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Association between lower extremity arterial calcification and coronary arterial calcification in a population at increased risk of cardiovascular disease

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ABSTRACT

Introduction There is conflicting evidence whether lower extremity arterial calcification coincides with coronary arterial calcification (CAC). The aims of this study were to investigate the associations between (1) femoral and crural calcification with CAC, and (2) femoral and crural calcification pattern with CAC.

Research design and methods This cross-sectional study included 405 individuals (74% men, 62.6±10.9 years) from the ARTEMIS cohort study at high risk of cardiovascular disease (CVD) who underwent a CT scan of the femoral, crural and coronary arteries. High CVD risk was defined as history/presence of cerebrovascular disease, coronary artery disease, abdominal aortic aneurysm, renal artery stenosis, peripheral artery disease or CVD risk factors: diabetes mellitus type 2, hypertension, hyperlipidemia. Calcification score within each arterial bed was expressed in Agatston units. Dominant calcification patterns (intimal, medial, absent/indistinguishable) were determined via a CT-guided histologically validated scoring algorithm. Multivariable-adjusted multinomial logistic regression analyses were used. Replication was performed in an independent population of individuals with diabetes mellitus type 2 (Early-HFpEF cohort study).

Results Every 100-point increase in femoral and crural calcification score was associated with 1.23 (95% CI=1.09 to 1.37, p<0.001) and 1.28 (95% CI=1.11 to 1.47, p=0.001) times higher odds of having CAC within tertile 3 (high) versus tertile 1 (low), respectively. The association appeared stronger for crural versus femoral arteries. Moreover, the presence of femoral intimal (OR=10.81, 95% CI=4.23 to 27.62, p<0.001), femoral medial (OR=10.37, 95% CI=3.92 to 27.38, p<0.001) and crural intimal (OR=6.70, 95% CI=2.73 to 16.43, p<0.001) calcification patterns were associated with higher odds of having CAC within tertile 3 versus tertile 1, independently from concomitant calcification score. This association appeared stronger for intimal versus medial calcification patterns. The replication analysis yielded similar results.

Conclusions Higher femoral and crural calcification scores were associated with higher CAC. Moreover, the presence of femoral intimal, femoral medial and crural intimal calcification patterns was associated with increased CAC. It appears that arterial calcification is a systemic process which occurs simultaneously in various arterial beds.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Individuals with diabetes mellitus type 2 or other cardiovascular disease (CVD) risk factors have a high burden of arterial calcifications, which pose them at high risk of atherosclerotic events, such as infarction and stroke, and arteriosclerotic events such as lower limb amputation.

WHAT THIS STUDY ADDS

⇒ This study shows that arterial calcification within the lower extremities coincides with coronary arterial calcification, which indicates that arterial calcification is a systemic process which occurs simultaneously throughout the whole body. This may explain why individuals with diabetes mellitus type 2 or other CVD risk factors with arterial calcification have a threefold to fourfold increased risk of CVD compared with individuals without arterial calcification.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This research underlines the importance of curbing the burden of arterial calcification within individuals with diabetes mellitus type 2 or other CVD risk factors in order to reduce CVD mortality and morbidity. Future research should focus on prevention and treatment of arterial calcification, while practice should focus on detection, prevention and treatment.

BACKGROUND

Arterial calcification in any arterial wall accounts for a threefold to fourfold increased risk of cardiovascular disease (CVD) and mortality.[1] The majority of research on calcification morphology has been devoted to the coronary arteries, carotid arteries and aorta, but less is known on calcification morphology in arteries of the lower extremities and their contribution to total calcification burden. It

is known that calcification of the intimal layer is more often observed in the coronary arteries, common carotid arteries and aorta, and is associated with atherosclerotic outcomes such as myocardial infarction and stroke.[2] In contrast, calcification of the medial layer is more often observed in arteries of the breast, carotid siphon and lower extremities, and is associated with vascular stiffness which may give rise to lower limb amputation among others.[3]

So far, it is unclear whether lower extremity arterial calcification (LEAC) coincides with coronary arterial calcification (CAC). A few studies investigated the association between LEAC and CAC. However, the validity of these study results was limited by the small sample size[4–7] and the use of specific study populations such as hemodialysis patients and patients with type 1 diabetes mellitus (T1DM).[5 6] Moreover, no prior studies investigated whether the presence of either intimal arterial calcification (IAC) or medial arterial calcification (MAC) patterns in the lower extremities was associated with CAC, independently from concomitant calcification score. Consequently, there is a need for a large-scale study investigating the association between LEAC, in terms of score and pattern, with CAC in a population at high risk of CVD. Understanding these associations may help to establish whether LEAC contributes to calcification burden and CVD risk independently from CAC. In addition, it may shed light on identification of individuals with pronounced CAC based on the presence of LEAC.

It is hypothesized that shear stress and atherosclerotic risk factors (eg, smoking and hyperlipidemia) have systemic consequences and therefore calcification of the femoral and crural arteries should be associated with higher CAC. Moreover, since IAC and MAC share various risk factors, it is thought that the presence of either IAC or MAC in the lower extremities is associated with increased CAC, with the association being stronger for IAC compared with MAC.[8]

This study aims to investigate the associations between (1) femoral and crural calcification with CAC and (2) femoral and crural calcification patterns with CAC, independently from concomitant calcification score, in a population at high risk of CVD. Cross-sectional data will be used from the ARTEMIS Study, which is a substudy with participants from the ongoing Second Manifestations of ARTerial Disease (SMART) cohort study and the ongoing Hoorn Diabetes Care System (DCS) cohort study.

