

1 Carotid artery calcification as a predictor of systemic vascular events

2

3 Noritaka Sano, ^{1,2}; Tamaki Kobayashi, ^{1,3}; Hiroharu Kataoka, ¹; Akiko Ishigami, ⁴; Yusuke

4 Shimahara, ^{5,6}; Naomi Morita, ^{7,8}; Masanobu Yamada⁷; Kunihiro Nishimura, ⁹; Koji Iihara, ¹

5

6 ¹ Department of Neurosurgery, National Cerebral and Cardiovascular Center, Suita, Japan

7 ² Department of Neurosurgery, Kyoto University Hospital, Kyoto, Japan

8 ³ Department of Neurosurgery, Kyoto-Katsura Hospital, Kyoto, Japan

9 ⁴ Division of Cerebrovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Japan

10 ⁵ Department of Cardiovascular Surgery, National Cerebral and Cardiovascular Center, Suita, Japan

11 ⁶ Department of Cardiovascular Surgery, Tokyo Medical University Hospital, Tokyo, Japan

12 ⁷ Department of Radiology, National Cerebral and Cardiovascular Center, Suita, Japan

13 ⁸ Department of Radiology, Jinsenkai MI Clinic, Toyonaka, Japan

14 ⁹ Department of Preventive Medicine and Epidemiologic Informatics, National Cerebral and

15 Cardiovascular Center, Suita, Japan

16

17 **Corresponding author**

18 Hiroharu Kataoka,

19 Department of Neurosurgery, National Cerebral and Cardiovascular Center, Suita, Japan

1 National Cerebral and Cardiovascular Center,
2 5-7-1, Fujishiro-dai, Suita, Osaka 565-8565, Japan.
3 Telephone: (81) 661701070
4 E-mail: hkataoka@ncvc.go.jp
5

6 **Acknowledgments:** Editing assistance was provided by Editage (editage.com).

7 **Declaration of conflicting interest:**

8 **Funding statement:** This study was supported by the Intramural Research Fund (22-1-7) for
9 Cardiovascular Diseases of the National Cerebral and Cardiovascular Center.

10 **Ethical approval and informed consent statements:** The study protocol was approved by our local
11 institutional review board (M23-021-2). All patients provided voluntary written informed consent for
12 participation in the study and publication of their data.

13 **Data availability statement:** The data supporting the findings of this study are available from the
14 corresponding author upon reasonable request.
15

1 **Abstract**

2 **Background:** While coronary calcification is a well-known predictor of coronary events, the impact
3 of carotid artery calcification on systemic vascular events (e.g., cerebral ischemic, coronary, and
4 peripheral artery events) remains unclear. The aim of this study was to determine whether carotid
5 calcification can be used to predict systemic vascular events.

6 **Methods:** This single-center, retrospective cohort study included 194 patients who had a history of
7 vascular disease, including carotid stenosis or occlusion, coronary artery disease, valvular heart
8 disease, ischemic stroke, or transient ischemic attack. We collected data pertaining to risk factors and
9 laboratory parameters. Calcification of the carotid arteries was assessed via whole-body computed
10 tomography, and the modified carotid Agatston calcium score (CCS) was determined. Participants
11 were divided into two CCS groups according to the cut-off value determined via receiver operating
12 characteristic curve analysis. Coronary, ischemic cerebrovascular and peripheral vascular events
13 were recorded over a 5-year follow-up period, and their incidence was compared between the groups
14 using Cox proportional hazards regression analysis.

15 **Results:** Older age, hypertension, and chronic kidney disease had a significant positive impact on the
16 CCS. Systemic vascular events (hazard ratio: 2.70, confidence interval [CI]: 1.07–6.79, $P = 0.022$),
17 coronary events (hazard ratio: 4.29, CI: 0.87–21.1, $P = 0.045$), and peripheral vascular events
18 (hazard ratio: incalculable, $P = 0.032$) were significantly more frequent in the high vs. low CCS
19 group.

Conclusion: The CCS may be a useful tool for predicting future systemic vascular events, including coronary and peripheral artery diseases.

Keywords: atherosclerosis, carotid arteries, peripheral artery disease, vascular calcification

Background

Systemic atherosclerotic plaques continuously progress and calcify over time to form advanced lesions if left untreated.¹ Calcification of the coronary arteries and distal aorta starts at an early age, and calcification of the carotid arteries and other vascular beds occurs subsequently.² Coronary artery calcification correlates with plaque volume. The Agatston calcium score (ACS) of the coronary arteries has been used to predict coronary heart disease in White populations,^{3,4} and the MESA study group has applied this score to multiethnic populations.⁵

Whether calcification of other major vessels, such as the aorta and carotid artery, predicts vascular events has been investigated. For example, calcification of the abdominal aorta has been shown to be a risk factor for symptomatic aneurysms and subsequent rupture.^{6,7} Some studies suggest that calcium deposits in the cervical carotid artery can predict symptomatic and asymptomatic strokes,^{8,9} whereas others contend that they are not good predictors because they are associated with plaque stability.¹⁰ Owing to these contradictory claims, the relationship between carotid artery calcification and stroke remains controversial.

The effect of carotid artery calcification on systemic vascular events, especially in the population who have already undergone treatment for systemic artery disease has not been investigated to date. Therefore, the present study aimed to elucidate the relationship between carotid Agatston calcium score (CCS) and future vascular events in patients with advanced vascular diseases.

Materials and Methods

Study participants

After the protocol was approved by our local institutional review board (M23-021-2), we provided explanations regarding this study to patients hospitalized at National Cerebral and Cardiovascular Center between June 2011 and March 2013. The target patients included those admitted to the Department of Neurosurgery for elective surgeries for carotid artery stenosis or occlusion, those admitted to the Division of Cerebrovascular Medicine for ischemic cerebrovascular disease or transient ischemic attack (TIA) and those admitted to the Department of Cardiovascular Surgery for elective surgeries for atherosclerotic valvular disease or coronary artery disease. A total of 199 patients who fully understood the significance of this study, which included whole-body CT scans, blood tests, and urine analyses conducted purely for research purposes, and who provided voluntary consent, were enrolled (Fig.1). The initial prospective study did not include plans for long-term follow-up. However, five years after the conclusion of the enrollment period, a retrospective study was conducted to investigate the incidence of vascular events over a five-year period,

1 following a new internet based opt-out process and approval of local institutional review board.

2 Patient demographic and clinical data (including risk factors for atherosclerosis) were collected at
3 the time of enrollment. The diagnostic criteria were as follows: blood pressure $\geq 130/85$ mmHg or
4 current use of antihypertensive drugs for hypertension;¹¹ fasting plasma glucose ≥ 7.0 mmol/L,
5 hemoglobin A1C ≥ 48 mmol/mol, or previous diagnosis with or without treatment for diabetes
6 mellitus;¹² and serum cholesterol ≥ 5.172 mmol/L or current use of cholesterol-regulating agents for
7 hyperlipidemia.¹ An estimated glomerular filtration rate of <60 mL/minute/1.73 m² was indicative of
8 chronic kidney disease. Smoking was defined as the current use of tobacco or a past smoking habit
9 that had persisted for more than 10 years. Laboratory data and calcium scores (obtained via whole-
10 body computed tomography) were collected within 1 week after the enrollment.

