Carotid Calcification Shape, Size, and Lumen Proximity Are Associated with Ischemic Events

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Rationale and Objectives: While calcification is a highly prevalent component in atherosclerotic extracranial carotid arteries and is known to impact plaque stability, the link between carotid calcification and ischemic events is yet to be identified. We aimed to investigate the associations of geometric features of carotid calcifications, and their temporal changes, with ischemic events.

Materials and Methods: We retrospectively analyzed 128 mildly stenotic carotid arteries (Plaque At Risk study) from 64 patients with recent ischemic event, using multi-detector computed tomography angiography data at baseline and after 2 years. The 3D artery and calcification geometries were reconstructed with a semi-automatic pipeline, and an in-depth calcification morphometric assessment was performed. We examined the distribution of the calcification morphometrics and their temporal changes and investigated their associations with ischemic events at the time of inclusion, using generalized linear mixed models.

Results: At baseline, compared to contralateral asymptomatic arteries, symptomatic carotids had more calcification bodies (mean [95%CI]: 1.9 [1.4–2.6] vs. 1.6 [1.2–2.2]). These calcifications were smaller (mean area [95%CI]: 3.7 mm^2 [2.9–5.1] vs. 4.5 mm^2 [3.5–5.8]) and narrower (mean width [95%CI]: 2.7 mm [2.3–3.4] vs. 3.1 mm [2.5–3.6]). At 2-year follow-up, adjusting for baseline measurements, these calcifications were smaller (mean width [95%CI]: 2.9 mm [2.5–3.5] vs. 3.3 mm [2.7–3.7]) and longer (mean [95%CI]: 8.6 mm [7.1–10.5] vs. 7.5 mm [6.3–9.5]) compared to asymptomatic side.

Conclusion: Symptomatic carotid arteries presented more and smaller calcifications with a tendency to grow more in the longitudinal artery direction, providing insights into the role of carotid calcifications in ischemic events.

Key Words: Atherosclerosis; Carotid artery; Calcification; Computed tomography angiography; Ischemic events.

Abbreviations: **CVRF** Cardiovascular Risk Factors, **GLMM** Generalized Linear Mixed Models, **MDCTA** Multi-detector Computed Tomography Angiography, **PARISK** Plaque At Risk, **TIA** transient ischemic attack

INTRODUCTION

Traditionally, the primary causal link between carotid atherosclerosis and ischemic events has been attributed to high-grade carotid artery stenosis (1–3). However, mounting evidence indicates that the composition of carotid plaques may have a pivotal role in ischemic events, as it impacts the carotid plaque stability (4).

Calcification is a highly prevalent structural component in atherosclerotic carotid arteries (5–9). Increased calcification volume in carotid arteries was suggested as a stabilizing factor (10,11). Specifically, macro-calcifications in carotid lesions were shown to be associated with a transcriptional profile typical in stable plaques (10), and the volume proportion of carotid plaque calcification to be inversely associated with ischemic neurological symptoms (11). On the other hand, several other studies (12,13) demonstrated no correlation between the volume or presence of calcification in the carotid arteries and neurological symptoms.

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Previous biomechanical analyses (14) have shown that carotid plaque rupture can trigger an ischemic event. Although calcifications in the carotid arteries are considered to enhance the mechanical stability by stiffening the artery, unlike the "softer" plaque components (15), they were also shown to potentially increase the risk of plaque rupture by creating localized stress concentration areas within the artery, which depends on the shape, size, and location of the calcification (16). Furthermore, a higher number of calcifications within carotid plaques has been associated with intraplaque hemorrhage (17,18), a recognized marker of plaque instability. The obscurity in understanding the role of carotid calcification in ischemic event may be clarified by detailed calcification morphometric analyses. It is only recently that a few studies went beyond the traditional calcification presence, volume, or score measurements, and evaluated the size and location of carotid calcifications (17,19). Yet, we still lack a detailed description and comprehension of carotid calcification morphometry, its temporal changes, and its association with ischemic events.

In the present study, the association of detailed carotid calcification morphometrics, and their change over time, with ischemic events at the time of study inclusion was examined. A symptomatic patient cohort with non-stenotic carotids and their longitudinal multi-detector computed tomography angiography (MDCTA) carotid data were utilized.

METHODS

Study Population

The present study included participants (n = 244), from the Plaque At RISK cohort (PARISK) (20). Participants in the PARISK study experienced recent (< 3 months) large artery ischemic event, such as transient ischemic attack (TIA), minor stroke or amaurosis fugax, and had an ipsilateral carotid plaque of at least 2 mm thick with mild-to-moderate carotid artery stenosis (< 70%) at the time of study enrollment. An incident of brief, localized brain dysfunction of vascular origin that lasted little more than 24 h and did not cause any long-term neurological impairments was referred to as a "TIA." A non-disabling stroke or a brief, localized brain dysfunction of vascular origin that lasted more than 24 hours and had a modified Rankin Scale score of three or less was considered a "minor stroke." The definition of "amaurosis fugax" was a sudden, monocular loss of vision that was considered to have a vascular cause. Patients who had experienced a severe stroke at the time of inclusion were excluded from the PARISK study, as they would undergo interventional procedures such as carotid endarterectomy or stenting, which would alter or remove plaque composition. A likely cause of a heart embolism (such as atrial fibrillation), renal clearance less than 30 mL/min, coagulation disorders, severe comorbidities, and known allergies to contrast agents were among the exclusion criteria (20).

The baseline and 2-year follow-up MDCTA imaging was scheduled for 118 patients, but due to logistical problems (i.e., CT scanners were either under maintenance or experiencing technical issues, and some patients were unwilling to undergo the CT scan on that day) (n = 13), contra-indications for contrast material (n = 11), informed consent withdrawal (n = 9) and patient death (n = 3), the MDCTA scans were present for 82 patients. Information on cardiovascular risk factors (CVRF), including hypertension, hypercholesterolemia, diabetes mellitus, smoking status, body mass index, and patients' medication was obtained at the baseline (Supplementary Data). Written informed permission was provided by each patient, and Institutional Ethical Review Board (IRB) authorization (MEC 09–2-082) was acquired. The research centers' ethics committees had previously authorized the study procedure, which complied with the 1975 Declaration of Helsinki's ethical standards. Comprehensive details regarding the study's design have already been released (20). The reporting of this study adheres to the STROBE guidelines.