MATERIALS AND METHODS

Study population and design

Data were used from two ongoing cohort studies, the DCS cohort[9] and the SMART cohort,[10] together forming the ARTEMIS Study.[8]

The DCS cohort consists of over 15 000 individuals aged 50–75 years with type 2 diabetes mellitus (T2DM) from the West-Friesland region in the Netherlands. These individuals were referred to the DCS Study centre by their general practitioner. The DCS Study centre provides monitoring of glycemic control and diabetes-related risk factors and complications. Details of the DCS are described elsewhere.[9] Individuals from the DCS cohort are representative of Western-European semiurban individuals with T2DM and are therefore considered to be at increased risk of CVD.[9]

The SMART cohort consists of over 13 000 individuals aged 18–79 years who were referred to the University Medical Centre Utrecht since 1996 with or at high risk of CVD. High risk of CVD was defined as having a history of cerebrovascular disease, coronary artery disease (CAD), abdominal aortic aneurysm, renal artery stenosis, peripheral artery disease (PAD) or having CVD risk factors such as T2DM, hypertension, hyperlipidemia, renal insufficiency and/or family history of CVD. Details of the SMART cohort are described elsewhere.[10] Attributable to the wide age range and the liberal definition of high CVD risk, individuals from the SMART cohort are representative for a nationwide or even Western-European population of individuals at high CVD risk.[11]

A total of 718 individuals (DCS N=198, SMART N=520) were included in the ARTEMIS Study as they underwent a high-resolution quantitative CT (HR-qCT) scan of the femoral arteries and crural arteries in both lower limbs between 2015 and 2017. Because of logistic and financial reasons, only a subset of individuals were invited to participate in the ARTEMIS Study. However, baseline characteristics were similar for SMART and SMART-ARTEMIS participants,[8 11] as well as for DCS and DCS-ARTEMIS participants.[9 12] Of these 718 individuals, 405 individuals (DCS N=198, SMART N=207) also received an HR-qCT scan of the coronary arteries and were included in the analysis. Individuals with bilateral lower limb amputation at the moment of inclusion into the ARTEMIS Study were excluded. Figure 1 portrays the flow diagram.

In a replication analysis, CT data of 600 individuals from the Early-HFpEF Study were used. The Early-HFpEF Study consists of 848 individuals with T2DM ranging 50–75 years. These participants also originated from the DCS cohort and are therefore at high risk of CVD. Main reasons for replication were the availability of reliability data on calcification measurements and validation of conclusions drawn from the main analyses.

Measurement of calcification score

The calcification score was derived from a CT scan of the femoral, crural and coronary arteries using a locally developed software tool (iX Viewer, Utrecht University Medical Centre). All participants underwent an unenhanced CT scan of either the full body or the legs (slice thickness 1 mm, increment 0.7 mm, resampled to 5 mm slices with 4 mm increment). A calcific lesion was considered as calcification when the CT density exceeded 130 Hounsfield units. The amount of calcification in the coronary arteries was measured using the Agatston method.[13] The Agatston method is the most commonly accepted

