Journal of the American Heart Association

ORIGINAL RESEARCH

Predictors for Rapid Progression of Coronary Calcification: An Optical Coherence Tomography Study

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BACKGROUND: The role of coronary calcification in cardiovascular events and plaque stabilization is still being debated, and factors involved in the progression of coronary calcification are not fully understood. This study aimed to identify the predictors for rapid progression of coronary calcification.

METHODS AND RESULTS: Patients with serial optical coherence tomography imaging at baseline and at 6 months were selected. Changes in the calcification index and predictors for progression of calcification were studied. Calcification index was defined as the product of the mean calcification arc and calcification length. Rapid progression of calcification was defined as an increase in the calcification index above the median value. Among 187 patients who had serial optical coherence tomography imaging, 235 calcified plaques were identified in 105 patients (56.1%) at baseline. After 6 months, the calcification index increased in 95.3% of calcified plaques from 132.0 to 178.2 (P<0.001). In multivariable analysis, diabetes mellitus (odds ratio [OR], 3.911; P<0.001), chronic kidney disease (OR, 2.432; P=0.037), lipid-rich plaque (OR, 2.698; P=0.034), and macrophages (OR, 6.782; P<0.001) were found to be independent predictors for rapid progression of coronary calcification. Interestingly, rapid progression of calcification was associated with a significant reduction of inflammatory features (thin-cap fibroatheroma; from 21.2% to 11.9%, P=0.003; macrophages; from 74.6% to 61.0%, P=0.001).

CONCLUSIONS: Diabetes mellitus, chronic kidney disease, lipid-rich plaque, and macrophages were independent predictors for rapid progression of coronary calcification. Baseline vascular inflammation and subsequent stabilization may be related to rapid progression of calcification.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01110538.

Key Words: calcification ■ inflammation ■ lipid-rich plaque ■ macrophage ■ optical coherence tomography

Coronary artery calcification can be detected by various imaging modalities. Previous studies using computed tomography showed that the degree and the progression of coronary artery calcification were strongly associated with an increased incidence of cardiovascular events.[1,2] Some studies reported that spotty calcification was associated with plaque instability.[3,4] On the other hand, calcium density is inversely associated with cardiovascular risk.[5] Recent studies reported that statin stabilizes the plaque by promoting coronary atheroma calcification independent of plaque regression effects.[6,7] The role of calcification in cardiovascular events and plaque stabilization is still being debated, and factors involved in the progression of coronary calcification are not fully understood.

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Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.019235

For Sources of Funding and Disclosures, see page 12.

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CLINICAL PERSPECTIVE

What Is New?

- Diabetes mellitus, chronic kidney disease, lipid-rich plaque, and macrophages were independent predictors for rapid progression of coronary calcification.
- Rapid progression of calcification was associated with a significant reduction of inflammatory features of plaques (thin-cap fibroatheroma and macrophages).
- Majority of precursor plaques of de novo calcified plaques are lipid-rich plaque and have macrophage infiltration.

What Are the Clinical Implications?

- The data from the study support the notion that coronary calcification represents an advanced, but stable stage of atherosclerosis.

Nonstandard Abbreviations and Acronyms

DM	diabetes mellitus
LRP	lipid-rich plaque
TCFA	thin-cap fibroatheroma
VSMC	vascular smooth muscle cell

Some studies using computed tomography or intravascular ultrasound (IVUS) have evaluated the factors associated with progression of calcification.[7,8] However, computed tomography is limited in its spatial resolution and ability to evaluate details of plaque calcification. IVUS is also limited in evaluating calcification because of its poor spatial resolution and high reflection.[9] Optical coherence tomography (OCT) is an effective tool for the evaluation and quantification of coronary calcification because, unlike IVUS, light can penetrate calcification.[10,11] OCT can also evaluate microstructures such as fibrous cap, macrophages, and microvessels.[12] However, there are limited data on evaluation of coronary calcification using OCT.[13] This study was conducted to identify the predictors for rapid progression of coronary calcification.

METHODS

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

Study Population

Patients who had serial OCT imaging at baseline and at 6 months (5–7 months) were selected from the "Massachusetts General Hospital OCT registry" (ClinicalTrials.gov: NCT01110538). All patients provided written informed consent, and this study was conducted in compliance with the Declaration of Helsinki. A total of 194 eligible patients who were enrolled between September 2010 and January 2014 were identified. Among these, 5 patients were excluded because of poor imaging quality and 2 patients because of limited imaging of the stented segment only. Consequently, 187 patients with 321 vessels were included in the final analysis. Using serial OCT images, we evaluated the chronological change of calcified plaque and tried to identify baseline predictors for rapid progression of calcification. Moreover, we identified de novo calcified plaques in follow-up OCT images, and analyzed baseline patient characteristics and precursor plaque characteristics of de novo calcified plaques.

Definitions

The detailed definitions of clinical presentation, diabetes mellitus (DM), and chronic kidney disease are described in Data S1.

