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ORIGINAL RESEARCH

Cross-Sectional Validation of a Novel Computed Tomography-Based Carotid Mean Calcium Density Measurement

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BACKGROUND: The purpose of this study was to validate a technique for measuring mean calcium density and to determine associations of cardiovascular risk factors with carotid calcium density.

METHODS AND RESULTS: We performed a cross-sectional study in a random sample of 100 stroke-free participants from the population-based Rotterdam Study. The mean calcium density of the combined left and right carotid bifurcations was quantified with a threshold of 130 Hounsfield Units (HU) using a novel density technique. To validate the methodology, carotid calcium volumes acquired using the technique in the current study were compared with measurements computed using dedicated clinical software (semiautomatic technique based on a threshold of ≥130 HU). Next, we investigated the associations of participant demographics, total calcium volume, and known cardiovascular risk factors (hypertension, diabetes, hypercholesterolemia, obesity, and smoking status) with the newly derived mean carotid calcium density measurement using linear regression analyses. Calcium volumes obtained with the 2 methods showed a high agreement (intraclass correlation coefficient=0.99, $P<0.001$), underlining the validity of the density technique. The total calcium volume was statistically significantly associated with the mean calcium density (cardiovascular risk factors adjusted model (B: 0.48 [95% CI, 0.30–0.66], $P<0.001$). We also found an association between hypercholesterolemia and mean calcium density (0.46 [0.09–0.83], $P=0.017$). No other significant associations were found between participant demographics or cardiovascular risk factors and mean carotid calcium density.

CONCLUSIONS: We demonstrated the feasibility of a carotid calcium density measurement technique. The data warrant a subsequent longitudinal study to determine the association between carotid calcium density and the risk of cerebrovascular events.

Key Words: calcium ■ carotid ■ computed tomography ■ density ■ ischemic stroke

Carotid artery disease is a significant contributor to the occurrence of ischemic stroke.[1] Mechanisms for carotid atherosclerotic embolization include thrombotic (fibrous cap rupture-associated)[2] and calcific emboli.[3] Computed tomography (CT) is a well-established tool to detect and quantify macroscopic arterial calcium.[4] Since the amount of calcium correlates with overall plaque burden,[5] CT is used as an indicator of vascular disease. However, the association between carotid calcium and cerebrovascular ischemic events remains unclear.[6] Higher degrees and volumes of carotid calcium have been associated with decreased cerebrovascular symptoms[7,8] and increased plaque stability.[9] Conversely, other studies suggest that carotid calcium is not a stabilizing factor[10] and could represent a marker of luminal stenosis and ischemic

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RESEARCH PERSPECTIVE

What Is New?

- Carotid artery calcium density can be readily quantified using the measurement technique described in this study.
- Particularly large calcification volume and the presence of hypercholesterolemia are associated with a higher density of carotid artery calcium.

What Question Should Be Addressed Next?

- What is the link between carotid artery calcium density and the risk of ischemic stroke?

Nonstandard Abbreviations and Acronyms

HU Hounsfield unit

symptoms.[11] The discrepancy in the conclusions of these studies is likely connected to the morphology of the calcium.[6] Spotty calcifications are associated with unstable plaques[12,13] and microcalcifications in the fibrous cap can trigger plaque rupture.[14]

The interface between calcified and noncalcified plaque tissue is a high-risk region for rupture because of the compliance mismatch of the 2 tissues for both micro-[14,15] and macrocalcifications.[16] Therefore, the interfacial area or surface area of calcified and noncalcified tissue within a plaque is hypothesized to be a novel marker of plaque stability.[17–20] This hypothesis would account for the biphasic effect of calcium on plaque stability. It is now appreciated that calcium of distinct sizes and shapes may play different roles in plaque stability.[21] The limited spatial resolution of clinical CT, however, precludes the possibility of accurately quantifying these regions. Areas of calcium identified on CT scans span a wide range of attenuation values because of the partial volume averaging effects, leading to increased interest in the density of calcium, beyond merely the amount of calcium. As demonstrated in a test phantom, variations in the density of calcium hydroxyapatite deposits are attributable to CT measurement errors (partial volume averaging).[22] Preliminary evidence from micro-CT and scanning electron microscopy analysis of carotid endarterectomy samples also suggests that calcified and noncalcified tissue domains are incorporated in pixels within a low-density Hounsfield unit (HU) range (130–299 HU).[23] It is therefore reasonable to consider that high-density calcium is a surrogate marker for macrocalcifications while low-density calcium is a proxy for mixed tissue domains. Indeed, dense calcium appears to be closely linked to more stable plaques.[24] Most evidence supporting this theory comes from the field of coronary atherosclerosis, where it was shown that higher density calcium relates to a lower risk of cardiac events when adjusting for coronary calcium volume[25,26] and lower plaque instability scores.[27] Yet, whether a similar link also exists between carotid atherosclerosis and the risk of stroke remains unknown.