11 12 *Protocol for calcium score acquisition*

13 All assessments were conducted using a SOMATOM Definition Flash computed CT scanner
14 (Siemens). Whole-body CT for each patient was performed from the orbitomeatal line to the ischial
15 tuberosity in a single session, and arterial visualization was achieved without contrast. The slice
16 thickness was 3 mm, and the X-ray voltage was 120 kV with a 500-ms exposure time. The area of
17 interest was the bilateral carotid arteries (common, internal, and external carotid arteries; the region
18 30 mm below and above the carotid bifurcation).

19 All images were analyzed using AW Volume Share2 and Smart Score 3.5 (GE Medical Systems)

by an observer blinded to the patient's clinical history. The ACS was determined for each vessel according to the original ACS method.³ The calcification volume was defined in Hounsfield units (HUs) weighted by maximal signal intensity scalers within the area (scalar 1, 130–199 HU; scalar 2, 200–299 HU; scalar 3, 300–399 HU; scalar 4, ≥ 400 HU). For example, if a calcified area in a CT slice has a maximum attenuation value of 350 HU (scaler 3) and occupies a 9-mm² area, its calcium score is 27 (3×9). The scores for all slices were added together to obtain the CCS. The total scores for the carotid arteries were summed. If carotid endarterectomy or carotid stenting was performed before enrollment, the score was excluded from the analysis.

Follow-up

We conducted a 5-year investigation using medical records to track the occurrence of vascular events, vascular surgeries, interventions in the area of interest, and cause of death in all patients. Patients who discontinued follow-up at our hospital during the 5-year study period owing to relocation or transfer to another facility were surveyed via telephone.

The primary outcome was a symptomatic vascular event (e.g., an ischemic cerebrovascular, coronary, aortic, or peripheral artery event). Ischemic cerebrovascular events were defined as focal neurological deficits that resulted in disability for more than 24 h and were confirmed by magnetic resonance imaging (MRI). Coronary events were diagnosed when two or more of the following four conditions were satisfied: 1) typical chest pain; 2) serum creatinine kinase-MB accounting for more

1 than 5% of total creatinine kinase, with its level more than twice the upper limit of the normal range;
2 3) typical electrocardiogram changes, including new ST or Q wave changes; and 4) a new cardiac
3 motion dyssynchrony on echocardiography continuing for more than 24 h. Symptomatic aortic
4 events included ruptures and new symptomatic dissecting aneurysms of the aorta or its major
5 branches. Symptomatic peripheral artery diseases included acute and chronic limb ischemia
6 presenting typical symptoms including pain, intermittent claudication, and pallor or chronic limb-
7 threatening ischemia due to stenosis or occlusion of the iliac artery or its major branches that was not
8 noted at the time of enrollment, intervention, or surgery. Lesions were confirmed by angiography or
9 CT angiography.

10 The secondary outcome was the elective procedure for asymptomatic lesions; these procedures
11 included carotid stenting, carotid endarterectomy, coronary artery stenting, endovascular aortic
12 repair, and lower extremity revascularization. Patients with symptomatic vascular lesions and related
13 vascular events that occurred prior to study enrollment were excluded from the analysis of future
14 vascular events. Deaths related to complications of elective surgery, which was scheduled at the time
15 of enrollment was not included in the symptomatic vascular events. However, all symptomatic
16 vascular events that occurred after enrollment were analyzed, regardless of whether they were
17 recurrences of the pre-enrollment vascular lesion or distinct from the initial disease.

18
19 *Statistical analysis*

JMP 17.0 software was used for statistical analysis of the data. The optimal cut-off value for the CCS were based on receiver operating characteristic curves for the occurrence of all CCS-related vascular events over the 5-year period (Fig. 2). Patients were accordingly divided into a high CCS group (a score of 126 or more) and low CCS group (a score of less than 126). Potential associations between the two groups and traditional vascular risk factors were evaluated using the Chi-square test. The relationship of the CCS as a continuous variable with age and blood and urine test data was assessed using Spearman's rank correlation coefficient and by determining *P*-values.

Differences in the frequency of the outcomes between the two CCS groups were analyzed using Fisher's exact test. To compare the frequencies of the primary outcomes, Kaplan–Meier cumulative event curves were generated, and Cox proportional hazards regression analysis was performed. The regression analysis was adjusted for background variables (e.g., hypertension, age >70 years-old, and chronic kidney disease) with *P* values <0.1 in a univariate analysis. In the regression analysis, only the first event in each vascular territory was considered. For all vascular events, only the earliest occurring event across the territories was considered.

All patients were included in the Kaplan–Meier and Cox proportional hazards regression analysis until their last visit or death, as long as either occurred within 5 years after their enrollment in the study. The elective procedure for asymptomatic lesions may introduce arbitrariness into the choice of timing and was therefore excluded from this analysis.

1 **Results**

2 *Demographic data*

3 Over a 24-month period, 199 patients were admitted with a history or the need for treatment of
4 the following: carotid artery stenosis or occlusion ($n = 96$), atherosclerotic valvular disease or
5 coronary disease ($n = 38$), or ischemic cerebral stroke or transient ischemic attack ($n = 65$). Five
6 patients were excluded from the analysis because they had a prior history of carotid artery
7 intervention or surgery. Of the 194 enrolled patients, 110 and 84 were assigned to the high and low
8 CCS groups, respectively (Fig. 1). Table 1 presents the baseline characteristics of the study cohort.

9 Age greater than 70 years ($P = 0.001$), hypertension ($P < 0.002$), and chronic kidney disease (P
10 < 0.001) were associated with high CCS. Based on examination of atherosclerosis-related test data
11 using Spearman's rank correlation coefficient, the CCS as continuous variable positively correlated
12 with age ($R = 0.314$, $P < 0.001$), serum phosphate level ($R = 0.158$, $P = 0.042$), and serum creatinine
13 level ($R = 0.353$, $P < 0.001$) and negatively correlated with urine creatinine level ($R = -0.262$, $P =$
14 0.001) and estimated glomerular filtration rate ($R = -0.320$, $P < 0.001$). Among patients with a history
15 of vascular disease, peripheral artery disease was slightly (although not significantly) associated with
16 high CCS ($P = 0.12$) (Table 2).

17

18

19 *Long-term outcomes*

Table 3 shows the outcomes for the 167 (86.1%) patients who completed the 5-year follow-up.

Fifteen (7.7%) patients died, 11 (10.0%) and four (4.8%) in the high and low CCS groups, respectively; there was no significant difference in the mortality rate between the groups ($P = 0.27$). Three patients died of perioperative complications of the elective cardiovascular surgery soon after enrollment, which was not included in the cardiovascular event. Three patients in the high CCS group died of acute coronary syndrome compared with none in the low CCS group; this difference was not significant. One patient in low CCS group died of Ischemic stroke. Eight patients died of causes unrelated to vascular disease: malignancy ($n = 3$), pneumonia ($n = 3$), and unknown ($n = 2$). Twelve patients (6.2%) dropped out during the follow-up period.