Calcification Morphometrics Assessment

At the time of inclusion and again after two years, patients underwent carotid artery contrast-enhanced MDCTA scans using the MDCTA system, following a previously outlined protocol (20). A trained reader (A.T.) performed lumen, vessel, and calcification segmentation of the ipsilateral and contralateral carotid arteries, relative to ischemic events, at both baseline and follow-up, using the QAngioCT software (Medis, version 3.2.0.13) (21,22). MDCTA scans were chosen to accurately segment the lumen, vessel wall, and calcification contours in carotid arteries and to optimize the detailed calcification morphometrics pipeline. A minimum of 30 horizontal CTA slices upstream and downstream of the carotid bifurcation were chosen in order to identify the region of interest (ROI). Then, QAngioCT performed longitudinal contouring, based on Hounsfield Units (HU) (Fig 1). The HU threshold for calcium detection was ≥600 HU to distinguish the calcifications from and the contrast agent (23). The reader and a second trained reader (F.F.) re-segmented and independently assessed a subset of 30 patients at baseline and follow-up (120 arteries) to investigate the intra-observer and inter-observer variability. The baseline and follow-up 2D contours of the lumen, vessel wall, and calcifications were transformed into 3D solid surfaces, co-registered longitudinally and circumferentially (MATLAB, v.2019B, Mathworks Inc., USA) (24,25). The 3D carotid geometries were partitioned into 2D cross-sectional slices perpendicular to the lumen centerline, with an inter-slice spacing of 0.5 mm (Vascular Modeling Toolkit 1.4.0, www.vmtk.org, and in-house developed MATLAB code) (26) (Fig 1).

At each cross-sectional slice, 2D calcification metrics were calculated based on the radial lines at every 1° originating from the lumen center. The 2D calcification metrics included calcification area (mm^2), relative calcification area (%) (i.e., calcification area divided by total vessel wall area*100%), thickness (mm), relative thickness (%), distance from the lumen (mm),

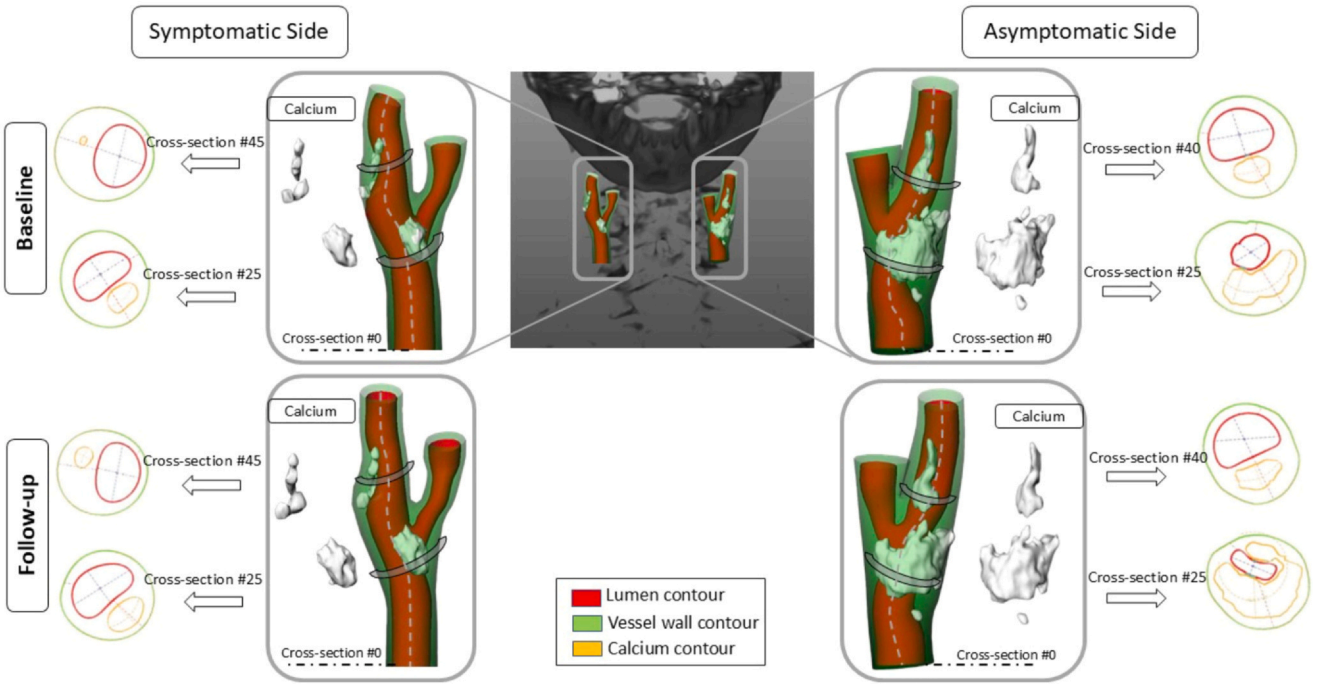


Figure 1. Demonstrative example of lumen, vessel wall, and calcification reconstruction and segmentation.

relative distance from the lumen (%), width (mm), arc angle (degree) and width-to-thickness ratio (i.e., circumferential arc width divided by maximum thickness). The calcifications were manually inspected to identify any lower-density calcifications connecting the segmented calcification areas. Once it was confirmed that the segmented calcifications were not in contact, even with lower-density calcifications, they were labeled as separate calcification bodies. For slices with multiple calcifications, the angle (degree) between the calcifications was measured as well. A calcification was identified as superficial if there was no tissue between the calcification and the lumen surface, and the length (mm) of the superficial calcifications was measured. The 3D (per artery) calcification metrics included the number of calcifications, calcification volume (mm^3), relative volume (%), calcification length (mm), and the contact area (mm^2) of superficial calcifications with the lumen. The number of spotty calcification bodies was identified if their axial length was < 3 mm and their arc $< 90°$ (19). A detailed description of the carotid calcification morphometrics analysis pipeline is presented in Figure S1 in Supplementary Data.

Statistical Analysis

Continuous variables are presented as mean with standard deviation or in case of skewed distribution, as median with interquartile range [Q1–Q3], and the categorical variables as absolute numbers with relative frequencies. The intra- and inter-observer variability was determined based on the intraclass correlation (Bland–Altman plots) of lumen, vessel wall, plaque burden, and calcification cross-sectional area measurements.

The carotid arteries were categorized as ipsilateral and contralateral based on ischemic events, with ipsilateral designated as symptomatic and contralateral as asymptomatic. Initially, we evaluated the distribution of calcification morphometrics at both baseline and follow-up. Then, the associations of calcification morphometric parameters with symptom presence at baseline were investigated utilizing the Generalized Linear Mixed Model (GLMM). In GLMM, the symptomatic and asymptomatic artery information was used as fixed factor, while adjusting for sex, hypertension, hypercholesterolemia, obesity, smoking status, diabetes mellitus, including patients' medication, patient's age, and vessel wall thickness at baseline by implementing them as fixed factors. Repeated comparisons of the p-values were corrected using the Bonferroni technique. Statistical analyses were conducted utilizing SPSS (IBM 27), and a two-tailed p-value < 0.05 was deemed significant. The artery and patient information were used as random factors to account for the intra-artery and within-patient dependency of the morphometric data. The GLMM at follow-up was also adjusted for baseline calcification metrics to present the change over time.