With current clinical software, it is only possible to measure the calcium volume. Recently, a novel methodology to quantify the density of calcium in carotid atherosclerosis was developed.[28] Until now, the density technique has only been applied to ex vivo carotid endarterectomy specimens.[28] In this manner, it is possible to perfect the acquisition, however, in vivo the acquisition is subject to motion artifacts and partial volume with surrounding physiological materials. The carotid calcium density measurements should therefore first be tested using in vivo CT scans.

The purpose of this study was to quantify the mean density of calcium within the carotid bifurcation in a community-dwelling population using the new density technique. As a first objective, to validate the density measurements, calcium volumes were also computed using the density technique to be compared against calcium volume measurements recorded using current dedicated clinical software. Provided that the 2 methods of quantifying the calcium volume were comparable, the second objective of the current study was to use the new density measurements to investigate the association of cardiovascular risk factors with mean carotid calcium density.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Setting

This study is embedded in the Rotterdam Study, the design and rationale of which have been described elsewhere.[29] The study was approved by an institutional review committee and all subjects gave informed consent. Between 2003 and 2004, all participants who visited the research center were asked to undergo a noncontrast CT scan as part of a project on the visualization of arterial calcification. From the 2524 participants who were scanned, we randomly selected 100 participants without prevalent stroke at the time of the CT for the current validation study.

Scan Protocol and Analysis of Calcium

Imaging parameters are described in detail elsewhere.[30] Briefly, noncontrast CT images were obtained using 16-slice multidetector CT scanners (Somatom Sensation 16; Siemens, Forchheim, Germany). A scan was performed which included the aortic arch and the carotid arteries. Three-millimeter thick slices were acquired, and images were reconstructed with effective slice width 1 mm, reconstruction interval 0.5, 120 mm field of view, and medium sharp convolution kernel (B35f). Carotid calcium volume in both left and right carotid arteries within 3 cm both proximal and distal of the bifurcation was quantified based on a threshold of 130 HU.

Carotid calcium was analyzed using ImageJ image processing software (ImageJ release 1.53c) to perform a calculation of calcium density. A single observer, blinded to the participant data, completed the calcium volume and density measurements from the CT scans using the custom density technique. One side (left or right) was analyzed at a time using the following steps. Areas of carotid calcium (both intimal and medial) 3 cm proximal and distal from the bifurcation were identified based on a threshold of 130 HU (Figure S1A). This threshold was selected (1) to be in line with established calcium scoring studies[31] and (2) to match the calcium threshold used in the dedicated calcium scoring software. The slice at which the carotid artery bifurcates is identified (Figure S1B). The image slices 3 cm both proximal and distal of the bifurcation were separated (Figure S1C). The region of interest containing the calcium is cropped using a rectangular-shaped ROI (Figure S1D and S1E). Areas of carotid calcium (≥130 HU) were segmented by eliminating any other high-density structures (≥130 HU) within the cropped stack (ie, jaw, teeth, and skull) (Figure S1F). Once the carotid bifurcation was successfully segmented (Figure S1G), a custom macro was applied to acquire a density histogram for the whole ROI (Figure S1H(i)). The density of the carotid calcium is isolated by only considering the frequency of pixels ≥130 HU (Figure S1H(ii)). These steps were then repeated for the other side (left or right). A combined frequency was obtained by adding the frequency of each calcified HU value from the left and right bifurcations. The total volume of calcium was calculated by multiplying the number of calcified pixels (≥130 HU; Figure S1H(ii)) by the known voxel volume of the scans. All calcified areas, even those smaller than 1 mm were included. For participants with no calcium, a calcium volume value of 0 mm^3 was used. The mean calcium density for each participant was also calculated as the mean of the combined density histogram from the left and right sides. For participants with no calcium, a mean calcium density value of 0 HU was used. Carotid calcium volume was analyzed for a second time using dedicated software (Syngo Calcium Scoring, Siemens, Forchheim, Germany). Here, the software identified adjacent pixels within the scans with attenuation ≥130 HU. In the same manner as the density technique, carotid calcium volume in both left and right carotid arteries within 3 cm both proximal and distal of the bifurcation was selected. The volume of calcium was calculated as the product of the area of a calcified lesion, the increment, and an isotropic interpolation factor.[30] All calcified areas, even those smaller than 1 mm, were included. The total volume of calcium was then calculated from the selected regions of interest. This semiautomatic methodology does not allow for mean calcium density to be assessed.