The total number of symptomatic vascular events was significantly higher in the high CCS group (23 [20.9%]) than in the low CCS group (6 [7.1%], $P = 0.008$). Ischemic cerebrovascular events occurred in 10 (9.1%) patients in the high CCS group and five (6.0%) in the low CCS group. Coronary events occurred in nine (8.2%) and two (2.4%) patients in the high and low CCS groups, respectively. No symptomatic aortic events occurred in either group. Five (4.6%) patients in the high CCS group (but none in the low CCS group) experienced symptomatic peripheral artery disease. The elective procedures for asymptomatic lesions were as follows: coronary artery stenting (two in each group) and endovascular aortic repair (two in the high CCS group and one in the low CCS group).

In the Kaplan–Meier analysis, all vascular events were more frequent in the high CCS group than in the low CCS group (hazard ratio: 2.70, confidence interval [CI]: 1.07–6.79, $P = 0.022$; Cox

proportional hazards regression analysis) (Fig. 3A). The percentage of ischemic cerebrovascular events was greater, although not significantly so, in the high CCS group (Fig 3B). In the Cox proportional hazards analysis, the cumulative incidence of symptomatic coronary events (hazard ratio: 4.29, CI: 0.87–21.1, $P = 0.045$) (Fig. 3C) and peripheral artery events ($P = 0.032$) (Fig. 3D) was greater in the high CCS group than in the low CCS group. The hazard ratio and confidence interval for the peripheral artery events could not be calculated because there were no peripheral artery events in the low CCS group.

Discussion

In the present study, the percentage of cumulative symptomatic vascular events was significantly greater in the high vs. low CCS group during the 5-year follow-up. The cumulative incidence of symptomatic coronary events and peripheral artery events was also higher in the high CCS group. Previous studies have often used carotid echocardiography^{14–16} and carotid MRI^{17–19} to describe the relationship between carotid artery atherosclerosis and systemic vascular events and between carotid artery stenosis¹⁴ or carotid plaque vulnerability^{17–19} and stroke or another cardiovascular event. Other studies examined the association between carotid artery calcification and future cardiovascular events using carotid ultrasonography;^{15,16} however, this procedure is subject to inter-evaluator variability and potential interpretative bias when classifying calcification as present or absent. The novelty of the current study is our use of objective carotid artery calcification scores (determined via

noncontrast-enhanced carotid CT) to predict systemic vascular events. We believe that this method can be easily incorporated into routine preoperative examinations, even in local facilities without magnetic resonance imagers. Because the ACS is calculated using automatic measuring software, interrater variation is unlikely to occur.

The percentage of symptomatic peripheral vascular events (common iliac, external iliac, or femoral artery stenosis) in a major aortic branch was significantly greater in the high vs. low CCS group ($P=0.032$). The percentage of cumulative coronary events tended to be higher in the high CCS group ($P=0.045$), perhaps owing to the close association of the CCS with the coronary calcification and aortic calcification. These results are supported by prior studies^{2,20,21} showing that several common risk factors affect carotid and aortic calcification in healthy adults, particularly men.^{21,22} Carotid calcification has been associated with a higher incidence of residual stenosis after stenting,²³ as well as a lower rate of restenosis after carotid endarterectomy.²⁴

Importantly, the results of the present study suggest that the CCS can be used to predict the occurrence of future vascular events. Notably, although the relationship between the CCS and stroke was not proportional, a high CCS may be a risk factor for coronary or peripheral artery disease. Previous studies investigating the association between carotid artery calcification and ischemic cerebrovascular disease have yielded mixed results, with some demonstrating a positive correlation^{8,9} and others a negative correlation.¹⁰ This discrepancy may reflect the opposing effects of carotid artery calcification, namely induction of stenosis and plaque stabilization, and is not resolved by the

findings of the current study. However, carotid artery calcification clearly correlates with calcification in the systemic arteries, including the coronary and peripheral arteries, and is considered a determinant of future vascular events. Coronary artery calcification has also been associated with subsequent coronary artery events,^{3,5} as well as carotid stenosis and ischemic cerebrovascular events.^{5,25–27}

In our study, older age, hypertension, and chronic kidney disease positively correlated with carotid artery calcification. In patients with hypertension or chronic kidney disease, bone matrix proteins are upregulated and α -smooth muscle actin is downregulated in the media via the renin-angiotensin and aldosterone pathways.^{28,29} Additionally, hypercalcemia and hyperphosphatemia have been reported to promote the progression of calcification in patients with chronic kidney disease.^{30,31} In the present study, and in agreement with a previous report,³⁰ calcification correlated with higher intact parathyroid hormone levels, while not reaching the statistical significance. Serum and urine creatinine levels, as well as the estimated glomerular filtration rate, directly reflect the stage of chronic kidney disease and have a strong association with carotid calcification.

Diabetes mellitus is a well-known risk factor for coronary artery calcification. The expression of bone matrix proteins in the medial layer of the arteries is increased in patients with diabetes mellitus,³² and hyperglycemia directly induces the transformation of vascular smooth muscle cells to an osteoblast-like phenotype.³³ However, in the present study, diabetes was not associated with carotid calcification (Table 1). The Rotterdam study suggested that the effect of diabetes on vascular

1 calcification varies depending on the specific vascular bed. Although coronary calcification tends to
2 be higher in all patients with diabetes, carotid artery calcification is apparently higher in female vs.
3 male patients.²¹ This difference may account for the lack of correlation between diabetes and carotid
4 calcification in the present study, as the majority of patients were men.

5 The presence of calcified atherosclerosis in a given vascular bed moderately correlates with the
6 presence of calcified atherosclerosis in other vascular beds, even in the early stages of vascular
7 disease.² The present study shows that the severity of calcification among the systemic large arteries
8 is very similar in patients with advanced vascular disease.

9 In a previous study, calcified atherosclerosis in the carotid artery and aorta was related to
10 mortality, especially when due to non-coronary causes, whereas coronary calcification was mainly
11 associated with deaths due to coronary causes.²⁰ In agreement, we found that the mortality rate
12 tended to be higher in the high ACS group than in the low CCS group. Further studies are required to
13 determine whether a high CCS is a risk factor for mortality in the advanced stages of atherosclerosis.

14 This study identifies the CCS as an indicator of systemic vascular calcification and a potential
15 predictor of future systemic vascular events, even in patients with advanced atherosclerosis. To lower
16 the risk of perioperative myocardial infarction, preoperative screening for coronary syndrome is
17 important when planning carotid revascularization procedures.³⁴ Indeed, multi-detector CT screening
18 of the carotid artery (with or without contrast enhancement) is routinely performed before carotid
19 revascularization to evaluate the operative risks, as well as the risks associated with the other

vascular beds, including the coronary and peripheral arteries. The possibility of vascular disease in patients with high CCS should be investigated to prevent future events. Furthermore, carotid stenosis should be considered as a local manifestation of systemic atherosclerosis.

Limitations

The main limitation of this study was the small sample size. Most of the participants had multiple vascular risk factors and either underwent elective surgery or an intervention for carotid, coronary, or valvular heart disease. And only a subset of inpatients during the study period provided voluntary consent to participate in this research, and the possibility of some selection bias cannot be ruled out. Therefore, our results may not apply to populations (e.g., young populations) with a low risk of vascular events. Furthermore, the decreased risk of ischemic cerebrovascular and coronary events after surgical intervention may have mitigated the difference between the high and low CCS groups.