RESULTS

Patient Characteristics

Among the 82 patients in the PARISK cohort who underwent MDCTA imaging at baseline and the 2-year follow-up, 10 were excluded from prospective analysis—two due to low-quality CTA scans and eight due to the absence of calcifications in both carotids at both time points. Of the

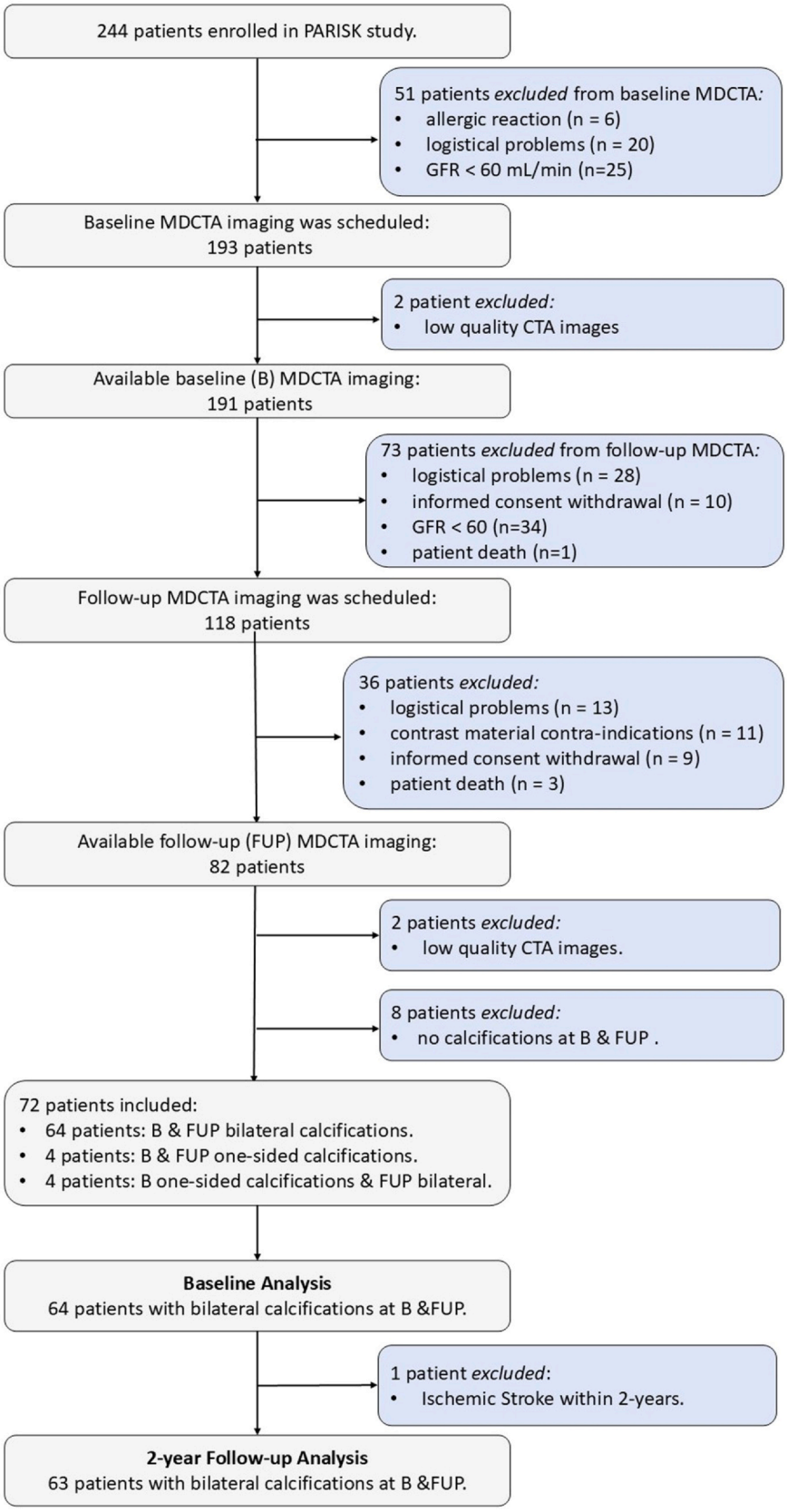


Figure 2. Study design flow chart.

TABLE 1. Patients' Characteristics at Time of Study Inclusion

Patients' Characteristics at Time of Study Inclusion (N = 64)	
Age (years, mean ± SD):	66 ± 8.3
Sex (men, *n* [%]):	48 (76%)
BMI (kg/m^2, mean ± SD):	26 ± 4.1
Hypertension, *n* (%):	45 (71%)
Hypercholesterolemia, *n* (%):	55 (87%)
Diabetes Mellitus, *n* (%):	12 (19%)
Current Smoking, *n* (%):	19 (30%)
Medication use at index event, *n*(%):	
Use of statins, *n* (%)	31 (49%)
Use of antihypertensive drugs, *n* (%)	38 (60%)
Use of antithrombotic drugs, *n* (%)	25 (39%)
Symptomatic right side, *n* (%):	35 (55%)

BMI, body mass index; SD, standard deviation.

remaining 72 patients, 64 had bilateral calcifications, and four had unilateral calcifications on the right side at both baseline and follow-up. The remaining four patients had unilateral calcifications on the right side at baseline, which progressed to bilateral calcifications at follow-up. In this study, 64 patients with bilateral calcifications were included (Fig 2). In Table 1 the patients' characteristics (n = 64) at the time of inclusion are listed. 48 patients (76%) were men and the mean age of the cohort was 66 ± 8. No significant cardiovascular risk factor differences were observed between men and women. A representative case example of the calcification morphometric analysis is illustrated in Figure 3.

Calcification Morphometrics Associations

The evaluation of intra- and inter-observer variability showed an excellent agreement, with an intraclass correlation coefficient greater than 0.92 and a coefficient of variation of 16.5% for lumen measurements, affected by the precise slice location of the bifurcation split, 8.2% for vessel wall, 3.1% for plaque burden, and 5.6% for calcification area in cross-sectional assessments (Supplementary Data: Table S1, Fig S2).