Assessment of Cardiovascular Risk Factors

Information on participant demographics cardiovascular risk factors and medication was collected by interview, physical examination, or blood sampling.[29] Participants' age and sex were recorded. Hypertension was defined as systolic and diastolic blood pressure ≥140 mm Hg and ≥90 mm Hg, respectively, or the use of blood pressure-lowering medication.[29] The Hexokinase method was used to determine glucose levels. Diabetes was defined as fasting serum glucose levels ≥7.0 mmol/L (or nonfasting serum glucose levels ≥11.1 mmol/L if fasting samples were unavailable) or the use of antidiabetic therapy.[29] Total cholesterol was measured using an automatic enzymatic procedure (Hitachi analyzer; Roche Diagnostics). Hypercholesterolemia was defined as total cholesterol concentration ≥ 6.2 mmol/L or the use of lipid-lowering medication.[29] Body mass index was calculated as weight (kg)/height $(m)^2$, and obesity was categorized as >30 kg/m^2.[29] Smoking behavior was categorized as "current smoking" and "nonsmoking."[29]

Statistical Analysis

To validate the density methodology, the calcium volumes computed using the density technique were compared with the volumes computed from the dedicated clinical software (Syngo Calcium Scoring, Siemens, Forchheim, Germany). The agreement between the 2 readings was assessed using Bland–Altman plots[32] and intraclass correlation coefficients. Then we assessed the association of standardized mean calcium density (per 1-SD increase) with participant age and sex, standardized total calcium volume (per 1-SD increase), and cardiovascular risk factors (hypertension, diabetes, hypercholesterolemia, obesity, and current smoking status) using linear regression. The regression analyses were conducted individually (unadjusted) and all in a single model (adjusted). All statistical analysis was conducted in SPSS version 25.

RESULTS

Study Population

The characteristics of the study population are shown in Table 1. The participants were 41% men with an age range of 67 (65–72) years; 72% had hypertension, 40% had hypercholesterolemia, and 8% had diabetes. Fifteen percent were classified as obese, and 17% were current smokers. Out of the 100 participants, 75 (75%) had calcium present in their carotid bifurcations. Table S1 presents the characteristics of the study population with those with no calcium excluded (n=25). To appreciate the range of calcium density >130 HU, Figure S2 presents a median frequency histogram for the calcium density values of all participants with carotid calcium (n=75).

Validation of Density Measurement Technique

There was a strong agreement between the 2 methods of quantifying calcium volume (intraclass correlation coefficient=0.991, $P<0.001$) (Figure). We therefore consider our method of calculating mean calcium density using the density technique as accurate. Figure S3 compares the 2 volume readings, excluding the 0 mm^3 volumes (intraclass correlation coefficient=0.991, $P<0.001$).

Cross-Sectional Association of Mean Carotid Calcium Density With Cardiovascular Risk Factors

Table 2 presents the associations between mean calcium density with participants' demographics and cardiovascular risk factors. There was a positive association between mean calcium density and total calcium volume (unadjusted, 0.51 [0.34–0.69], $P<0.001$; and adjusted, 0.48 [0.30–0.66], $P<0.001$). For every 1-SD increase in calcium volume, there is a 0.51-SD increase in calcium density. Of note, the calculation of both of these measurements is based on the identification of pixels ≥130 HU. We also found a positive association between mean calcium density and hypercholesterolemia (unadjusted, 0.62 [0.23–1.01], $P=0.002$; and adjusted, 0.46 [0.09–0.83], $P=0.017$). Participants with hypercholesteromia have 0.62-SD higher density calcium compared with those without hypercholesteromia. Of note, no other significant associations were found between mean carotid calcium density and participant age, sex, hypertension, diabetes, obesity, or smoking status. Table S2 presents the associations between mean calcium density with participants' demographics and cardiovascular risk factors excluding participants with no calcium (n=25).

