)	128 (18.2)	8 (1.1)	19 (3.8)	15 (6.7)

Data are expressed as mean ± SEM or n (%). CACS: Coronary artery calcium score. CV: Cardiovascular. FHS: Framingham Heart Study. IMT: Intima-media thickness. NS: Non-significant (p value >0.05). Supplemental Tables are calculated from a complete imaging dataset of PESA participants (n=4002).

Table S2. Relation between age, male gender and traditional risk factors and the extent of atherosclerosis

	No disease (n=1502)	**Focal disease (n=849)**	**Intermediate disease (n=1113)**	**Generalized disease (n=538)**	**P value**
Age	44.2 ± 3.77	45.4 ± 4.1	46.7 ± 4.13	48.8 ± 3.99	<0.001
Male gender	739 (49.2)	530 (62.4)	809 (72.7)	446 (82.9)	<0.001
Smoking	199 (13.2)	154 (18.1)	271 (24.3)	195 (36.2)	<0.001
Diabetes	8 (0.5)	14 (1.7)	18 (1.6)	34 (6.3)	<0.001
Dyslipidemia	442 (29.4)	318 (37.5)	548 (49.2)	345 (64.1)	<0.001
Hypertension	99 (6.6)	82 (9.7)	158 (14.2)	120 (22.3)	<0.001
Family history	221 (14.7)	121 (14.3)	182 (16.4)	110 (20.4)	<0.001

Data are expressed as mean ± SD or n (%).

Supplemental Figures

Figure S1. Distribution of multi-territorial extent of subclinical atherosclerosis