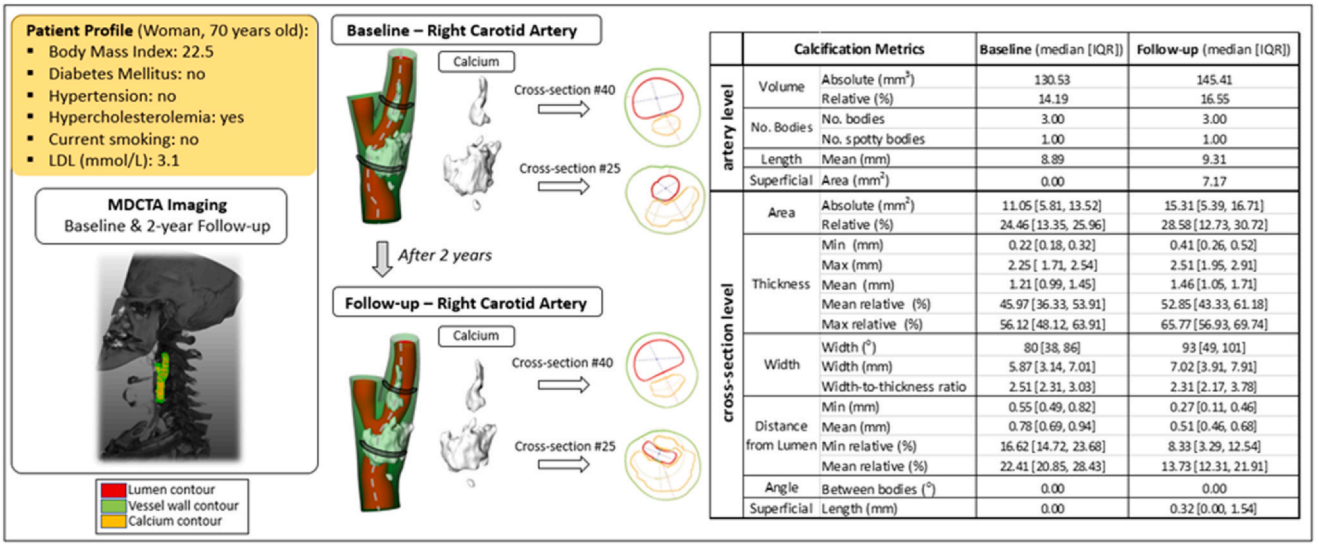
Baseline Analysis

At baseline, cross-sections containing at least one calcification body were selected for analysis, totaling 1961 out of 6505 cross-sections (30%). The absolute calcification volume was 35.8 mm^3 (IQR: 11.7–113.9 mm^3), while the median absolute calcification area and thickness were 3.2 mm^2 (IQR: 1.2–7.8 mm^2) and 0.9 mm (IQR: 0.6–1.4 mm), respectively. A detailed description of the calcification morphometrics measured at baseline is provided in Table 2 and Supplementary Data: Table S2 and Fig S3.

Figure 4 illustrates the GLMM analysis results at baseline. Compared to asymptomatic arteries, the symptomatic (ipsilateral to ischemic events) arteries had calcifications with smaller absolute and relative areas ($p < 0.001$). Moreover, symptomatic arteries had calcifications shorter in width ($p < 0.05$) and contained more calcification bodies ($p < 0.001$). Regarding the proximity of the calcifications to the lumen, symptomatic arteries presented calcifications with a larger absolute and relative distance from the lumen ($p < 0.001$) and a smaller superficial calcification area ($p < 0.001$).

Follow-up Analysis

After excluding one patient who experienced a recurrent ischemic stroke before the 2-year follow-up to ensure the inclusion of patients with identical clinical outcomes and to prevent alterations in plaque morphology due to rupture, 63 patients were examined in the follow-up evaluation (Fig 2). In the 2-year follow-up analysis, cross-sections containing at least one calcification body at baseline and/or during follow-



	Calcification Metrics		Baseline (median [IQR])	Follow-up (median [IQR])
artery level	Volume	Absolute (mm^3)	130.53	145.41
		Relative (%)	14.19	16.55
	No. Bodies	No. bodies	3.00	3.00
		No. spotty bodies	1.00	1.00
	Length	Mean (mm)	8.89	9.31
	Superficial	Area (mm^2)	0.00	7.17
cross-section level	Area	Absolute (mm^2)	11.05 [5.81, 13.52]	15.31 [5.39, 16.71]
		Relative (%)	24.46 [13.35, 25.96]	28.58 [12.73, 30.72]
	Thickness	Min (mm)	0.22 [0.18, 0.32]	0.41 [0.26, 0.52]
		Max (mm)	2.25 [1.71, 2.54]	2.51 [1.95, 2.91]
		Mean (mm)	1.21 [0.99, 1.45]	1.46 [1.05, 1.71]
		Mean relative (%)	45.97 [36.33, 53.91]	52.85 [43.33, 61.18]
		Max relative (%)	56.12 [48.12, 63.91]	65.77 [56.93, 69.74]
	Width	Width (°)	80 [38, 86]	93 [49, 101]
		Width (mm)	5.87 [3.14, 7.01]	7.02 [3.91, 7.91]
		Width-to-thickness ratio	2.51 [2.31, 3.03]	2.31 [2.17, 3.78]
	Distance from Lumen	Min (mm)	0.55 [0.49, 0.82]	0.27 [0.11, 0.46]
		Mean (mm)	0.78 [0.69, 0.94]	0.51 [0.46, 0.68]
		Min relative (%)	16.62 [14.72, 23.68]	8.33 [3.29, 12.54]
		Mean relative (%)	22.41 [20.85, 28.43]	13.73 [12.31, 21.91]
	Angle	Between bodies (°)	0.00	0.00
	Superficial	Length (mm)	0.00	0.32 [0.00, 1.54]

Figure 3. A representative case example of an asymptomatic carotid artery illustrating the calcification morphometric analysis pipeline.

TABLE 2. Baseline and Follow-up Carotid Calcification Morphometric (Median, Interquartile Range) Measurements

Baseline Calcification Metrics	All arteries (*N* = 128)	Symptomatic Arteries (*N* = 64)	Asymptomatic Arteries (*N* = 64)
Volume (mm^3)	35.82 [11.77, 113.91]	51.35 [16.39, 143.53]	27.28 [11.54, 96.28]
No. bodies	2.00 [1.00, 3.00]	3.00 [2.00, 4.00]	2.00 [1.00, 3.00]
Mean length (mm)	5.63 [3.32, 8.05]	5.63 [3.69, 8.05]	5.50 [2.96, 8.04]
Superficial Area (mm^2)	3.11 [0.22, 8.57]	3.08 [0.17, 7.57]	3.14 [0.22, 9.86]
Area (mm^2)	3.18 [1.19, 7.79]	3.14 [1.20,7.17]	3.30 [1.28, 8.38]
Mean thickness (mm)	0.93 [0.59, 1.41]	0.95 [0.61, 1.50]	0.93 [0.57, 1.33]
Width (mm)	2.32 [1.36, 4.62]	2.20 [1.26, 4.13]	2.37 [1.34, 4.28]
Width-to-thickness ratio	1.62 [1.21, 2.53]	1.64 [1.21, 2.61]	1.60 [1.20, 2.42]
Mean distance from lumen (mm)	0.86 [0.46, 1.53]	1.00 [0.52, 1.79]	0.73 [0.42, 1.27]
Angle Between bodies (°)	60.00 [30.00, 91.00]	57.00 [27.00, 87.00]	68.00 [42.00, 95.00]
Follow-up Calcification Metrics	*All arteries (N = 126)*	*Symptomatic Arteries (N = 63)*	*Asymptomatic Arteries (N = 63)*
Volume (mm^3)	63.57 [22.75, 137.25]	82.25 [22.21, 175.31]	55.43 [22.75, 103.53]
No. bodies	3.00 [2.00, 4.00]	3.00 [2.00, 5.00]	3.00 [2.00, 4.00]
Mean length (mm)	5.88 [3.87, 8.06]	6.21 [4.42, 7.89]	4.75 [3.33, 8.35]
Superficial Area (mm^2)	5.44 [2.17, 18.68]	5.68 [2.41, 21.11]	4.63 [1.11, 14.35]
Area (mm^2)	3.68 [1.25, 8.71]	3.72 [1.37, 9.32]	3.66 [1.13, 8.13]
Mean thickness (mm)	0.98 [0.61, 1.49]	1.00 [0.63, 1.54]	0.95 [0.59, 1.44]
Width (mm)	2.76 [1.42, 5.01]	2.71 [1.49, 5.14]	2.76 [1.33, 4.87]
Width-to-thickness ratio	1.69 [1.22, 2.62]	1.67 [1.21, 2.61]	1.72 [1.24, 2.71]
Mean distance from lumen (mm)	0.76 [0.37, 1.41]	0.75 [0.37, 1.49]	0.76 [0.38, 1.32]
Angle between bodies (°)	59.00 [29.00, 92.00]	62.00 [31.00, 94.00]	53.00 [27.00, 89.00]