Table 1. Characteristics of the Participant Population

Participant variables	(N=100)
Sex, n men (%)	41 (41)
Age, median (25–75th)	67 (65–72)
Calcium, n (%)	75 (75)
Volume, mm^3, median (25–75th)	18 (0–93)
Mean density, HU, median (25–75th)	213 (98–325)
Maximum density, HU, median (25–75th)	499 (99–1273)
Hypertension, n (%)	72 (72)
Diabetes, n (%)	8 (8)
Hypercholesterolemia, n (%)	40 (40)
Obesity, n (%)	15 (15)
Smoking, n (%)	17 (17)

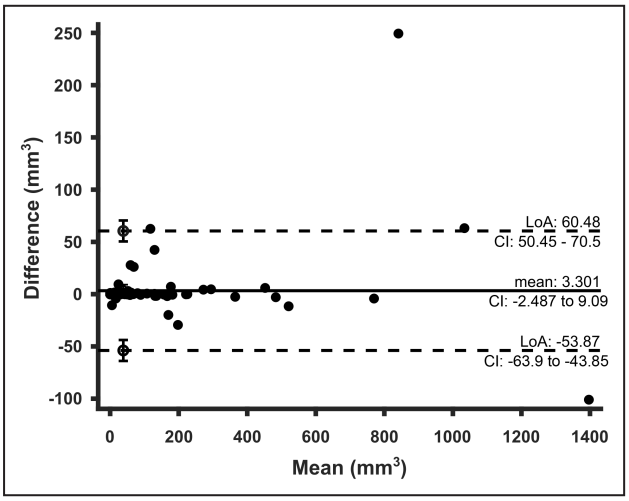

Figure. Bland–Altman plot of calcium volumes (mm^3) acquired using dedicated software versus the density technique used in the current study.
LoA indicates limits of agreement.

DISCUSSION

In the current study, we validated a density technique to compute the mean calcium density measurement in the carotid bifurcation using in vivo noncontrast CT scans. In a cross-sectional association of mean calcium density with participant demographics and cardiovascular risk factors, we showed that calcium density in the carotid bifurcation is positively associated with calcium volume and hypercholesterolemia. The higher volume of carotid calcium present in the carotid bifurcation, the higher the mean calcium density. Additionally, those with hypercholesteremia (prescribed lipid-lowering medication) are more likely to have higher mean calcium density. No other significant associations were found between mean carotid calcium density and participant age, sex, hypertension, diabetes, obesity, or smoking status.

Table 2. Cross-Sectional Association of Participant Demographics and Cardiovascular Risk Factors With Standardized Mean Calcium Density in the Carotid Bifurcation

	Unadjusted			Adjusted		
	Constant	Regression coefficient (B) (95% CI)	*P* value	Constant	Regression coefficient (B) (95% CI)	*P* value
Sex*	−0.08	0.14 (−0.27 to 0.54)	0.50	0.28	0.15 (−0.22 to 0.52)	0.41
Age, y	−0.14	0.002 (−0.03 to 0.04)	0.91		−0.01 (−0.04 to 0.02)	0.59
Standardized volume	0	0.51 (0.34 to 0.69)	<0.001		0.48 (0.30 to 0.66)	<0.001
Hypertension	−0.23	0.33 (−0.11 to 0.77)	0.15		0.08 (−0.33 to 0.49)	0.69
Diabetes	−0.02	−0.28 (−0.46 to 1.01)	0.45		−0.002 (−0.70 to 0.70)	1
Hypercholesterolemia	−0.25	0.62 (0.23 to 1.01)	0.002		0.46 (0.09 to 0.83)	0.02
Obesity	−0.06	0.40 (−0.16 to 0.95)	0.16		0.19 (−0.31 to 0.70)	0.45
Smoking	−0.01	0.06 (−0.47 to 0.59)	0.83		−0.17 (−0.68 to 0.34)	0.51

The unadjusted model presents the individual linear regression analysis results. The adjusted model presents the results for all variables in 1 linear regression model. Unstandardized coefficients are presented.

*Men are the reference category.