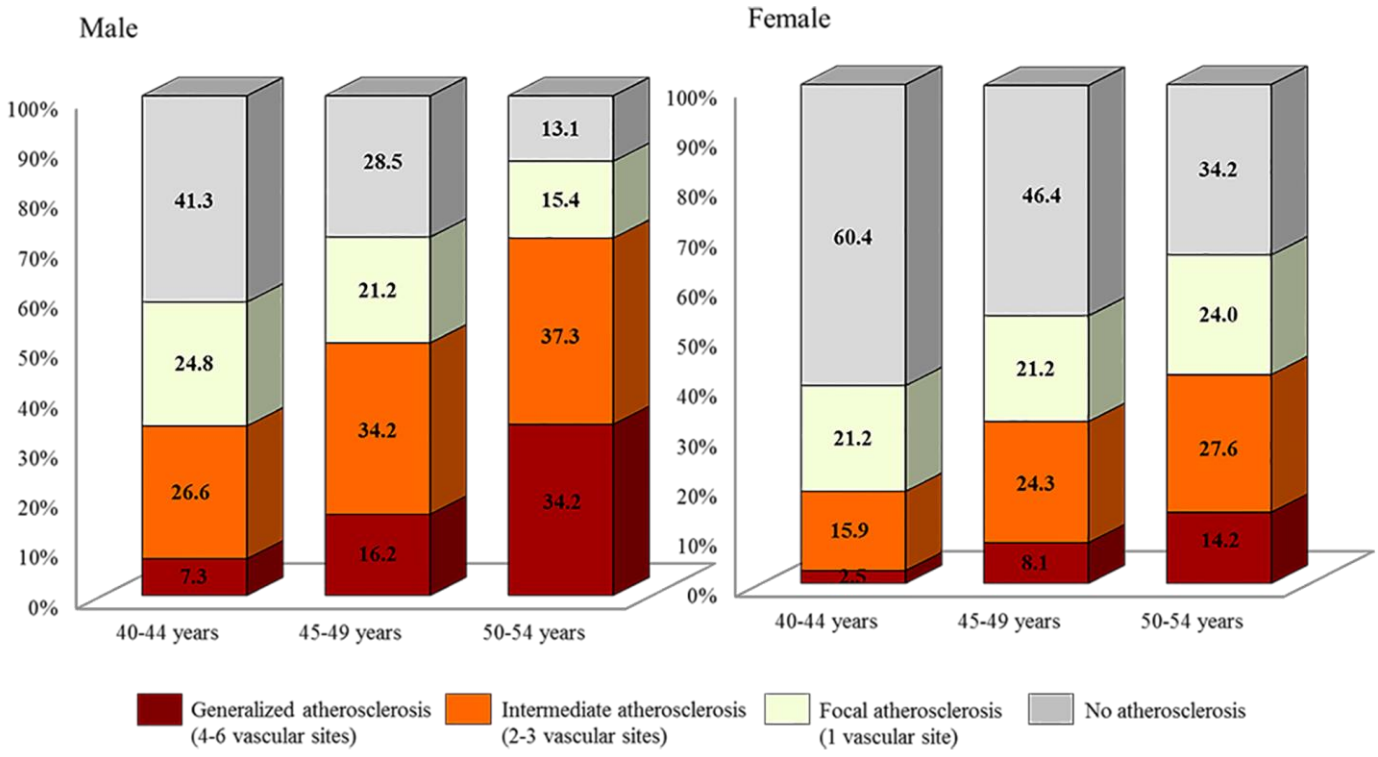


Figure S2. Association of subclinical atherosclerosis with the 10-year Framingham Heart Study risk score according to vascular territory

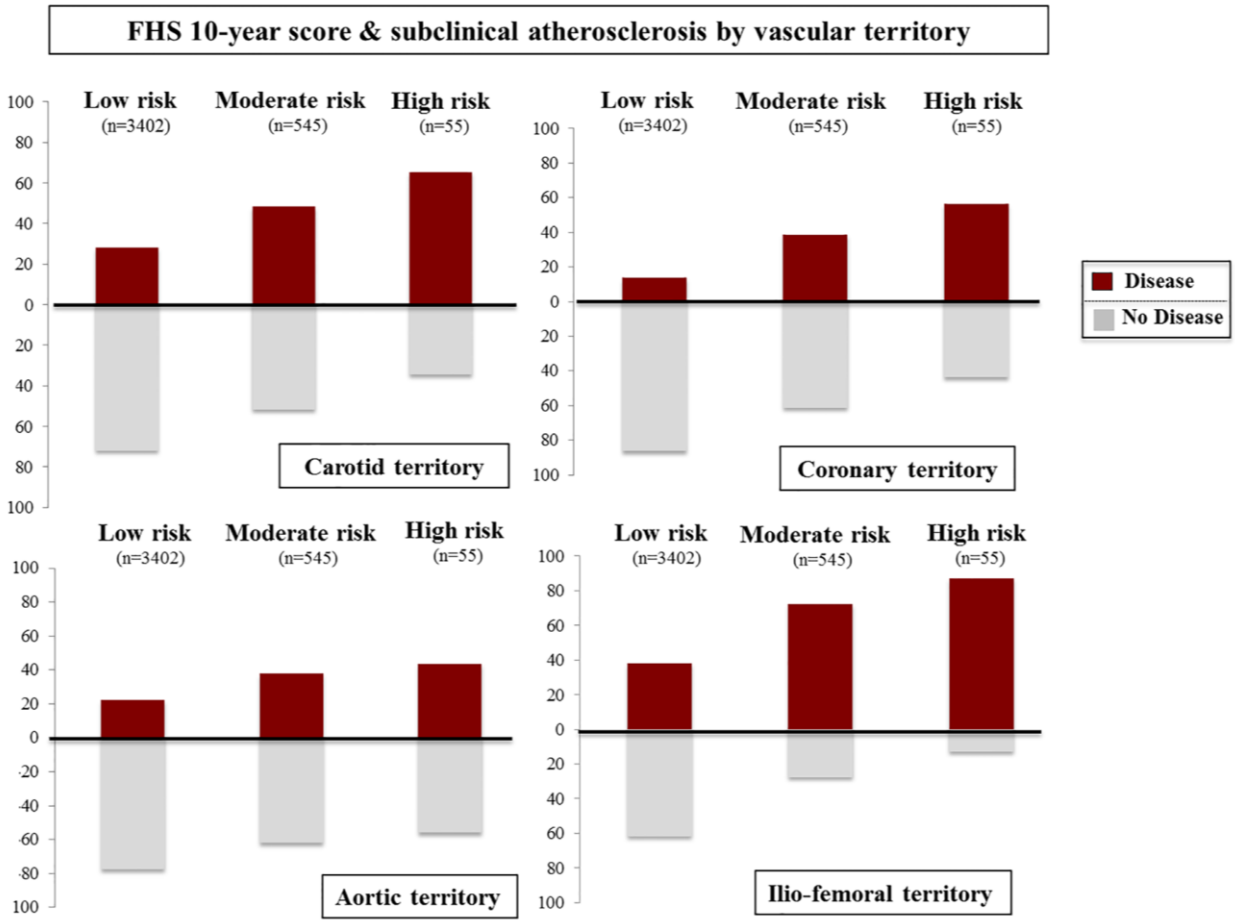


Figure S3. Association of subclinical atherosclerosis with the 30-year Framingham Heart Study risk score according to vascular territory