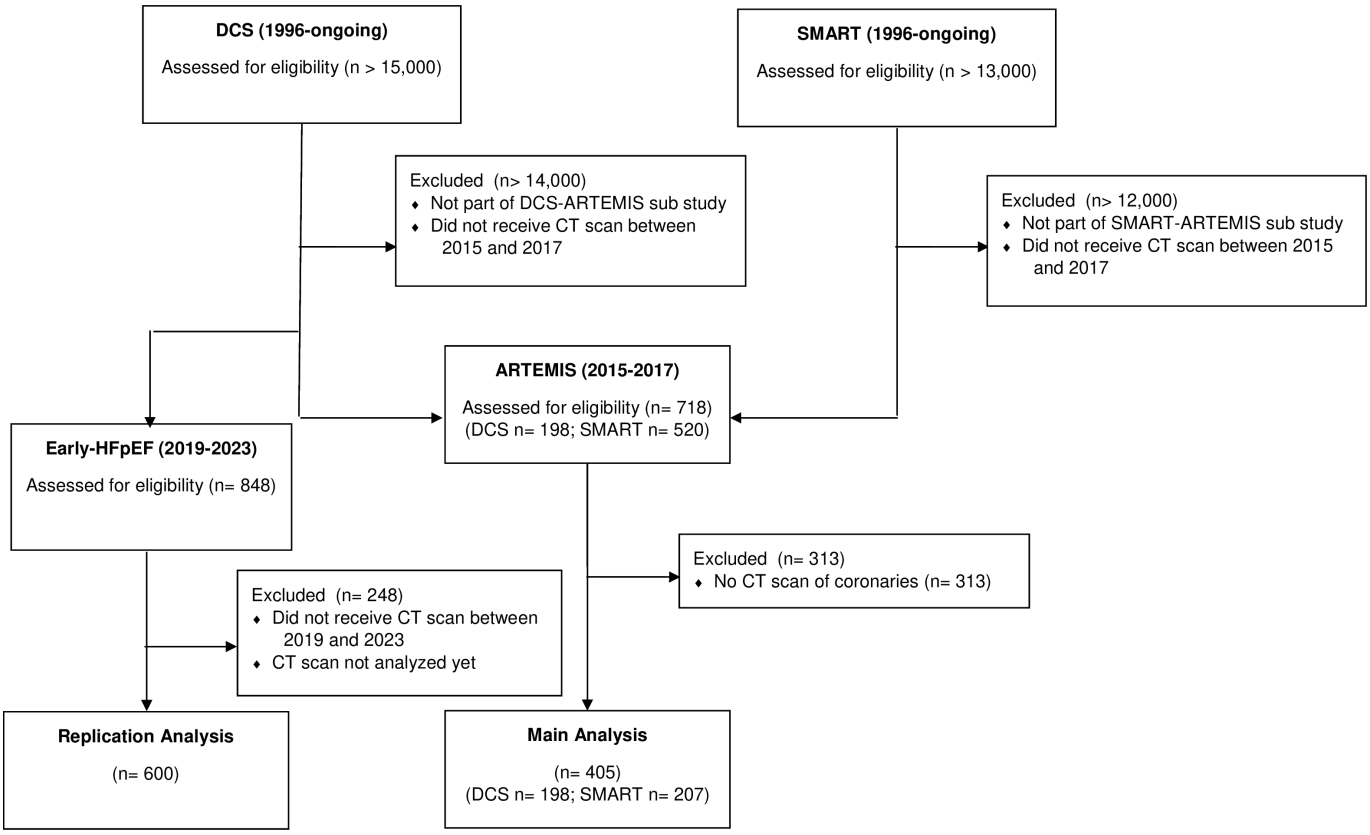

Figure 1 Flow diagram of the Diabetes Care System (DCS) and Second Manifestations of ARTerial Disease (SMART) cohorts forming the ARTEMIS cohort study with replication by the Early-HFpEF cohort study.

method for determination of CAC. Femoral and crural calcification scores were also measured using the Agatston method after resampling to a higher slice thickness. Subsequently, the calcification scores of the left and right femoral arteries were averaged, just as the calcification scores of the left and right crural arteries. The main outcome of this study was CAC and due to the non-normal distribution of calcification scores, CAC scores were normalized by categorization into tertiles. The quantitative scoring of LEAC and CAC was performed by an experienced researcher (EJB) who was blinded to patient characteristics. This researcher was trained by an experienced radiologist (PAdJ). Data on reliability parameters within the ARTEMIS Study were unknown.

Within the Early-HFpEF Study, determination of LEAC score was performed by a researcher (RM) and two research assistants who were trained by PAdJ. The inter-rater correlation coefficient was 0.922 (0.853–0.959) for the femoral arteries and 0.890 (0.793–0.942) for the crural arteries, which both reflect excellent reliability. Finally, determination of CAC score was done by a researcher (RM) and a research assistant, who were trained by PAdJ. The inter-rater correlation coefficient of the CAC measurement was 0.997 (0.994–0.999), which reflects nearly perfect reliability.

Measurement of calcification pattern

The calcification pattern was derived from the femoral and crural arteries. A CT-guided scoring algorithm was used to identify the pattern of calcification in these arteries.[14] Points were assigned to three domains of the calcification: circularity (0=absent, 1=dot, 2=<90°, 3=90–270° or 4=270–360°), thickness (0=absent, 1=≥1.5 mm, or 3=<1.5 mm) and morphology (0=indistinguishable, 1=irregular/patchy or 4=continuous). The points per domain were summed for determination of the total pattern score. Dominant calcification pattern was categorized as: 'absent' (0 points), 'indistinguishable' (0 points for morphology, regardless of points for circularity and thickness), 'dominant IAC' (<7 points) or 'dominant MAC' (≥7 points). The qualitative scoring of calcifications was performed by PAdJ who was blinded to patient characteristics. The scoring algorithm was developed by Kockelkoren *et al* and was validated in the intracranial internal carotid artery.[14] This study showed that the inter-rater reliability was 0.72 (0.60–0.84) and the intrarater reliability was 0.82 (0.73–0.89).[14] In 2022, the scoring algorithm was validated versus histology in the crural arteries and more than 70% of the arteries were correctly classified as 'absent', 'dominant IAC' or 'dominant MAC'.[15] Moreover, the absolute agreement for IAC was 0.47, while this was 0.42 for MAC.[15]

Within the Early-HFpEF Study, determination of calcification pattern was done by a researcher (RM) and two research assistants who were trained by PAdJ. The inter-rater kappa was 0.59 (0.55–0.64) and 0.57 (0.53–0.62) for the femoral and crural arteries, respectively. Both agreements reflect moderate reliability.

Covariates

Medical history on cerebrovascular disease, CAD, aneurysm of the abdominal aorta and PAD (all yes/no) was obtained via a comprehensive screening at baseline (SMART) or via self-report during routine care visits (DCS). Smoking status was self-reported (never/former/current smoker). Moreover, data on the use of antihypertensive medication and lipid-lowering medication (both yes/no) were collected via self-report and medication lists. Data on diabetes status (yes/no) were collected in the SMART cohort when there was a referral diagnosis or self-report of diabetes mellitus, the participant used glucose-lowering medication and/or when there was a baseline fasting plasma glucose level ≥7 mmol/L. Physical examinations were performed in order to obtain weight, height, systolic blood pressure (SBP) and diastolic blood pressure (DBP). The weight (kg) and height (m) were used to calculate the body mass index (BMI) (kg/m^2). The SBP (≥140 mm Hg) and DBP (≥90 mm Hg) were used along with the use of antihypertensive medication (yes/no) to define hypertension (yes/no) if at least one of these three criteria was fulfilled.

Within the Early-HFpEF Study, only data on age and sex were available, as the study was still ongoing in 2023.