Conclusion

We identified the CCS as a useful tool for predicting future systemic vascular events, including coronary and peripheral artery disease.

Acknowledgments: Editing assistance was provided by Editage (editage.com).

1 **Statements and declarations**

2 **Ethical considerations:** The study protocol was approved by our local institutional review board
3 (M23-021-2).

4 **Consent to participate and for publication:** All patients provided voluntary written informed
5 consent for participation in the study and publication of their data.

6 **Funding:** This study was supported by the Intramural Research Fund (22-1-7) for Cardiovascular
7 Diseases of the National Cerebral and Cardiovascular Center.

8 **Conflict of interests:** Iihara reports lecture fees from Daiichi Sankyo. Co. Ltd., and stockholder in
9 Megwel.co.jp.

10 **Data availability statement:** The data supporting the findings of this study are available from the
11 corresponding author upon reasonable request.

12

13 **References**

- 14 1 Stary HC. The Sequence of Cell and Matrix Changes in Atherosclerotic Lesions of Coronary
15 Arteries in the First Forty Years of Life. *Eur Heart J.* 1990;11:3–19.
- 16 2 Allison MA, Criqui MH, Wright CM. Patterns and Risk Factors for Systemic Calcified
17 Atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2004;24:331–336.
- 18 3 Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Detrano R. Quantification
19 of Coronary Artery Calcium Using Ultrafast Computed Tomography. *J*

1 4 Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary Artery
2 Calcium Area by Electron-Beam Computed Tomography and Coronary Atherosclerotic
3 Plaque Area: A Histopathologic Correlative Study. *Circulation*. 1995;92:2157–2162.

4 5 Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, Liu K, Shea S, Szklo M,
5 Bluemke DA, et al. Coronary Calcium as a Predictor of Coronary Events in Four Racial or
6 Ethnic Groups. *N Engl J Med*. 2008;358:1336–1345.

7 6 Buijs RVC, Willems TP, Tio RA, Boersma HH, Tiellu IFJ, Slart RHJA, Zeebregts, CJ.
8 Calcification as a Risk Factor for Rupture of Abdominal Aortic Aneurysm. *Eur J Vasc*
9 *Endovasc Surg*. 2013;46:542–548.

10 7 Li ZY, U-King-Im J, Tang TY, Soh E, See TC, Gillard JH. Impact of Calcification and
11 Intraluminal Thrombus on the Computed Wall Stresses of Abdominal Aortic Aneurysm. *J*
12 *Vasc Surg*. 2008;47:928–935.

13 8 Fanning NF, Walters TD, Fox AJ, Symons SP. Association Between Calcification of the
14 Cervical Carotid Artery Bifurcation and White Matter Ischemia. *Am J Neuroradiol*.
15 2006;27:378–383.

16 9 Nandalur KR, Baskurt E, Hagspiel KD, Finch M, Phillips CD, Bollampally SR, Kramer CM.
17 Carotid Artery Calcification on CT May Independently Predict Stroke Risk. *Am J Roentgenol*.
18 2006;186:547–552.

19 10 Shaalan WE, Cheng H, Gewertz B, McKinsey JF, Schwartz LB, Katz D, Cao D, Desai T,

1 Glagov S, Bassiouny HS. Degree of carotid plaque calcification in relation to symptomatic
2 outcome and plaque inflammation. J Vasc Surg. 2004 Aug;40(2):262-9.

3 11 Deedwania PC. Hypertension and diabetes: new therapeutic options. Arch Intern Med.
4 2000;160(11):1585-94.

5 12 Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its
6 complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a
7 WHO consultation. Diabet Med. 1998 Jul;15(7):539-53.

8 13 National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and
9 Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of
10 the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation,
11 and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report.
12 Circulation. 2002;106(25):3143-421.

13 14 Ihle-Hansen H, Vigen T, Berge T, Walle-Hansen MM, Hagberg G, Ihle-Hansen H,
14 Thommessen B, Ariansen I, Røsjø H, Rønning OM, Tveit A, Lyngbakken M. Carotid Plaque
15 Score for Stroke and Cardiovascular Risk Prediction in a Middle-Aged Cohort From the
16 General Population. J Am Heart Assoc. 2023 Sep 5;12(17):e030739

17 15 Vigili de Kreutzenberg S, Fadini GP, Guzzinati S, et al. Carotid plaque calcification predicts
18 future cardiovascular events in type 2 diabetes. Diabetes Care 2015; 38: 1937–44.

19 16 Prabhakaran S, Singh R, Zhou X, et al. Presence of calcified carotid plaque predicts vascular

events: the Northern Manhattan Study. *Atherosclerosis* 2007; 195: e197-201.

17 Brunner G, Virani SS, Sun W, Liu L, Dodge RC, Nambi V, Coresh J, Mosley TH, Sharrett AR, Boerwinkle E, Ballantyne CM, Wasserman BA. Associations Between Carotid Artery Plaque Burden, Plaque Characteristics, and Cardiovascular Events: The ARIC Carotid Magnetic Resonance Imaging Study. *JAMA Cardiol.* 2021 Jan 1;6(1):79-86

18 van der Toorn JE, Bos D, Ikram MK, Verwoert GC, van der Lugt A, Ikram MA, Vernooij MW, Kavousi M. Carotid Plaque Composition and Prediction of Incident Atherosclerotic Cardiovascular Disease. *Circ Cardiovasc Imaging.* 2022 Mar;15(3):e013602

19 Bos D, Arshi B, van den Bouwhuijsen QJA, Ikram MK, Selwaness M, Vernooij MW, Kavousi M, van der Lugt A. Atherosclerotic Carotid Plaque Composition and Incident Stroke and Coronary Events. *J Am Coll Cardiol.* 2021 Mar 23;77(11):1426-1435.

20 Allison MA, Hsi S, Wassel CL, Morgan C, Ix JH, Wright CM, Criqui MH. Calcified Atherosclerosis in Different Vascular Beds and the Risk of Mortality. *Arterioscler Thromb Vasc Biol.* 2012;32:140–146.

21 Odink AE, van der Lugt A, Hofman A, Hunink MGM, Breteler MMB, Krestin GP, Witteman JCM. Risk Factors for Coronary, Aortic Arch and Carotid Calcification; The Rotterdam Study. *J Hum Hypertens.* 2010;24:86–92.

22 Mizuhashi S, Kataoka H, Sano N, Ideguchi M, Higashi M, Miyamoto Y, Iihara K. Impact of Diabetes Mellitus on Characteristics of Carotid Plaques and Outcomes After Carotid

1 Endarterectomy. *Acta Neurochir (Wien)*. 2014;156:927–933.

2 23 Katano H, Yamada K. Analysis of Calcium in Carotid Plaques With Agatston Scores for
3 Appropriate Selection of Surgical Intervention. *Stroke*. 2007;38:3040–3044.