Coronary artery calcium scoring is a well-defined estimate of the risk of myocardial infarction,[33] the same is not true for carotid calcium and ischemic stroke. Despite the more time-consuming nature of the density technique,[28] information can be obtained about the density of the calcium as well as the calcium volume, which is not possible using current dedicated software. The current study validates its use with clinical noncontrast CT scans. A further study is planned to examine the association between mean carotid calcium density and incident stroke in a longitudinal population-based cohort. If proven, an automated technique should be developed to perform these measurements easily and routinely in the clinic.

Unsurprisingly, there is an association between mean calcium density and total calcium volume. Both measures originate from the identification of pixels ≥130 HU. Additionally, calcified particles coalesce with the progression of calcium, reducing the number of calcified and noncalcified tissue interfaces that would decrease the density values attributable to the partial volume effect.[18] Additionally, larger calcified particles have been shown to have higher maximum density values.[34] In the field of coronary atherosclerosis, calcium volume is a positive predictor of coronary heart disease, whereas calcium density is inversely associated with coronary heart disease (when adjusting for calcium volume).[35] The results of this study suggest that carotid calcium density is associated with carotid calcium volume, but it may carry independent information useful in the prediction of stroke, akin to the relationship between coronary calcium volume and density and coronary heart disease.

Lipid-lowering medications (or statins) are prescribed for hypercholesterolemia.[36] In the current study, hypercholesterolemia was defined as the use of lipid-lowering medication or total cholesterol concentration ≥6.2 mmol/L. Statins have been shown to promote both coronary and carotid plaque calcium,[37,38] contributing to plaque stabilization[39] and a reduced risk of stroke.[40] It had been hypothesized that statins may increase coronary calcium density, along with a lower risk of coronary and cardiovascular events.[25,33] Recently, it was confirmed that statin treatment alters the microarchitecture (surface area) of aortic calcium deposits in mice[41] and densifies coronary calcium as assessed by serial coronary computed tomography angiography.[42] In the current study, we report a positive association between mean carotid calcium density and hypercholesterolemia (prescription of lipid-lowering medication). We hypothesize that the prescription of statins in these participants may have altered the microarchitecture of the carotid calcium, resulting in increased mean calcium density values, with a possible reduction in the risk of stroke.

Interestingly, we did not observe any significant associations between mean carotid calcium density and participants' demographics (age and sex) or other cardiovascular risk factors besides hypercholesterolemia (hypertension, diabetes, obesity, and smoking status). Carotid calcium volume is known to progress with age and is more associated with men than women,[43,44] however there is no known association between calcium density and demographics. A similar conclusion can be drawn for the remaining cardiovascular risk factors apart from hypercholesterolemia.

A strength of this study includes the measurement of both calcium volume and density. The decision was taken to compute the combined mean calcium density of both the left and right carotid bifurcations as this value is thought to produce a more accurate estimation of the calcified particle distribution,[34,45] heterogeneous

patterns of calcium,[46,47] and has a higher signal-noise ratio compared with other measurements such as the maximum density.[48] Moreover, mean calcium density values in the current study are measured on a continuous scale, as recommended by previous studies which computed average calcium density grades.[25] In this regard, mean calcium density measurements may also be considered a limitation, as the density technique requires manual input and is more time-consuming than the dedicated clinical software.

This study has some other limitations that require consideration. First, microcalcifications are frequent in atherosclerotic plaques and a known source of plaque rupture.[14] However, the resolution of clinical CT scanners likely precludes the influence of microcalcifications on the overall density of the tissue. The critical size of microcalcifications required to be detected on clinical CT scanners should be verified in histopathological studies. In this regard, the threshold of 130 HU for identifying calcium should be examined. Second, further information would be required about statin treatment such as dosage and duration to conduct further analysis on the association of carotid calcium density and statin intake. Additionally, information on which lipid-lowering therapies were prescribed other than statins was not available for the participants in the current study. Third, it is not possible to visualize the noncalcified portions of the tissue with noncontrast CT. Nevertheless, calcium reflects the underlying disease burden.[49] Fourth, emboli causing strokes originate from a wide range of sources. However, up to 18% of ischemic strokes are caused by carotid artery disease[1] and calcification is typically confined to the carotid bifurcation.[50]

SUMMARY

Here we validated the use of a density measurement technique in the carotid bifurcation and investigated the association of mean carotid calcium density with known cardiovascular risk factors. This method has the potential to be automated for routine clinical use. Future studies should examine the utility of mean carotid calcium density in the prediction of stroke.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S2
Figures S1–S3

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Supplemental Material

Table S1. Characteristics of the participant population, excluding those with no calcium in their carotid bifurcation.