Biochemical measurements

Fasting blood samples were drawn and analyzed for measurement of total cholesterol (mmol/L), high-density lipoprotein-cholesterol (mmol/L), low-density lipoprotein (LDL)-cholesterol (mmol/L), triglycerides (mmol/L), glucose (mmol/L), HbA1c (mmol/mol), C reactive protein (mg/L) and creatinine levels (µmol/L) using standard laboratory techniques.[9 10] The CKD-EPI formula was used to estimate the glomerular filtration rate (eGFR) (mL/min/1.73 m^2).[16] Hyperlipidemia was dichotomously defined as having a total cholesterol ≥5.0 mmol/L, an LDL-cholesterol ≥3.2 mmol/L and/or using lipid-lowering medication.

Statistical analysis

Baseline characteristics were described for the full cohort and per CAC tertile. Continuous variables were expressed as mean (SD) or median (IQR) depending on their distribution. Categorical variables were presented as frequencies (group percentage). Differences between these CAC tertiles were tested with a X^2 test for categorical variables and with an analysis of variance (ANOVA) (or Kruskal-Wallis test in case of violation of assumptions for ANOVA) for continuous variables.

The associations between femoral and crural calcification scores with CAC score were examined using a multinomial logistic regression analysis. Calcifications in the femoral and crural arteries were modeled continuously with increments of 100 points in calcification score, while CAC score was normalized by categorization into tertiles 1 (low; reference), 2 (medium) and 3 (high). No normalization of femoral and crural scores was needed as multinomial regression analysis does not require normalization of exposure variables.

The associations between calcification pattern in the femoral and crural arteries with CAC score were evaluated using a multinomial logistic regression analysis. Both the femoral and crural calcification patterns were regarded as exposure and were categorized as 'absent/indistinguishable' (reference), 'dominant IAC' and 'dominant MAC'. Again, CAC score was categorized into tertiles.

Confounders were selected based on literature. Model 1 adjusted for age, sex, cohort, and femoral and crural Agatston score (per 1-point increment; only for analysis on calcification pattern). Model 2 included adjustment for variables in model 1 along with smoking status and BMI. Finally, model 3 included adjustment for model 2 along with diabetes mellitus, hypertension, hyperlipidemia and eGFR (per 1-point increment).

Effect modification by sex, smoking status, history of CVD, history of diabetes, use of antihypertensive medication and use of lipid-lowering medication was checked. This was evaluated by adding an interaction term to the final model. If the p-for-interaction values were below 0.1, then the analysis was stratified accordingly.

Effect estimates consisted of ORs, the respective 95% CIs, and the corresponding p values. Moreover, goodness of fit of the final model was tested using the Hosmer-Lemeshow test and described with the McFadden's R^2.

The two main analyses were replicated using data from the Early-HFpEF Study. Only adjustment for age and sex was performed as data on all other covariates were still unavailable within the Early-HFpEF Study at moment of data analysis.

The correlation between calcification scores in each arterial bed was described using the Spearman's rank-order correlation test and was visually presented with two-way scatterplots. Moreover, the correlation between calcification pattern in the femoral arteries with calcification pattern in the crural arteries was described using the Cramér's V test. For the latter correlation, the calcification patterns were categorized as 'absent', 'indistinguishable', 'dominant IAC' and 'dominant MAC'.

Imputation of calcification scores was considered infeasible as most of the missing scores were attributable to technical issues or presence of prostheses. As such, complete case analysis was applied on data from participants who had calcification data on both femoral and coronary arteries (n=383), on both crural and coronary arteries (n=395) and on all three arteries (n=380). Two participants were excluded because they had missing data on smoking status and BMI. All analyses were carried out using STATA software (V.17.0, StataCorp, College Station, USA). A probability value of 0.05 was used as a cut-off value to assess statistical significance, unless stated otherwise.

Table 1 Baseline characteristics described for the full cohort and per coronary arterial calcification (CAC) category