4 24 Katano H, Mase M, Nishikawa Y, Yamada H, Yamada K. Analysis of Recurrent Stenosis
5 After Carotid Endarterectomy Featuring Primary Plaque Calcification. *Neurosurgery*.
6 2017;80:863–870.

7 25 Kim BJ, Choi SY, Lee SH, Kim CK, Ryu WS, Han MK, Oh BH, Bae HJ, Yoon BW.
8 Advanced Coronary Artery Calcification is Associated With Ischemic Stroke. *Cerebrovasc*
9 *Dis*. 2010;30:93–100.

10 26 Lee KB, Budoff MJ, Zavodni A, Polak JF, Jeffrey Carr J, Burke GL, Herrington DM.
11 Coronary Artery Calcium is Associated With Degree of Stenosis and Surface Irregularity of
12 Carotid Artery. *Atherosclerosis*. 2012;223:160–165.

13 27 Hermann DM, Gronewold J, Lehmann N, Moebus S, Jöckel K-H, Bauer M, Erbel R, Nixdorf
14 H, Recall Study Investigative Group. Coronary Artery Calcification is an Independent Stroke
15 Predictor in the General Population. *Stroke*. 2013;44:1008–1013.

16 28 Armstrong ZB, Boughner DR, Drangova M, Rogers KA. Angiotensin II type 1 Receptor
17 Blocker Inhibits Arterial Calcification in a Pre-Clinical Model. *Cardiovasc. Res*.
18 2011;90:165–170.

19 29 Savoia C, Burger D, Nishigaki N, Montezano A, Touyz RM. Angiotensin II and the Vascular

Phenotype in Hypertension. *Expert Rev Mol Med*. 2011;13:e11.

Block G, Hulbert-Shearon T, Levin N, Port F. Association of Serum Phosphorus and Calcium x Phosphate Product With Mortality Risk in Chronic Hemodialysis Patients: A National Study. *Am J Kidney Dis*. 1998;31:607–617.

Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung H, Emerick A, Greaser L, et al. Coronary-Artery Calcification in Young Adults With End-Stage Renal Disease Who Are Undergoing Dialysis. *N Engl J Med*. 2000;342:1478–1483.

Chen NX, Duan D, O'Neill KD, Moe SM. High Glucose Increases the Expression of Cbfa1 and BMP-2 and Enhances the Calcification of Vascular Smooth Muscle Cells. *Nephrol Dial Transplant*. 2006;21:3435–3442.

Boström KI, Jumabay M, Matveyenko A, Nicholas SB, Yao Y. Activation of Vascular Bone Morphogenetic Protein Signaling in Diabetes Mellitus. *Circ Res*. 2011;108:446–457.

Mantese VA, Timaran CH, Chiu D, Begg RJ, Brott TG. The Carotid Revascularization Endarterectomy Versus Stenting Trial (CREST): Stenting Versus Carotid Endarterectomy for Carotid Disease. *Stroke*. 2010;41:S31–S34.

Figure legends

Fig. 1 The study flow diagram

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Fig. 2 Receiver operating characteristic curve for predicting cumulative systemic vascular events over a 5-year post-enrollment period based on the carotid Agatston calcium score (CCS)

The optimal cut-off value was determined at the point of the asterisk (CCS: 126, sensitivity: 47.3%, specificity: 79.3%)

Fig. 3 Kaplan–Meier cumulative event curves and adjusted cox proportional hazards regression analysis for vascular events in patients with high and low carotid calcium scores

(A) The rates for all vascular events. (B) The rates for ischemic cerebrovascular events. (C) The rates for coronary events. (D) The rates for peripheral artery events. The differences in the curves in panels A, C and D are significant, whereas those in panels B are not significant. The *P* values were calculated via Cox proportional hazards regression analysis, and the analysis was adjusted for background variables (age >70 years old, hypertension, and chronic kidney disease) with *P* values <0.1 in a univariate analysis.

199 patients admitted to national cerebral and cardiovascular center
because of carotid stenosis or occlusion (n=96)
valvular or coronary disease (n=38)
ischemic cerebral stroke or transient ischemic attack (n=65)
from June 2011 to March 2013, and obtained whole body CT

Excluded:

5 patients with previous carotid stent
or carotid endarterectomy

194 patients whose carotid ACS was obtained
(median carotid ACS =166,
cut off value determined based on ROC curve=126)