Participant Variables	(n = 75)
Sex, n males (%)	27 (36)
Age, median [25-75th]	67 [66 – 72]
Volume (mm^3), median [25-75th]	43 [13 – 150]
Mean Density (HU), median [25-75th]	264 [202 – 373]
Maximum Density (HU), median [25-75th]	905 [429 – 1507]
Hypertension, n (%)	57 (76)
Diabetes, n (%)	7 (9)
Hypercholesterolemia, n (%)	33 (44)
Obesity, n (%)	13 (17)
Smoking, n (%)	14 (19)

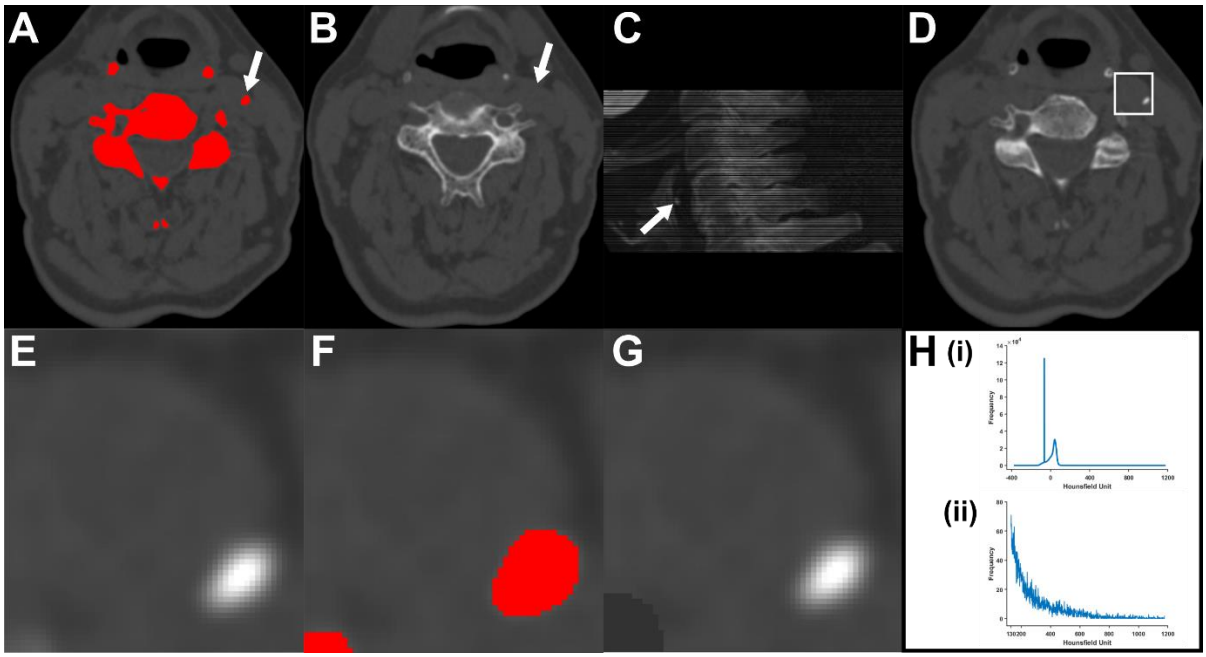
Table S2. Standardised mean calcium density in the carotid bifurcation and cross-sectional association with participant demographics and cardiovascular risk factors, excluding those with no calcium in their carotid bifurcation.

	Unadjusted			Adjusted		
	Constant	**Regression Coefficient (B) (95% CI)**	***P*-value**	**Constant**	**Regression Coefficient (B) (95% CI)**	***P*-value**
***Sex**	0.19	-0.29 (-0.77 0.19)	0.23	**1.16**	-0.37 (-0.79 0.05)	0.09
Age	-0.14	0.002 (-0.04 0.05)	0.93		-0.01 (-0.05 0.03)	0.47
Standardised Volume	**0**	**0.51 (0.31 0.71)**	**<0.001**		**0.49 (0.29 0.68)**	**<0.001**
Hypertension	-0.09	0.11 (-0.43 0.65)	0.68		-0.26 (-0.74 0.22)	0.29
Diabetes	0	0.05 (-0.75 0.84)	0.91		-0.15 (-0.90 0.60)	0.69
Hypercholesterolemia	**-0.32**	**0.72 (0.29 1.16)**	**<0.001**		**0.79 (0.38 1.20)**	**<0.001**
Obesity	-0.04	0.25 (-0.36 0.86)	0.42		0.03 (-0.51 0.56)	0.92
Smoking	0.04	-0.19 (-0.78 0.41)	0.54		-0.51 (-1.09 0.07)	0.08

*In the variable Sex, males are the reference category.