	Total study population	CAC tertile 1 (low) (0–87)	CAC tertile 2 (medium) (94–514)	CAC tertile 3 (high) (516–6853)	P value
n	718	135	135	135	
Personal characteristics					
Male sex (%)	552 (76.9)	84 (62.2)	99 (73.3)	116 (85.9)	<0.001
Age (years)	62.0 (10.6)	56.7 (11.1)	63.4 (9.2)	67.7 (9.4)	<0.001
Lifestyle variables					
Current smoker (%)	136 (19.0)	29 (21.6)	33 (24.4)	16 (11.9)	0.001
Body mass index (kg/m^2)	28.0 (4.4)	29.2 (4.8)	28.0 (4.3)	28.6 (4.8)	0.119
Diabetes mellitus	279 (38.9%)	68 (50.4%)	71 (52.6%)	85 (63.0%)	0.085
Diabetes mellitus type 2 (%)	276 (38.4)	68 (50.4)	71 (52.6)	84 (62.2)	0.115
Hypertension	428 (59.6%)	72 (53.3%)	88 (65.2%)	98 (72.6%)	0.004
Systolic blood pressure (mm Hg)	133 (17)	130 (15)	134 (17)	136 (19)	0.019
Diastolic blood pressure (mm Hg)	78 (9)	80 (8)	79 (8)	77 (9)	0.077
Antihypertensive medication (%)	405 (56.6)	65 (48.2)	82 (60.7)	91 (67.9)	0.004
Hyperlipidemia	248 (34.5%)	57 (42.2%)	69 (51.1%)	77 (57.0%)	0.050
Total cholesterol (mmol/L)	4.2 (3.5–4.9)	4.3 (3.6–5.1)	4.1 (3.7–5.0)	4.0 (3.4–4.6)	0.045
LDL-cholesterol (mmol/L)	2.3 (1.8–2.8)	2.4 (1.7–3.0)	2.2 (1.8–2.7)	2.1 (1.6–2.6)	0.053
HDL-cholesterol (mmol/L)	1.2 (1.0–1.4)	1.2 (1.0–1.4)	1.2 (1.0–1.5)	1.1 (1.0–1.4)	0.366
Lipid-lowering medication (%)	580 (80.9)	89 (65.9)	114 (84.4)	123 (91.8)	<0.001
Triglyceride level (mmol/L)	1.4 (1.0–1.9)	1.4 (1.0–2.0)	1.4 (1.1–2.0)	1.4 (1.0–1.9)	0.922
Other clinical variables					
eGFR (mL/min/1.73 m^2)	85 (25)	80 (22)	81 (25)	84 (25)	<0.001
C reactive protein level (mg/L)	1.6 (0.8–3.7)	1.5 (0.7–3.8)	1.7 (0.8–3.7)	1.5 (0.9–3.2)	0.960
Glucose level (mmol/L)	6.2 (5.6–7.7)	6.5 (5.7–8.7)	6.6 (5.8–8.0)	6.9 (6.0–8.6)	0.268
HbA1c level (mmol/mol)	38 (35–48)	40 (34–51)	41 (37–51)	44 (37–55)	0.052
Creatinine level (µmol/L)	83 (72–94)	78 (67–86)	81 (72–93)	86 (74–102)	<0.001
Manifest cardiovascular disease	518 (72.1%)	74 (54.8%)	76 (56.3%)	88 (65.2%)	0.173
Cerebrovascular disease (%)	107 (14.9)	31 (23.0)	13 (9.6)	14 (10.4)	0.002
Coronary artery disease (%)	408 (56.8)	42 (31.1)	57 (42.2)	79 (58.5)	<0.001
Abdominal aortic aneurysm (%)	26 (3.6)	2 (1.5)	4 (3.0)	2 (1.5)	0.600
Peripheral artery disease (%)	37 (5.2)	4 (3.0)	5 (3.7)	5 (3.7)	0.929

Differences were tested with a X^2 test for categorical variables and ANOVA (Kruskal-Wallis in case of violation of assumptions) for continuous variables.
ANOVA, analysis of variance; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

RESULTS

Baseline and calcification characteristics

The total cohort consisted of 76.9% males with a mean age of 62.0±10.6 years (table 1). Moreover, 59.6% of the cohort had hypertension, while 34.5% had hyperlipidemia. Across increasing CAC categories, the percentage of male sex and mean age increased. Within the full cohort, 38.0% and 27.9% showed a dominant IAC and a dominant MAC pattern in the femoral arteries, respectively (table 2). The prevalence of a dominant IAC and a dominant MAC pattern was somewhat lower in the crural arteries (30.4% and 25.2%, respectively). The prevalence of both IAC and MAC increased across increasing CAC categories in both the femoral and crural arteries. Furthermore, the full cohort showed a median calcification score of 143 (16–645) and 74 (9–364) in the femoral and crural arteries, respectively. Both calcification scores showed a tendency to increase along increasing CAC categories.

The study population characteristics of the Early-HFpEF cohort are shown in online supplemental table 1. The Early-HFpEF Study population (61.5% males, mean age 67.8±6.1 years) had slightly less males and a slightly higher age compared with the study participants from the ARTEMIS Study. The prevalence of IAC (femoral=22.0%; crural=12.2%) was lower, while the prevalence of MAC

Table 2 Femoral and crural calcification characteristics described for the full cohort and per coronary arterial calcification (CAC) category

	Total study population	CAC tertile 1 (low) (0–87)	CAC tertile 2 (medium) (94–514)	CAC tertile 3 (high) (516–6853)	P value
n	713	133	133	134	
Femoral calcification pattern*					<0.001
Absent (%)	162 (22.7)	72 (54.4)	16 (12.0)	7 (5.2)	
Dominant IAC (%)	271 (38.0)	20 (15.0)	57 (42.9)	61 (45.5)	
Dominant MAC (%)	199 (27.9)	17 (12.8)	39 (29.3)	61 (45.5)	
Indistinguishable (%)	81 (11.4)	24 (18.1)	21 (15.8)	5 (3.7)	
n	532	56	109	123	
Femoral calcification quantity†‡§					
Femoral mean Agatston score (unitless)	143 (16–645)	30 (2–154)	119 (27–461)	567 (153–2445)	<0.001
n	718	135	135	135	
Crural calcification pattern					<0.001
Absent (%)	215 (29.9)	87 (64.4)	34 (25.2)	11 (8.2)	
Dominant IAC (%)	218 (30.4)	13 (9.6)	42 (31.1)	52 (38.5)	
Dominant MAC (%)	181 (25.2)	14 (10.4)	28 (20.7)	62 (45.9)	
Indistinguishable (%)	104 (14.5)	21 (15.6)	31 (23.0)	10 (7.4)	
n	490	46	96	121	
Crural calcification quantity†‡§					
Crural mean Agatston score (unitless)	74 (9–364)	31 (6–255)	49 (8–275)	332 (74–1172)	<0.001

Differences were tested with a X^2 test for categorical variables and Kruskal-Wallis test for continuous variables.
*Missing calcification pattern data in femoral artery (n=5).
†Excluding CT scans with 'absent' in this arterial bed.
‡Using a CT density threshold of 130 Hounsfield units.
§Missing calcification score data in femoral artery (n=19) and crural artery (n=13).
IAC, intimal arterial calcification; MAC, medial arterial calcification.