OCT Imaging Acquisition and Analysis

OCT imaging was performed using a frequency-domain (C7/C8, OCT Intravascular Imaging System, St. Jude Medical, St. Paul, Minnesota) or time-domain (M2/M3 Cardiology Imaging Systems; Light Lab Imaging Inc) OCT system after intracoronary administration of 100 to 200 μg of nitroglycerin. All OCT images were submitted to the core laboratory at Massachusetts General Hospital for offline analysis. Analysis was performed by 2 independent investigators who were masked to the clinical, angiographic, and laboratory data, using an offline review workstation (Ilumien Optis, St. Jude Medical). Previously stented coronary segments and coronary segments undergoing stent placement during the index procedure were excluded from OCT analysis. Several landmarks, including stent edges and anatomical landmarks such as side branches, pericardium, calcified plaque position and configuration, lumen shape, and/or positional or directional relationships among all these landmarks were used to identify the target calcified plaque.[13]

Calcification was identified as heterogeneous areas of high and low reflectivity, with low signal attenuation and sharply demarcated borders.[12,14] Calcified plaque was defined as a plaque that contained calcium.[12] Cross-sectional OCT images were quantitatively analyzed at 1-mm intervals.[15] Calcification arc and calcification thickness were evaluated in each cross-sectional view. Calcification length was measured in the longitudinal view. The calcification index

was defined as the product of the mean calcification arc and calcification length. Minimal calcification depth was defined as the minimum distance from the lumen to the superficial calcification edge.[14] Because of lack of established OCT criteria for superficial calcification, we used calcification depth thresholds of 65 μm and 100 μm to define calcifications as superficial-65 and superficial-100, respectively.[14] Microcalcification was defined as maximal calcium length <1 mm and maximal calcium arc <22.5°, spotty calcification was defined as calcium length ranging from 1 mm to 4 mm or maximal calcium arc ranging from 22.5° to 90°, and macrocalcification was defined as maximal calcium length >4 mm or maximal calcium arc >90°.[14,16] Additional plaque analysis was performed according to previously established criteria and described in Data S1.[12]

Statistical Analysis

Categorical data are presented as counts and percentages and were compared using the Chi-square test or Fisher exact test, as appropriate. Continuous data are presented as mean±SD or median (25th–75th percentile), as appropriate depending on the normality of distribution tested by the Kolmogorov–Smirnov test. Between-group comparisons were performed using independent-sample *t*-tests or Mann–Whitney *U* tests, as appropriate. Tests for the within-group longitudinal changes were performed using paired-sample *t* tests, Wilcoxon signed rank tests, or McNemar tests, as appropriate. A comparison of calcified plaque characteristics among different groups was performed using generalized estimating equations to consider the potential cluster effects of multiple calcified plaques in a single patient. Predictors for rapid progression of calcification were assessed with a multivariable logistic regression model. Independent variables with a $P<0.10$ in the univariate test were entered into the multivariable modeling. All analyses were performed with SPSS (version 25 for Windows; SPSS, Inc., Chicago Illinois).

RESULTS

Baseline Patient Characteristics

Baseline characteristics are shown in Table 1. The median follow-up duration was 184 days. The mean age was 59.2 years, 70.6% of patients were men, and 40.1% of patients presented with acute coronary syndrome (ACS). Among the 187 patients, calcified plaque was identified in 105 patients (56.1%) at baseline. Patients with calcified plaque had significantly higher prevalence of prior percutaneous coronary intervention (PCI) and DM. There was a trend toward higher prevalence of chronic kidney disease (CKD) in patients with calcified plaque. Nobody was on warfarin.

Serial OCT Findings

The results of serial OCT analysis of calcified plaques are shown in Table 2 and representative images are depicted in Figure 1. A total of 235 calcified plaques were identified in 105 patients at baseline. Among 235 calcified plaques, 112 (47.7%) calcified plaques were identified in patients with ACS and 123 (52.3%) were identified in patients with stable angina pectoris (SAP). Baseline and follow-up calcification index were not significantly different between patients with ACS and patients with SAP (baseline, 127.6 [58.8–315.7] versus 132.0 [57.7–263.8]; $P=0.836$, follow-up; 177.6 [90.0–362.9] versus 178.2 [81.0 versus 432.0]; $P=0.908$). During the 6-month period, calcification burden increased in the majority of patients (95.3%). At baseline, 52.3% of calcified plaques also met the criteria for lipid-rich plaque (LRP). The prevalence of thin-cap fibroatheroma (TCFA) and macrophages significantly decreased at follow-up (from 13.6% to 6.8%, $P<0.001$ and from 46.4% to 40.4%, $P=0.022$, respectively). In calcified plaques which met the criteria for LRP, lipid index significantly decreased and fibrous cap thickness significantly increased at follow-up.

Excellent intraobserver and interobserver agreement was noted in the OCT identification of calcified plaque (κ, 0.956 and 0.911, respectively), LRP (κ, 0.957 and 0.911), and macrophages (κ, 0.919 and 0.876).