up were selected, amounting to 2598 out of 6375 cross-sections (41%). The absolute calcification volume was 63.5 mm^3 (IQR: 22.7–137.2 mm^3) and the absolute calcification area had a median of 3.7 mm^2 (IQR: 1.3–8.7 mm^2), while the median absolute calcification thickness was 1 mm (IQR: 0.6–1.5 mm). A comprehensive overview of the calcification morphometrics measured at baseline is presented in Table 2 and Supplementary Data: Table S2 and Figure S3.

The results of the GLMM analysis for follow-up data, adjusting for baseline measurements are shown in Figure 5. Specifically, symptomatic (ipsilateral to ischemic events) arteries had calcifications with a smaller width ($p < 0.05$) and width-to-thickness ratio ($p < 0.01$) change over time compared to asymptomatic arteries. The calcification bodies within symptomatic arteries were longer ($p < 0.001$) and more spread with larger arc angle between calcification bodies ($p < 0.001$) over time. In contrast with baseline results, the superficial area of calcifications in symptomatic arteries was larger compared to asymptomatic arteries ($p < 0.05$).

DISCUSSION

A comprehensive morphometric assessment of carotid calcifications and their changes over time was reported in this study. This assessment enabled us to compare the morphometric features of calcifications in the symptomatic and contralateral asymptomatic carotid arteries. The main findings of this study are as follows: (1) although not their volume, some geometric features of calcifications are significantly different between symptomatic and asymptomatic carotids, (2) symptomatic carotids present more calcification bodies, and these calcifications are smaller and further away from the lumen (baseline analysis), and (3) the calcifications in symptomatic carotids grow less circumferentially and more longitudinally compared to the ones in asymptomatic carotids (follow-up analysis, adjusting for baseline measurements).

The potential role of carotid calcification in carotid plaque stability and subsequent ischemic event is still unclear. Whereas fewer stroke symptoms in patients with calcified carotid plaques, and greater and denser calcification content for stable carotid plaques were reported (27–29), some other studies failed to show any significant association of the calcification volume and the presence in carotid arteries with ischemic events (12,13).

From a biomechanical standpoint, an ischemic event can be triggered by a carotid plaque rupture. (14). Previous biomechanical studies highlighted that carotid calcifications confer stability by stiffening the atherosclerotic carotid artery (15,30–32). However, it was also demonstrated that carotid calcification location, shape, and size can destabilize the plaque by creating stress concentration points in the plaque (16). Hence, a better understanding of the role of carotid calcifications in ischemic event necessitates local calcification morphometric assessment, as in the current study.