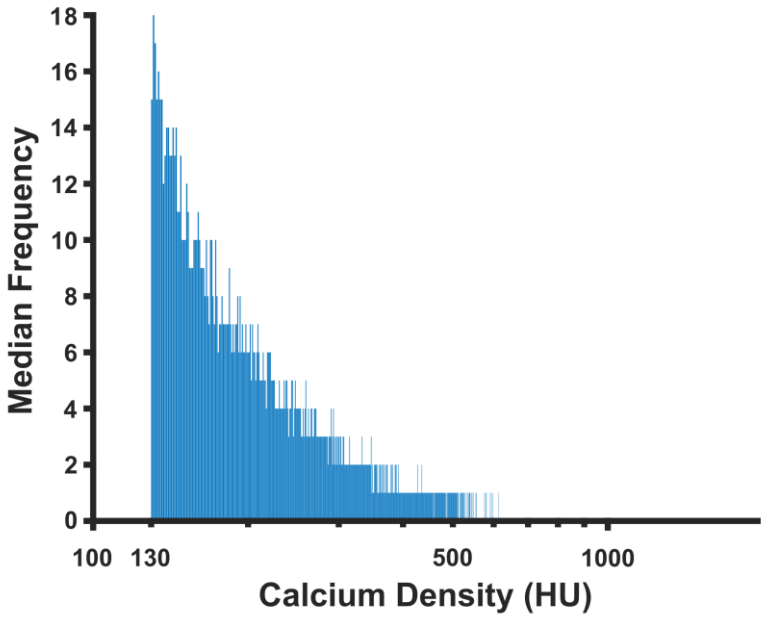
Unadjusted presents the individual linear regression analysis results. The adjusted model presents the results for all variables in one linear regression model. Unstandardised coefficients are presented.

Figure S1. Workflow and sample histogram.



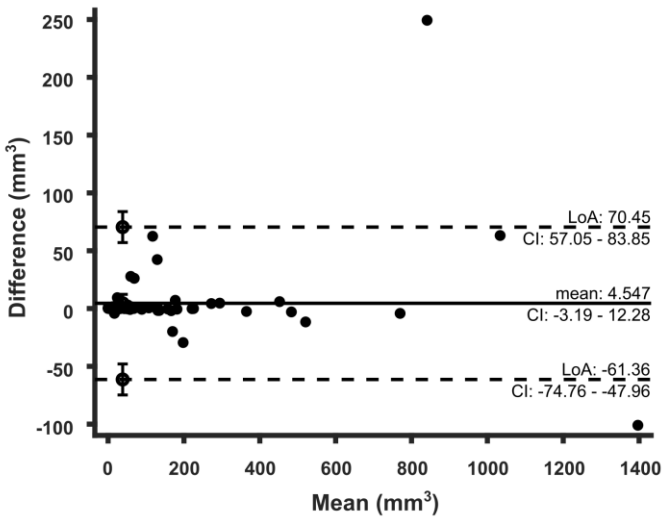
(A) Calcium in the left carotid bifurcation identified. (B) Left carotid bifurcation identified. (C) Image slices 3cm proximal and distal to the bifurcation separated. (D) Rectangular region of Interest (ROI) identified. (E) ROI cropped. (F) Interfering high density structures removed. (G) Area of calcium segmented. (H) (i) Example histogram of the segmented ROI stack. (ii) Example histogram with density ≥ 130 HU area isolated. Steps repeated for the right side.

Figure S2. Histogram of median calcium density values for all participants with carotid calcium (n = 75).



Note x-axis is logarithmic scale.

Figure S3. Bland-Altman plot of calcium volumes (mm^3) acquired using dedicated software versus the density technique employed in the current study, excluding the 0 calcium readings.



LoA: Limits of Agreement. CI: Confidence Interval.