(femoral=40.0%; crural=28.2%) and median calcification scores (femoral=658 (197–2269); crural=223 (69–985)) were higher in the Early-HFpEF Study compared with the ARTEMIS Study.

Association between femoral and crural calcification scores with CAC score

Adjusted according to model 3, every 100-point increase in femoral calcification score was significantly associated with 1.17 (95% CI 1.05 to 1.31, p=0.004) and 1.23 (95% CI 1.09 to 1.37, p<0.001) times higher odds of having a CAC score within middle and highest tertile, versus lowest tertile, respectively (table 3). The Hosmer-Lemeshow p value was 0.419 and the McFadden's R^2 was 0.214, indicating a good fit.

Similarly, every 100-point increase in crural calcification score was associated with 1.25 (95% CI 1.08 to 1.44, p=0.002) and 1.28 (95% CI 1.11 to 1.47, p=0.001) times higher odds of having a CAC score within middle and highest tertile, versus lowest tertile, respectively (table 3: model 3). The Hosmer-Lemeshow p value was 0.946 and the McFadden's R^2 was 0.189, again indicating a good fit.

No effect modification by sex, smoking status, manifest CVD, history of diabetes, use of antihypertensive medication and use of lipid-lowering medication was observed (data not shown).

Association between femoral and crural calcification patterns with CAC score

Significant associations were observed for calcifications of both the femoral and crural arteries, where the effect estimates were slightly stronger, although not significantly, for IAC compared with MAC (table 4).

Having an IAC or an MAC pattern in the femoral arteries was significantly associated with higher odds of having a CAC score within tertile 3 (high) versus tertile 1 (low) (OR=10.81; 95% CI 4.23 to 27.62, p<0.001; OR=10.37, 95% CI 3.92 to 27.38, p<0.001) in model 3, respectively. These effect estimates were weaker, although still statistically significant when comparing IAC and MAC in tertile 2 (medium) with tertile 1. The Hosmer-Lemeshow p value was 0.174 and the McFadden's R^2 was 0.267, indicating a good fit.

Similar associations were observed in the crural arteries. The presence of an IAC or an MAC pattern in the crural arteries was significantly associated with higher odds of having a CAC score within tertile 3 versus tertile 1 (OR=6.70; 95% CI 2.73 to 16.43, p<0.001; OR=5.10, 95%

Table 3 Associations between every 100-unit increase in calcification score within the femoral arteries (upper half) and within the crural arteries (lower half) with coronary arterial calcification (CAC) categories

			Coronary*					
			T2 (medium)			T3 (high)		
		n	OR	95% CI	P value	OR	95% CI	P value
Femoral†	Model 1‡	381	1.19	1.07 to 1.33	0.002	1.24	1.11 to 1.38	<0.001
	Model 2§	381	1.18	1.06 to 1.31	0.003	1.23	1.11 to 1.37	<0.001
	Model 3¶	381	1.17	1.05 to 1.31	0.004	1.23	1.09 to 1.37	<0.001
Crural†	Model 1‡	393	1.27	1.10 to 1.47	0.001	1.30	1.12 to 1.50	0.001
	Model 2§	393	1.26	1.09 to 1.45	0.002	1.29	1.11 to 1.49	0.001
	Model 3¶	393	1.25	1.08 to 1.44	0.002	1.28	1.11 to 1.47	0.001

*The reference is T1 (low).
†Per increase of 100 units in calcification score.
‡Adjusted for age (years), sex (male/female) and cohort (SMART/DCS).
§Adjusted for model 1+smoking status (current/former/never) and body mass index (kg/m^2).
¶Adjusted for model 2+diabetes mellitus type 2 (yes/no), hypertension (yes/no), hyperlipidemia (yes/no) and estimated glomerular filtration rate (mL/min/1.73 m^2).
DCS, Diabetes Care System; SMART, Second Manifestations of ARTerial Disease; T, tertile.

CI 2.03 to 12.80, p=0.001) in model 3, respectively. When comparing tertile 2 with tertile 1, only the association for IAC remained significant (OR=2.35; 95% CI 1.05 to 5.29, p=0.038), whereas this was statistically insignificant for MAC (OR=1.54; 95% CI 0.63 to 3.74, p=0.339). Again, effect estimates were stronger for IAC compared with MAC. The Hosmer-Lemeshow p value was 0.235 and the McFadden's R^2 was 0.250, again indicating a good fit.

No effect modification by sex, smoking status, manifest CVD, history of diabetes, use of antihypertensive medication and use of lipid-lowering medication was observed (data not shown).

Correlations between femoral, crural and coronary calcification scores

The Spearman's rank-order correlation test showed that femoral calcification score was strongly correlated with CAC score (r=0.627; p<0.001) and with crural calcification score (r=0.758; p<0.001). Moreover, crural calcification score was positively associated with CAC score (r=0.575; p<0.001). The corresponding two-way scatterplots are shown in online supplemental figure 1.

Correlation between femoral calcification pattern and crural calcification pattern

The Cramér's V test showed that femoral calcification pattern was strongly correlated with crural calcification pattern. The corresponding Cramér's V was 0.517.