High carotid ACS group (n=110)
Carotid ACS ≥ 126

Low carotid ACS group (n=84)
Carotid ACS < 126

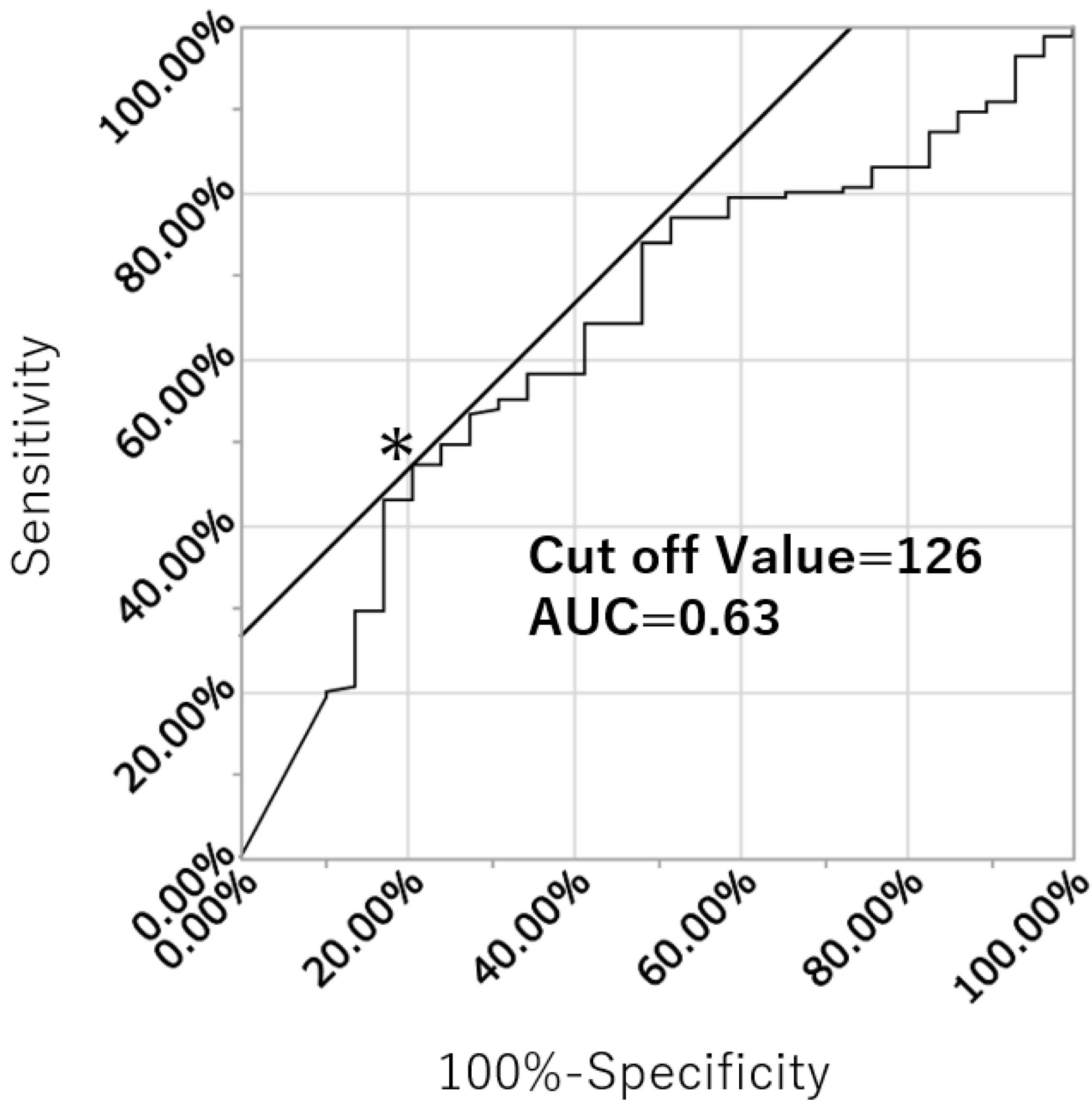


Table 1 Relationships between Clinical Characteristics and CCS

Carotid Calcium Score Median (25percentile-75percentile)	Yes	No	P value*
Age >70 years old	265 (31-792)	118 (1-330)	0.001
Sex (Male)	176 (15-637)	130 (8-561)	0.328
Hypertension	226(29-727)	29 (0-166)	0.002
Diabetes Mellitus	206 (26-673)	161 (5-589)	0.386
Hyperlipidemia	223 (25-628)	124 (5-658)	0.216
Smoking	172 (8-555)	162 (28-708)	0.846
Chronic Kidney Disease	313 (69-789)	79 (1-332)	<0.001

Carotid Calcium Score	All participants (n=194)	R**	P value
Age	70.1±8.5	0.314	< 0.001
C-reactive protein (mg/dL)	0.37±0.72	0.151	0.046
HbA1c (NGSP, %)	6.12±0.84	0.128	0.143
Serum LDL (mmol/L)	2.56±0.88	-0.083	0.285
Serum HDL (mmol/L)	1.25±0.88	-0.022	0.778
Serum triglycerides (mmol/L)	1.47±0.79	-0.143	0.064
Serum calcium (mmol/L)	2.34±0.11	0.129	0.096
Serum phosphate (mmol/L)	1.10±0.21	0.158	0.042
Intact PTH (pmol/L)	5.73±3.37	0.150	0.059
Serum Creatinine (mmol/L)	0.10±0.11	0.353	< 0.001
Urine creatinine (mmol/L)	8.50±5.25	-0.262	0.001
eGFR (ml/min)	62.4±21.1	-0.320	< 0.001

Data are presented as mean±SD

*Mann-Whitney's U test

** Spearman's rank order correlation coefficient

SD = standard deviation, NGSP = National Glycohemoglobin Standardization Program, LDL = low density lipoprotein, HDL=high density lipoprotein, PTH = parathyroid hormone, eGFR = estimated glomerular filtration rate

Table 2. Baseline Past history

Carotid calcium score	High (n=110)	Low (n=84)	P value *
Past history, n (%)			
Ischemic cerebrovascular disease	48 (43.6)	38 (45.2)	0.88
Coronary heart disease	40 (36.4)	25 (30.0)	0.36
Peripheral artery disease	13 (11.8)	4 (4.8)	0.12
Any history of above	78 (70.9)	56 (66.7)	0.53