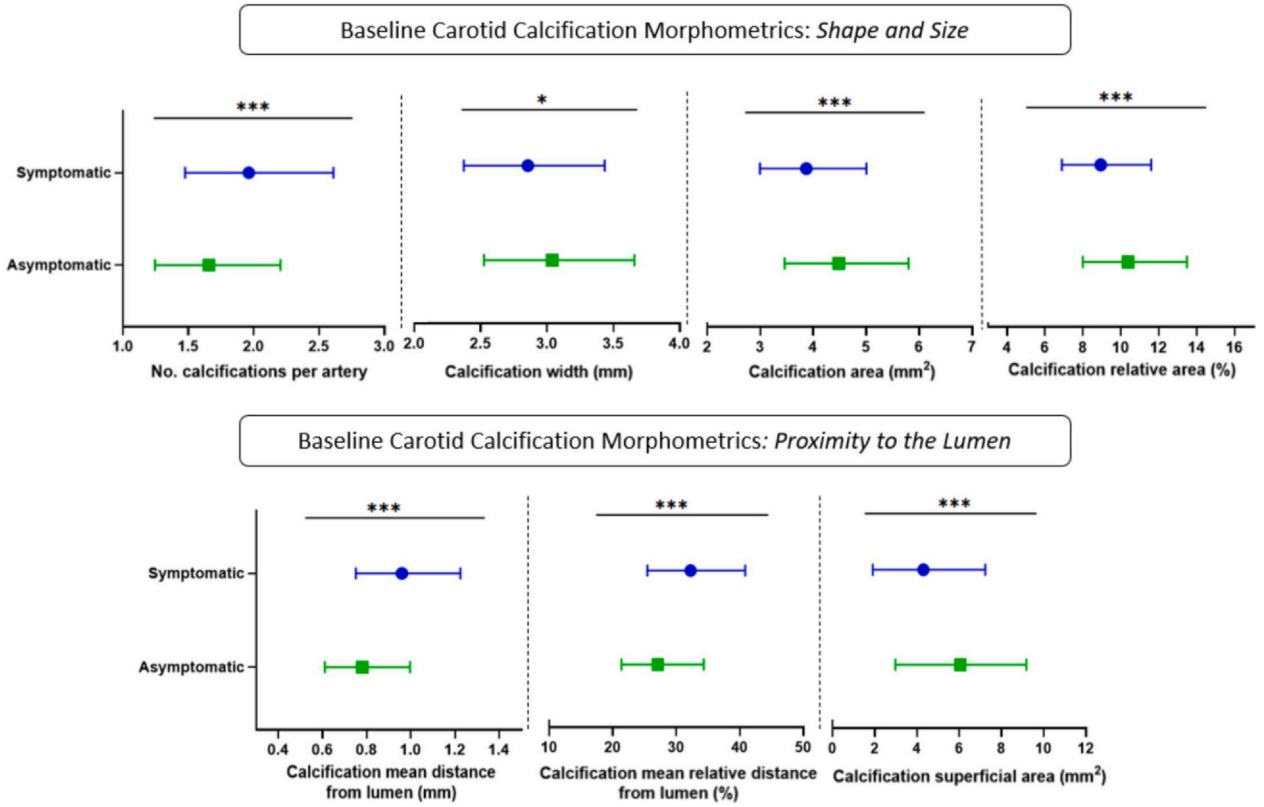


Figure 4. Baseline analyses: associations of calcification metrics with asymptomatic vs symptomatic (i.e., contralateral vs ipsilateral to ischemic events) arteries based on GLMM (95% CI), CI, confidence interval; GLMM, generalized linear mixed models. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Our finding of no significant difference in calcification volume or its change over time between symptomatic and asymptomatic carotids contrasts with some previous studies (27–29) but is consistent with a recent meta-analysis from the PARISK study (33). Although there was no difference in total calcification volume, symptomatic carotid arteries analyzed in our study presented greater number of calcifications. Multiple calcifications within the arteries possibly create scattered points of stress concentration and hence a higher risk of plaque rupture (17,34). Previously, higher number of calcifications in carotid plaques were also associated with the presence of intra-plaque hemorrhage (17,18), a plaque vulnerability marker. From a biological perspective, inflammation in atherosclerotic plaques suppresses calcification by inhibiting vascular smooth muscle cells' osteogenic differentiation through cytokine-mediated blockade of pathways (35,36). This pro-inflammatory environment leads to matrix degradation and plaque instability, while stable plaques exhibit reduced inflammation and greater calcification due to active osteogenesis (37,38). Symptomatic (ipsilateral to ischemic events) carotids in our study presented smaller calcifications, evident from the cross-sectional area and width measurements. These calcifications were also further away from the lumen and had a smaller superficial area. This may imply a greater soft component content, such as lipid-rich necrotic core or intra-plaque hemorrhage, in symptomatic carotids located closer to the lumen. However, this requires further confirmatory evidence to be collected in the future with imaging modalities that are more appropriate for this than MDCTA used in our study.

We have observed that the calcifications in symptomatic arteries had a smaller width-to-thickness ratio at follow-up, indicating that the calcification growth was more radial than circumferential in the symptomatic carotids. In line with our finding, calcifications that are elongated in the circumferential direction around the lumen were previously suggested to enhance plaque mechanical stability (39–43). Earlier, the carotid calcification length was reported to be associated with secondary major adverse events (44). In our study, symptomatic arteries presented also longer calcification bodies in the axial direction of the artery. Our findings suggest that a more detailed, in-depth morphometric analysis may offer better insight into the stabilizing effects of carotid calcifications, compared to traditional calcification volume and presence. Ultimately, these calcification metrics could serve as indicators of the risk for ischemic event recurrence, the occurrence of a first-ever event, or even as factors influencing treatment decisions in subclinical populations.

Our study has some limitations. First, we segmented calcifications greater than 600 HU using CTA scans, which probably led to an underestimation of the overall calcification volume and missed smaller or low-density calcifications.

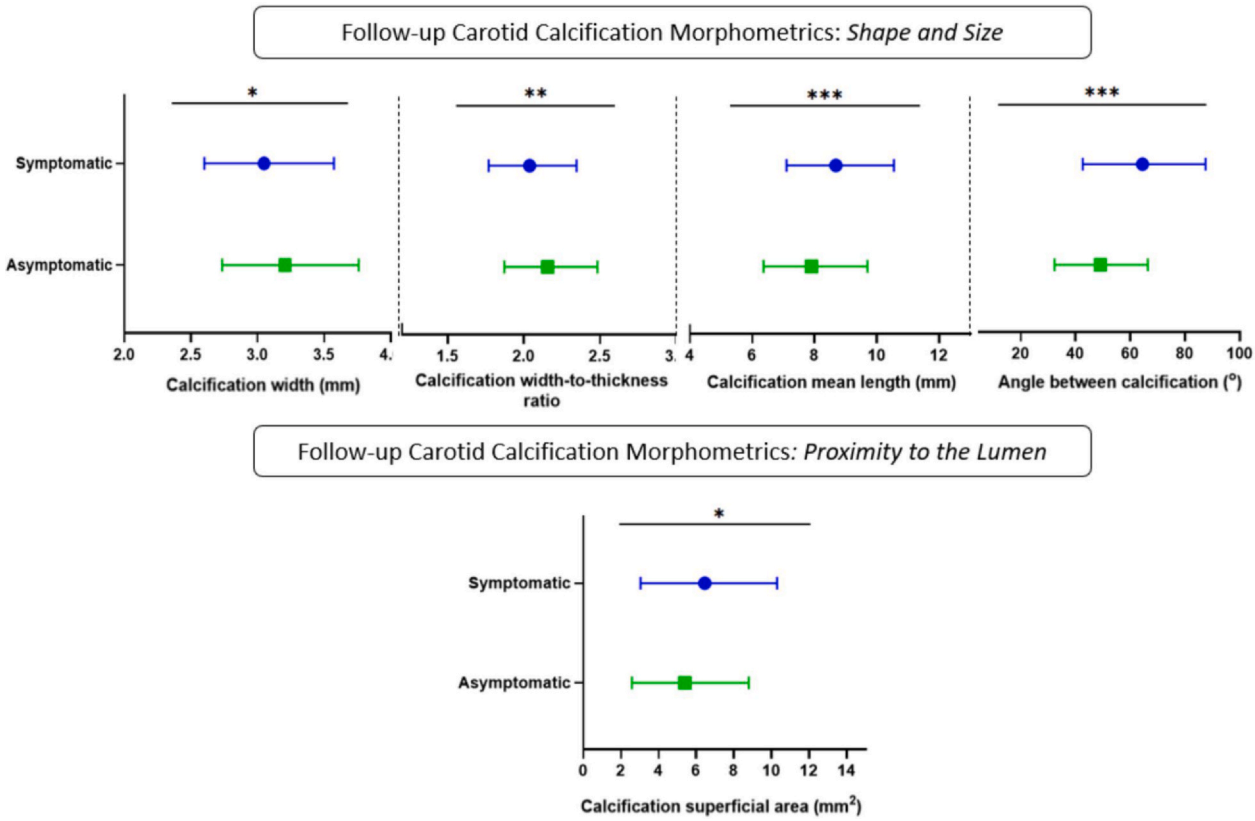


Figure 5. Follow-up analyses: associations of calcification metrics with asymptomatic vs symptomatic (i.e., contralateral vs ipsilateral to ischemic events) arteries based on GLMM (95% CI) adjusting for baseline measurements, CI, confidence interval; GLMM, generalized linear mixed models. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Alternatively, non-contrast CT scans could help address this underestimation, but CTA scans were essential for our in-depth carotid calcification morphometry assessment (e.g., proximity to lumen calculation). Second, our study focused on carotid calcifications, making standard clinical CTA scans adequate for our needs. However, recent advances in CT imaging, such as photon-counting computed tomography (45), also offer insights into soft plaque components and their association with calcifications and ischemic events. Finally, our study was limited to minor ischemic events at the time of study inclusion, as patients with major strokes typically undergo carotid endarterectomy or stenting procedures, excluding calcified cerebral embolism as a cause of ischemic events due to the mild-moderate carotid artery stenosis. While earlier research focused on ischemic stroke exclusively, we opted to broaden our investigation by including TIA, minor stroke, and amaurosis fugax as continuum subgroups (46).