Replication analysis

According to the Early-HFpEF data, every 100-point increase in femoral calcification score was significantly associated with 1.04 (95% CI 1.02 to 1.06, p<0.001) and 1.07 (95% CI 1.05 to 1.09, p<0.001) times higher odds of having a CAC score within second and third tertile versus first tertile (online supplemental table 2: model 1). Similarly, every 100-point increase in crural calcification score was significantly associated with 1.07 (95% CI 1.02 to 1.11, p=0.003) and 1.09 (95% CI 1.04 to 1.14, p<0.001) times higher odds of having a CAC score within second and third tertile versus first tertile (online supplemental table 2: model 1). Similar to the ARTEMIS data, these associations were stronger within the crural compared with the femoral arteries.

Having an IAC or an MAC pattern in the femoral arteries was significantly associated with higher odds of having a CAC score within tertile 3 versus tertile 1 (OR=6.26; 95% CI 3.02 to 12.97, p<0.001; OR=3,45, 95% CI 1.81 to 6.56, p<0.001), respectively (online supplemental table 3: model 1). These effect estimates were weaker, although still statistically significant, when comparing IAC and MAC in tertile 2 (medium) with tertile 1. Similar associations were observed in the crural arteries. The presence of an IAC or an MAC pattern in the crural arteries was significantly associated with higher odds of having a CAC score within tertile 3 versus tertile 1 (OR=13.41; 95% CI 4.30 to 41.84, p<0.001; OR=2.68, 95% CI 1.36 to 5.28, p=0.005), respectively. When comparing tertile 2 with tertile 1, only the association for IAC remained significant (OR=4.65; 95% CI 1.50 to 14.41, p=0.008), whereas this was statistically insignificant for MAC (OR=0.78; 95% CI 0.40 to 1.54, p=0.479). Similar to the ARTEMIS data, effect estimates were stronger for IAC compared with MAC.

DISCUSSION

Main findings

This study showed that increased calcification in both the femoral and crural arteries was associated with increased CAC, with the association appearing stronger within the

Table 4 Associations between calcification pattern (IAC, MAC, absent/indistinguishable) within the femoral and crural arteries with coronary arterial calcification (CAC) categories

				Coronary*					
				T2 (medium)			T3 (high)		
			n	OR	95% CI	P value	OR	95% CI	P value
Femoral†	IAC	Model 1‡	378	4.30	2.06 to 8.93	<0.001	9.15	3.79 to 22.06	<0.001
		Model 2§	378	3.78	1.76 to 8.09	0.001	9.88	3.94 to 24.76	<0.001
		Model 3¶	378	4.14	1.89 to 9.05	<0.001	10.81	4.23 to 27.62	<0.001
	MAC	Model 1‡	378	3.44	1.54 to 7.68	0.003	9.21	3.63 to 23.36	<0.001
		Model 2§	378	3.90	1.70 to 8.96	0.001	9.22	3.58 to 23.72	<0.001
		Model 3¶	378	4.51	1.91 to 10.64	0.001	10.37	3.92 to 27.38	<0.001
	IAC vs MAC	Model 1‡	378	1.25	0.53 to 2.96	0.610	0.99	0.42 to 2.37	0.987
		Model 2§	378	0.97	0.38 to 2.44	0.944	1.07	0.42 to 2.74	0.886
		Model 3¶	378	0.92	0.36 to 2.36	0.858	1.04	0.40 to 2.73	0.932
Crural†	IAC	Model 1‡	378	2.65	1.21 to 5.77	0.014	6.62	2.79 to 15.68	<0.001
		Model 2§	378	2.42	1.09 to 5.36	0.029	6.91	2.86 to 16.71	<0.001
		Model 3¶	378	2.35	1.05 to 5.29	0.038	6.70	2.73 to 16.43	<0.001
	MAC	Model 1‡	378	1.27	0.54 to 3.00	0.585	4.72	1.92 to 11.56	0.001
		Model 2§	378	1.43	0.60 to 3.42	0.426	4.60	1.86 to 11.35	0.001
		Model 3¶	378	1.54	0.63 to 3.74	0.339	5.10	2.03 to 12.80	0.001
	IAC vs MAC	Model 1‡	378	2.08	0.77 to 5.60	0.146	1.40	0.53 to 3.70	0.493
		Model 2§	378	1.70	0.61 to 4.70	0.310	1.50	0.55 to 4.10	0.425
		Model 3¶	378	1.53	0.54 to 4.29	0.421	1.31	0.47 to 3.65	0.600

*The reference is T1 (low).
†The reference is 'absent/indistinguishable'.
‡Adjusted for age (years), sex (male/female), cohort (SMART/DCS), femoral Agatston score (unitless) and crural Agatston score (unitless).
§Adjusted for model 1+smoking status (current/former/never) and body mass index (kg/m^2).
¶Adjusted for model 2+diabetes mellitus type 2 (yes/no), hypertension (yes/no), hyperlipidemia (yes/no) and estimated glomerular filtration rate (mL/min/1.73 m^2).
DCS, Diabetes Care System; IAC, intimal arterial calcification; MAC, medial arterial calcification; SMART, Second Manifestations of ARTerial Disease; T, tertile.

crural versus femoral arteries. Moreover, the presence of either a dominant IAC pattern or a dominant MAC pattern in the femoral arteries and crural arteries was associated with increased CAC, independently of concomitant calcification score, with associations appearing stronger for IAC compared with MAC.