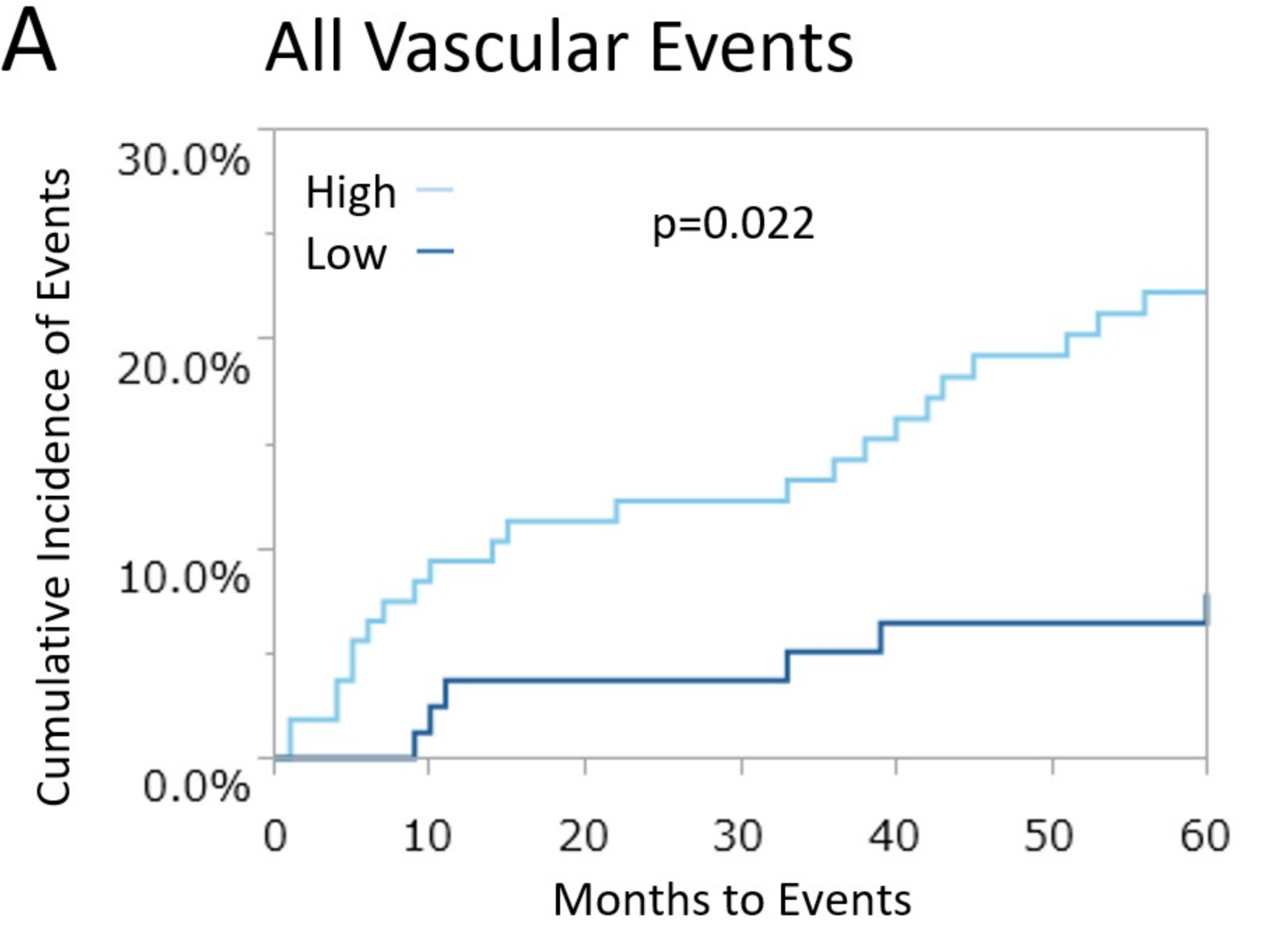
* Fisher’s exact test

Table 3. Long-term outcomes

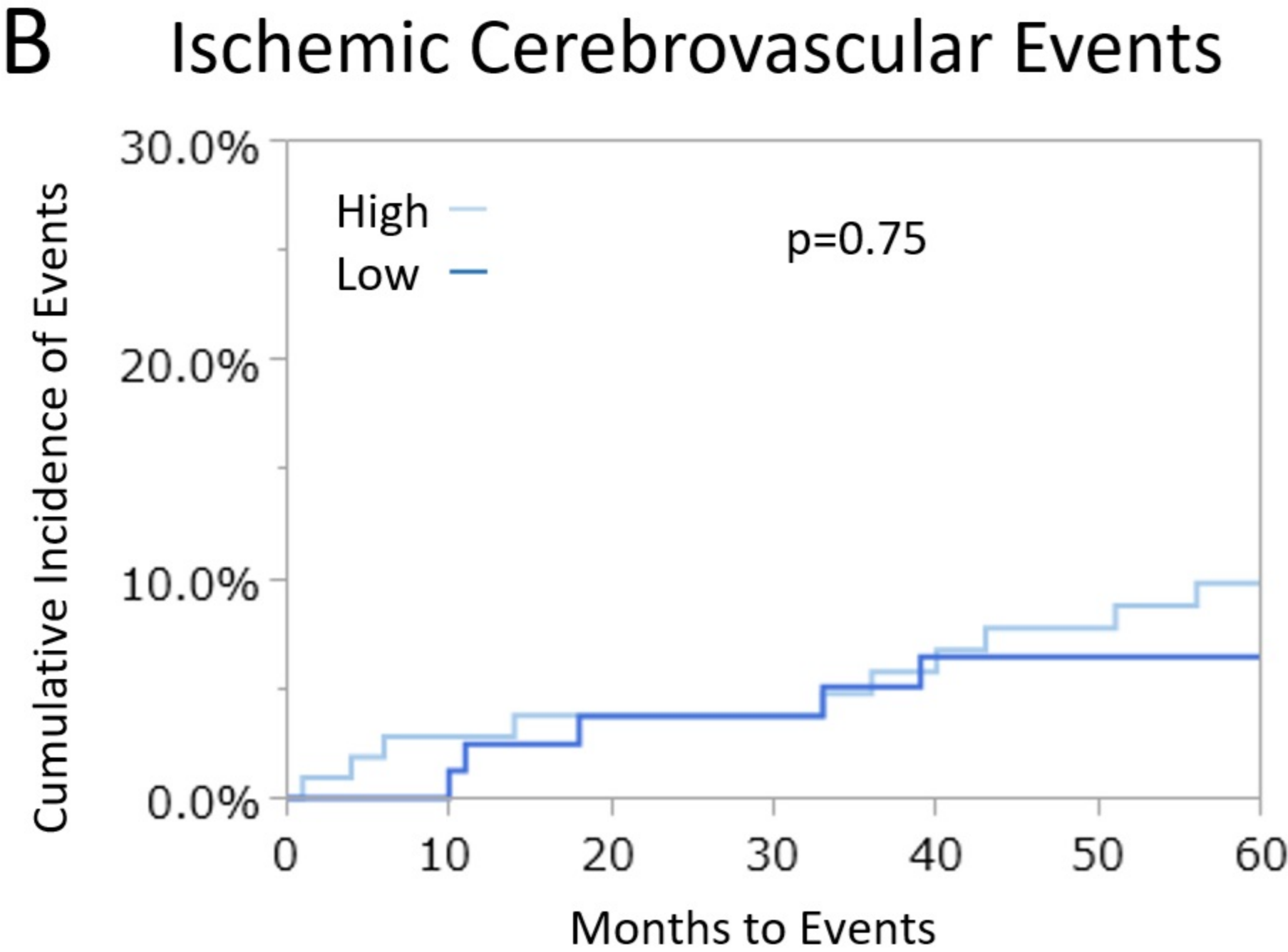
Carotid calcium score	High (n=110)	Low (n=84)	P value *
Death, n (%)	11 (10.0)	4 (4.8)	0.27
Operative Complication Related	3 (2.7)	0	0.26
Ischemic stroke Related	0	1(1.2)	0.43
Acute Coronary Syndrome Related	3 (2.7)	0	0.26
Aorta and Its Major Branch Related	0	0	1.0
Others [§]	5 (4.5)	3 (3.6)	1.0
All (symptomatic)Vascular Events	23 (20.9)	6 (7.1)	0.008
Ischemic Cerebrovascular Events, n (%)	10 (9.1)	5 (6.0)	0.59
Coronary Events, n (%)	9 (8.2)	2 (2.4)	0.12
Aortic Events (%)	0	0	1.0
Peripheral Artery Events (%)	5 (4.6)	0	0.071
Elective procedure for asymptomatic lesion			
Carotid stenting or Carotid Endarterectomy	0	0	1.0
Coronary artery stenting (%)	2 (1.8)	2 (2.4)	1.0
Endovascular aortic repair (%)	2 (1.8)	1 (1.2)	1.0
Lower extremity revascularization (%)	0	0	1.0

Fisher's exact test [§] Death irrelevant to vascular disease such as infectious disease, trauma or malignancy