CONCLUSION

Our study provided a detailed morphometric assessment of calcifications in mildly stenotic carotid arteries and demonstrated the association of some morphometric features of calcifications and their temporal changes with large artery ischemic events at the time of study inclusion. Compared to contralateral ones, carotids ipsilateral to ischemic events presented more and smaller calcifications, located further away from the lumen, with a tendency to grow more in the longitudinal artery direction. These findings highlight the importance of measuring the geometric features of calcifications to better describe and understand their potential role in ischemic events. These calcification metrics may ultimately act as indicators for the risk of ischemic event occurrence, or as determinants guiding treatment decisions in subclinical populations.

ETHICAL APPROVAL

The study received Institutional Review Board (MEC 09–2-082) approval, and all participants provided written informed consent. The study protocol adhered to the ethical guidelines of the 1975 Declaration of Helsinki and had previously received approval from the ethics committees of the participating research centers (clinical trials.gov NCT01208025).

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CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Aikaterini Tziotziou: data analysis, software development, writing – original draft; Federica Fontana: data analysis, writing – review & editing; Suze-Anne Korteland: software development, writing – review & editing; Juul Bierens: data collection, writing – review & editing; Paul J; Nederkoorn: data collection, writing – review & editing; Pim A de Jong: data collection, writing – review & editing; M; Eline Kooi: data collection, writing – review & editing; Aad van der Lugt: supervision, writing – review & editing; Antonius F.W; van der Steen: supervision, writing – review & editing; Jolanda J; Wentzel: conceptualization, writing – review & editing; Daniel Bos: supervision, conceptualization, writing – review & editing; Ali C; Akyildiz: supervision, conceptualization, writing – review & editing.

DATA AVAILABILITY

The data supporting this article cannot be shared publicly due to the privacy of individuals who participated in the study. The data can be made available by the corresponding author upon reasonable request.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REGISTRATION

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APPENDIX A. SUPPORTING INFORMATION

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.acra.2025.05.066.

REFERENCES

1. Naylor AR, Ricco JB, de Borst GJ, et al. Editor's choice—management of atherosclerotic carotid and vertebral artery disease: 2017 clinical practice guidelines of the European Society for Vascular Surgery (ESVS). Eur J Vasc Endovasc Surg 2018; 55:3–81.
2. Katan M, Luft A. Global burden of stroke. Semin Neurol 2018; 38:208–211.
3. Bonati LH, Kakkos S, Berkefeld J, et al. European Stroke Organization guideline on endarterectomy and stenting for carotid artery stenosis. Eur Stroke J 2021; 6:1–47. https://doi.org/10.1177/23969873211026990
4. Bos D, Arshi B, van den Bouwhuijsen QJA, et al. Atherosclerotic carotid plaque composition and incident stroke and coronary events. J Am Coll Cardiol 2021; 77(11):1426–1435. https://doi.org/10.1016/j.jacc.2021.01.038
5. Odink AE, van der Lugt A, Hofman A, et al. Association between calcification in the coronary arteries, aortic arch and carotid arteries: the Rotterdam study. Atherosclerosis 2007; 193(2):408–413. https://doi.org/10.1016/j.atherosclerosis.2006.07.007
6. van Dam-Nolen DHK, van Egmond NCM, Dilba K, et al. Sex differences in plaque composition and morphology among symptomatic patients with mild-to-moderate carotid artery stenosis. Stroke 2022; 53(2):370–378. https://doi.org/10.1161/STROKEAHA.121.036564
7. Ho JS, Cannaday JJ, Barlow CE, et al. Computed tomography detection of carotid calcium and subclinical carotid atherosclerosis. Int J Cardiovasc Imaging 2012; 28(6):1601–1607. https://doi.org/10.1007/s10554-011-9980-5
8. Denzel C, Lell M, Maak M, et al. Carotid artery calcium: accuracy of a calcium score by computed tomography-an in vitro study with comparison to sonography and histology. Eur J Vasc Endovasc Surg 2004; 28(2):214–220. https://doi.org/10.1016/j.ejvs.2004.05.004
9. Birenbaum D, Bancroft LW, Felsberg GJ. Imaging in acute stroke. West J Emerg Med 2011; 12(1):67–76.
10. Karlöf E, Seime T, Dias N, et al. Correlation of computed tomography with carotid plaque transcriptomes associates calcification with lesion-stabilization. Atherosclerosis 2019; 288:175–185. https://doi.org/10.1016/j.atherosclerosis.2019.05.005
11. Nandalur KR, Hardie AD, Raghavan P, et al. Composition of the stable carotid plaque: insights from a multidetector computed tomography study of plaque volume. Stroke 2007; 38(3):935–940. https://doi.org/10.1161/01.STR.0000257995.74834.92
12. Marquering HA, Majoie CB, Smagge L, et al. The relation of carotid calcium volume with carotid artery stenosis in symptomatic patients. AJNR Am J Neuroradiol 2011; 32(7):1182–1187. https://doi.org/10.3174/ajnr.A2519
13. Homssi M, Saha A, Delgado D, et al. Extracranial carotid plaque calcification and cerebrovascular ischemia: a systematic review and meta-analysis. Stroke 2023; 54(10):2621–2628. https://doi.org/10.1161/STROKEAHA.123.042807
14. Ahmed M, McPherson R, Abruzzo A, et al. Carotid artery calcification: what we know so far. Cureus 2021; 13(10):e18938. https://doi.org/10.7759/cureus.18938
15. Barrett HE, Van der Heiden K, Farrell E, et al. Calcifications in atherosclerotic plaques and impact on plaque biomechanics. J Biomech 2019; 87:1–12. https://doi.org/10.1016/j.jbiomech.2019.03.005
16. Gijsen FJH, Vis B, Barrett HE, et al. Morphometric and mechanical analyses of calcifications and fibrous plaque tissue in carotid arteries for plaque rupture risk assessment. IEEE Trans Biomed Eng 2021; 68(4):1429–1438. https://doi.org/10.1109/TBME.2020.3038038
17. Yang J, Pan X, Zhang B, et al. Superficial and multiple calcifications and ulceration associate with intraplaque hemorrhage in the carotid atherosclerotic plaque. Eur Radiol 2018; 28:4968–4977. https://doi.org/10.1007/s00330-018-5535-7
18. Lin R, Chen S, Liu G, et al. Association between carotid atherosclerotic plaque calcification and intraplaque hemorrhage: a magnetic resonance imaging study. Arterioscler Thromb Vasc Biol 2017; 37(6):1228–1233. https://doi.org/10.1161/ATVBAHA.116.308360
19. Zhang F, Yang L, Gan L, et al. Spotty calcium on cervicocerebral computed tomography angiography associates with increased risk of ischemic stroke. Stroke 2019; 50:859–866. https://doi.org/10.1161/STROKEAHA.118.023273
20. Truijman MTB, Kooi ME, van Dijk AC, et al. Plaque at RISK (PARISK): prospective multicenter study to improve diagnosis of high-risk carotid plaques. Int J Stroke 2014; 9:747–754. https://doi.org/10.1111/ijs.12167