Comparison with previous literature

The findings of the association between femoral and crural calcification score with CAC are partially in agreement with a previous study conducted by Aly *et al*. This research group investigated in patients with CAD whether calcification score in various arterial beds was correlated with the Gensini score, which is a measure of luminal narrowing within the coronary arteries (with a higher score reflecting more luminal narrowing).[7] This study showed that femoral calcification score positively correlated with Gensini score (r=0.3, p=0.007). However, the same study showed that there was no significant correlation between crural calcification score and Gensini score (r=0.1, p=0.386). Although the study by Aly *et al* did not specifically use CAC as outcome, the Gensini score may be considered a proxy for CAC as these two variables are found to be strongly correlated and both variables characterize coronary artery health.[17] However, Aly *et al* showed that the association between LEAC and CAC was stronger for the femoral arteries compared with the crural arteries, whereas within the present study, this was the opposite. This discrepancy may be attributable to differences in study populations and data collection methods.

Moreover, the study findings seem to be corroborated by a study conducted by Shin *et al* in patients with PAD.[4] This study showed that patients with PAD with single-vessel or multivessel CAD had significantly more calcification in the femoral arteries and crural arteries compared with individuals with non-significant CAD. Similar to our

study, the associations were stronger in the crural arteries compared with the femoral arteries.

In addition, a study by Patsch *et al* investigated whether crural calcification score correlated with CAC score in patients with chronic kidney disease treated with hemodialysis.[5] This study demonstrated that the crural calcification score highly correlated with CAC score (r=0.6, p<0.001), which was similar to our findings.

Finally, a longitudinal study showed that LEAC was positively but not significantly associated with CAC over a period of 6 years.[6] This study was conducted in patients with T1DM. However, no distinction was made between the upper and lower leg.

To our knowledge, this is the first study to examine the association between the presence of IAC and MAC-dominant calcification patterns in the lower extremities with CAC, independently from calcification score in the lower extremities. Therefore, we cannot compare the results of these analyses with previous literature. We have observed that the presence of a dominant IAC (in both femoral and crural arteries) and dominant MAC (only in the femoral artery) pattern was associated with increased CAC, independently of total calcification in these arteries. One study by Lilly *et al* showed that an elevated ankle-brachial index (>1.4), as a proxy for MAC, was associated with higher CAC in individuals with T2DM.[18] However, the study by Hoek *et al* showed that an elevated ankle-brachial index is associated with MAC but cannot be used as a proxy for the presence of MAC in a group of individuals at high risk of CVD.[19] Nevertheless, the current study implies that either the presence of IAC or MAC within the femoral and crural arteries is associated with increased CAC after calcification score has been taken into account. We have observed that the association between calcification pattern with CAC was slightly stronger for IAC compared with MAC, which seems to be corroborated by the fact that the coronary arteries show a dominant IAC pattern.[20]

Relevance

Results of this study may be useful in the search for new markers of CVD. Arterial calcification is often present in early stages of atherogenesis[21] and may thus pose as an early marker of atherosclerotic outcomes within the coronary arteries.[7] The study results imply that calcifications within the femoral arteries and crural arteries may act as a marker of increased CAC and thus an increased risk of coronary events. Moreover, the study results imply that the presence of a dominant IAC pattern in the upper and lower leg and a dominant MAC pattern in the upper leg may be indicative of increased CAC. Future research should point out whether there is indeed a causal association between calcifications in these two arterial beds and whether the assessment of LEAC is a valid tool for predicting CAC. In such instance, data obtained from CT scans of the lower extremities may provide vital information on CAC without subjecting the individual to additional testing. The results could, in addition to systemic atherosclerosis burden, also be explained by variation between persons in their tendency to calcify. Results from a study by Eelderink *et al* suggest that some individuals have a high tendency to calcify both in the intima and the media and in multiple vascular beds beyond atherosclerosis burden.[22]

Strengths and limitations

This study has several strengths. To start, this study had a large sample size of individuals with or at high risk of CVD. Another strength of the study was the measurement of calcification pattern and calcification score within the same population, which is rare in studies on arterial calcification. Moreover, we used a CT-guided scoring algorithm to characterize dominant calcification patterns, which was histologically validated in the femoral and crural arteries. Finally, there were comprehensive data on confounders (0.5% missing) and replication in an independent population.

This study also has limitations. First, this study used cross-sectional data, which provide no evidence for potential causal associations. Due to logistic reasons, 313 out of the 718 individuals in the ARTEMIS Study did not receive an HR-qCT scan of the coronary arteries, which limited the power for the analyses. Moreover, LEAC score data were missing in 25 individuals out of 405 with CAC data (6%), mostly attributable to insufficient quality of the scans (due to technical issues or scatter caused by prostheses), and data on reliability of the available LEAC score data were unknown. However, the low amount of missing data (6% of calcification data; 0.5% on confounder data) makes selection bias minimal. Finally, history of manifest CVD was self-reported along with smoking status and medication use. This data collection method is subjective to recall bias and social desirability bias. As such, true prevalences of these characteristics may be higher than observed.

CONCLUSION

In conclusion, this study showed that increased femoral and crural calcification were associated with higher CAC. Moreover, the presence of intimal or medial calcification patterns in the femoral arteries and the presence of an intimal calcification pattern in the crural arteries were associated with higher CAC, independently of calcification scores within these arteries. It appears that arterial calcification is a systemic process which is reflected by simultaneous calcification in various arterial beds. Additional research focusing on causality and the diagnostic importance of this association is warranted to establish the exact role of arterial calcifications within the lower extremities with regard to coronary calcification burden.

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