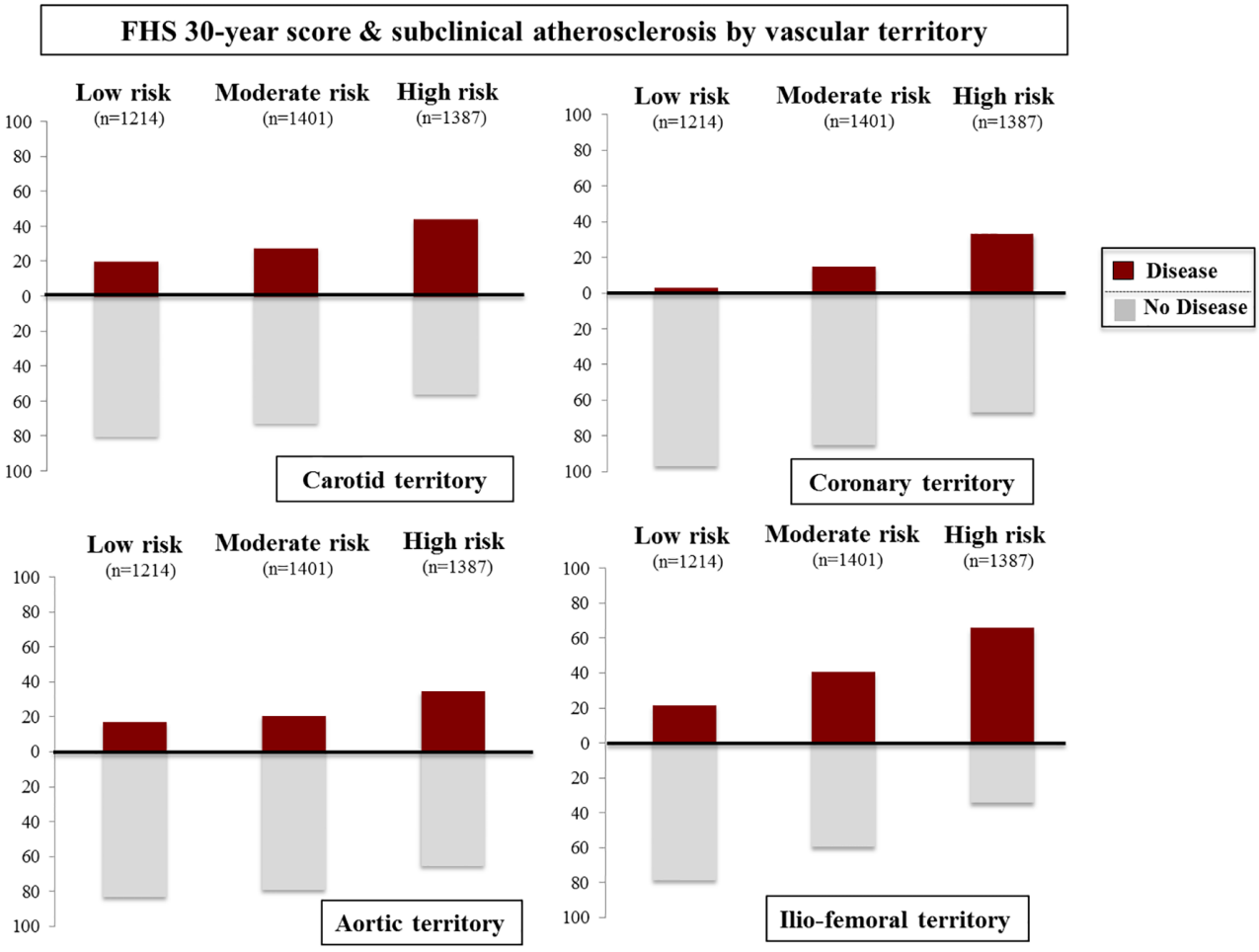


Figure S4. Association of subclinical atherosclerosis with European SCORE risk according to vascular territory