21. Lansky A, Tuinenburg J, Costa M, et al. Quantitative angiographic methods for bifurcation lesions: a consensus statement from the European Bifurcation Group. Catheter Cardiovasc Interv 2009; 73(2):258–266. https://doi.org/10.1002/ccd.21814
22. de Graaf MA, Broersen A, Kitslaar PH, et al. Automatic quantification and characterization of coronary atherosclerosis with computed tomography coronary angiography: cross-correlation with intravascular ultrasound virtual histology. Int J Cardiovasc Imaging 2013; 29:1177–1190. https://doi.org/10.1007/s10554-013-0194-x
23. Glodny B, Helmel B, Trieb T, et al. A method for calcium quantification by means of CT coronary angiography using 64-multidetector CT: very high correlation with Agatston and volume scores. Eur Radiol 2009; 19:1661–1668. https://doi.org/10.1007/s00330-009-1345-2
24. Groen HC, Gijsen FJ, van der Lugt A, et al. Plaque rupture in the carotid artery is localized at the high shear stress region: a case report. Stroke 2007; 38(8):2379–2381. https://doi.org/10.1161/STROKEAHA.107.484766
25. Wang Q, Tang D, Wang L, et al. Combining morphological and biomechanical factors for optimal carotid plaque progression prediction: an MRI-based follow-up study using 3D thin-layer models. Int J Cardiol 2019; 15:266–271. https://doi.org/10.1016/j.ijcard.2019.07.005
26. Antiga L, Piccinelli M, Botti L, et al. An image-based modeling framework for patient specific computational hemodynamics. Med Biol Eng Comput 2008; 46:1097–1112. https://doi.org/10.1007/s11517-008-0420-1
27. Hunt JL, Fairman R, Mitchell ME, et al. Bone formation in carotid plaques: a clinicopathological study. Stroke 2002; 33(5):1214–1219. https://doi.org/10.1161/01.str.0000013741.41309.67
28. Saba L, Nardi V, Cau R, et al. Carotid artery plaque calcifications: lessons from histopathology to diagnostic imaging. Stroke 2022; 53(1):290–297. https://doi.org/10.1161/STROKEAHA.121.035692
29. Fan ZX, Yuan SJ, Li XQ, et al. Preliminary study on the differentiation of vulnerable carotid plaques via analysis of calcium content and spectral curve slope by using gemstone spectral imaging. Exp Ther Med 2022; 23(5):325. https://doi.org/10.3892/etm.2022.11254
30. Shaalan WE, Cheng H, Gewertz B, et al. Degree of carotid plaque calcification in relation to symptomatic outcome and plaque inflammation. J Vasc Surg 2004; 40(2):262–269. https://doi.org/10.1016/j.jvs.2004.04.025
31. Buffinton CM, Ebenstein DM. Effect of calcification modulus and geometry on stress in models of calcified atherosclerotic plaque. Cardiovasc Eng Tech 2014:244–260. https://doi.org/10.1007/s13239-014-0186-6
32. Huang H, Virmani R, Younis H, et al. The impact of calcification on the biomechanical stability of atherosclerotic plaques. Circulation 2001; 103(8):1051–1056. https://doi.org/10.1161/01.cir.103.8.1051
33. van Dam-Nolen DHK, Truijman MTB, van der Kolk AG, et al. Carotid plaque characteristics predict recurrent ischemic stroke and TIA: The PARISK (Plaque At RISK) Study. JACC Cardiovasc Imaging 2022; 15(10):1715–1726. https://doi.org/10.1016/j.jcmg.2022.04.003
34. Wong KK, Thavornpattanapong P, Cheung SC, et al. Effect of calcification on the mechanical stability of plaque based on a three-dimensional carotid bifurcation model. BMC Cardiovasc Disord 2012; 12:7. https://doi.org/10.1186/1471-2261-12-7
35. Ikeda K, Souma Y, Akakabe Y, et al. Macrophages play a unique role in the plaque calcification by enhancing the osteogenic signals exerted by vascular smooth muscle cells. Biochem Biophys Res Commun 2012; 425(1):39–44. https://doi.org/10.1016/j.bbrc.2012.07.045
36. Barillari G, Albonici L, Incerpi S, et al. Inflammatory cytokines stimulate vascular smooth muscle cells locomotion and growth by enhancing alpha5beta1 integrin expression and function. Atherosclerosis 2001; 154(2):377–385. https://doi.org/10.1016/s0021-9150(00)00506-2
37. Shioi A, Ikari Y. Plaque calcification during atherosclerosis progression and regression. J Atheroscler Thromb 2018; 25(4):294–303. https://doi.org/10.5551/jat.RV17020
38. Lee HY, Lim S, Park S. Role of inflammation in arterial calcification. Korean Circ J 2021; 51(2):114–125. https://doi.org/10.4070/kcj.2020.0517
39. Mori H, Torii S, Kutyna M, et al. Coronary artery calcification and its progression: what does it really mean? JACC Cardiovasc Imaging 2018; 11(1):127–142. https://doi.org/10.1016/j.jcmg.2017.10.012
40. Shi X, Gao J, Lv Q, et al. Calcification in atherosclerotic plaque vulnerability: friend or foe? Front Physiol 2020; 11:56. https://doi.org/10.3389/fphys.2020.00056
41. Xu X, Hua Y, Liu B, et al. Correlation between calcification characteristics of carotid atherosclerotic plaque and plaque vulnerability. Ther Clin Risk Manag 2021; 17:679–690. https://doi.org/10.2147/TCRM.S303485
42. Barrett HE, Cunnane EM, Kavanagh EG, et al. On the effect of calcification volume and configuration on the mechanical behaviour of carotid plaque tissue. J Mech Behav Biomed Mater 2016; 56:45–56. https://doi.org/10.1016/j.jmbbm.2015.11.001
43. Benitez J, Fontanarosa D, Wang J, et al. Evaluating the impact of calcification on plaque vulnerability from the aspect of mechanical interaction between blood flow and artery based on MRI. Ann Biomed Eng 2021; 49(4):1169–1182. https://doi.org/10.1007/s10439-020-02655-1
44. Nonin S, Iwata S, Sugioka K, et al. Plaque surface irregularity and calcification length within carotid plaque predict secondary events in patients with coronary artery disease. Atherosclerosis 2017; 256:29–34. https://doi.org/10.1016/j.atherosclerosis.2016.11.008
45. Van der Bie J, van Straten M, Booij R, et al. Photon-counting CT: review of initial clinical results. Eur J Radiol 2013; 163:110829. https://doi.org/10.1016/j.ejrad.2023.110829
46. Koudstaal PJ, van Gijn J, Frenken CW, et al. TIA, RIND, minor stroke: a continuum, or different subgroups? Dutch TIA Study Group. J Neurol Neurosurg Psychiatry 1992; 55:95–97.