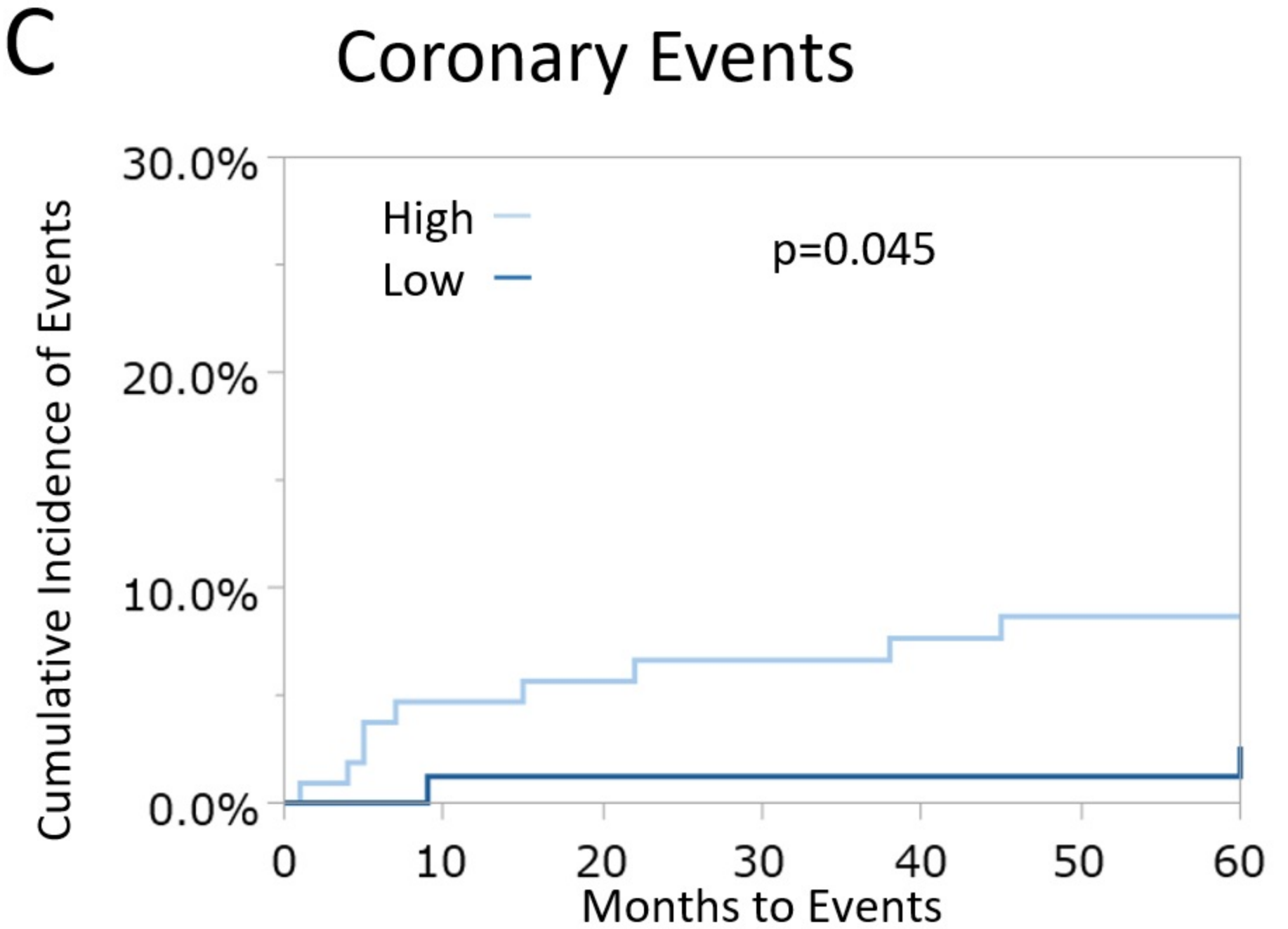
*



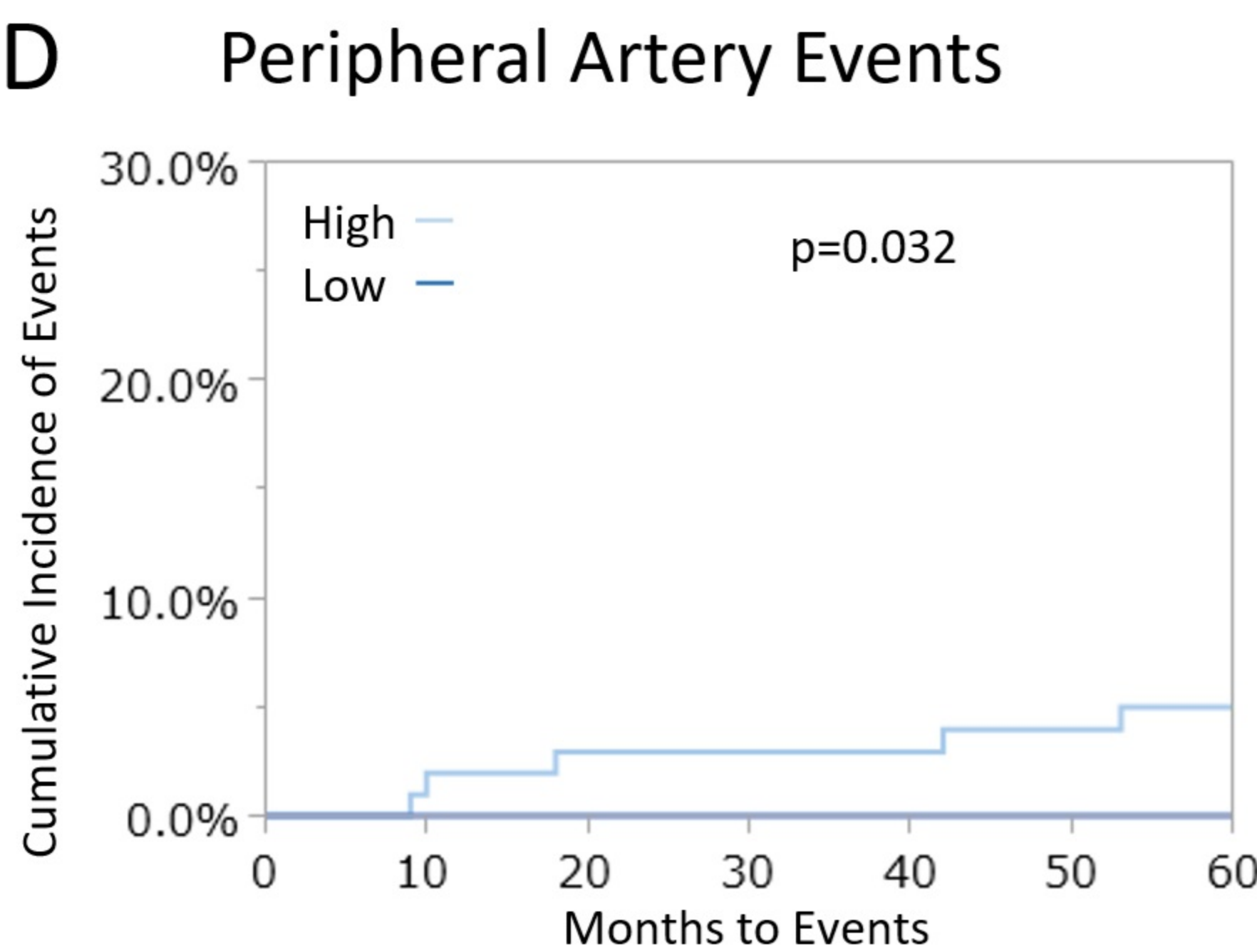
Months to events		10	20	30	40	50	60
High carotid calcium score	numbers at risk	96	94	90	86	81	76
	cumulative events	10	12	13	17	20	23
Low carotid calcium score	numbers at risk	80	75	73	69	68	66
	cumulative events	2	3	3	5	5	6



Months to events		10	20	30	40	50	60
High carotid calcium score	numbers at risk	102	101	98	95	90	86
	cumulative events	3	4	4	7	8	10
Low carotid calcium score	numbers at risk	82	76	74	70	70	68
	cumulative events	1	3	3	5	5	5



Months to events		10	20	30	40	50	60
High carotid calcium score	numbers at risk	102	98	94	92	90	88
	cumulative events	5	6	7	8	9	9
Low carotid calcium score	numbers at risk	81	77	76	73	72	71
	cumulative events	1	1	1	1	1	2



Months to events		10	20	30	40	50	60
High carotid calcium score	numbers at risk	102	100	97	96	93	90
	cumulative events	2	3	3	3	4	5
Low carotid calcium score	numbers at risk	82	78	77	74	73	72
	cumulative events	0	0	0	0	0	0