Predictors for Rapid Progression of Calcification

To find the predictors for rapid progression of calcification, calcified plaques were divided into 2 groups based on the degree of calcification index change from baseline to follow-up. Rapid progression of calcification was defined as an increase in calcification index above the median value (median calcification index change was 40.6). Characteristics of calcified plaques with rapid progression are shown in Figure 2. Calcified plaques with rapid progression of calcification, compared with those without rapid progression, had significantly higher prevalence of LRP, TCFA, macrophages, cholesterol crystals, and microvessels, both at baseline and follow-up. TCFA and macrophages significantly decreased with rapid progression.

Table 3 shows the results of univariable and multivariable analysis. In multivariable analysis, DM and CKD were found to be independent clinical predictors for rapid progression of calcification, while LRP and macrophages were found to be independent morphologic predictors for rapid progression of coronary calcification (additional data are shown in Tables S1–S3 and Figure S1).

Table 1. Baseline Patient Characteristics

	All Patients (n=187)	Patients With Calcified Plaque (n=105)	Patients Without Calcified Plaque (n=82)	*P* Value
Follow-up duration, d	184 (175–193)	187 (179–193)	185 (174–195)	0.840
Length of OCT observation, mm	53.0 (32.6–90.6)	55.0 (35.9–92.4)	44.2 (30.0–81.0)	0.067
No. of observed vessels	2.0 (1.0–2.0)	2.0 (1.0–2.0)	1.0 (1.0–2.0)	0.111
Age, y	59.2±10.3	61.5±10.1	57.7±9.9	0.617
Men, n (%)	132 (70.6)	77 (73.3)	55 (67.1)	0.351
Clinical presentation				0.505
STEMI, n (%)	13 (6.9)	8 (7.6)	5 (6.1)	
NSTE-ACS, n (%)	62 (33.2)	38 (36.2)	24 (29.3)	
SAP, n (%)	112 (59.9)	59 (56.2)	53 (64.6)	
Prior MI, n (%)	64 (34.2)	35 (33.3)	29 (35.4)	0.771
Prior PCI, n (%)	102 (54.5)	67 (63.8)	35 (43.7)	0.004
Hypertension, n (%)	115 (61.5)	66 (62.9)	49 (59.8)	0.665
Dyslipidemia, n (%)	134 (71.7)	78 (74.3)	56 (68.3)	0.367
Diabetes mellitus, n (%)	76 (40.6)	50 (47.6)	26 (31.7)	0.028
Insulin user, n (%)	47 (61.8)	28 (56.0)	19 (73.1)	0.146
CKD, n (%)	17 (9.1)	13 (12.4)	4 (4.9)	0.077
Family history of CAD, n (%)	10 (5.3)	6 (5.7)	4 (4.9)	0.801
Smoking				0.774
Current smoker, n (%)	45 (24.1)	25 (23.8)	20 (24.4)	
Past smoker, n (%)	41 (21.9)	25 (23.8)	16 (19.5)	
Never smoker, n (%)	101 (54.0)	55 (52.4)	46 (56.1)	
Creatinine, mg/dL	0.90 (0.79–1.03)	0.91 (0.80–1.04)	0.89 (0.77–1.01)	0.450
eGFR, mL/min per 1.73m^2	85.0 (72.8–97.6)	84.0 (71.5–100.1)	85.9 (74.1–96.1)	0.669
LDL-C, mg/dL	90.2±33.5	92.7±35.7	88.9±31.1	0.513
HDL-C, mg/dL	49.0±19.9	49.1±17.0	48.9±24.0	0.894
Triglyceride, mg/dL	139.5±111.5	133.5±110.9	137.0±73.5	0.322
HbA1c, %	6.0 (6.0–7.1)	6.0 (6.0–8.0)	6.0 (5.9–7.0)	0.859
Medication at baseline				
Aspirin, n (%)	140 (74.9)	82 (78.1)	58 (70.7)	0.249
Clopidogrel, n (%)	111 (59.4)	69 (65.7)	42 (51.2)	0.045
Statin, n (%)	123 (65.8)	76 (72.4)	47 (57.3)	0.031
ACEI/ARB, n (%)	56 (29.9)	36 (34.3)	20 (24.4)	0.143
Medication at discharge				
Aspirin, n (%)	183 (97.9)	104 (99.0)	79 (96.3)	0.224
Clopidogrel, n (%)	173 (92.5)	102 (97.1)	71 (86.6)	0.006
Statin, n (%)	182 (97.3)	103 (98.1)	79 (96.3)	0.461
ACEI/ARB, n (%)	66 (35.3)	43 (41.0)	23 (28.0)	0.067

Values are mean±SD, n (%), or median (interquartile range). ACEI/ARB indicates angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; NSTE-ACS, non–ST-segment–elevation acute coronary syndrome; OCT, optical coherence tomography; PCI, percutaneous coronary intervention; SAP, stable angina pectoris; and STEMI, ST-segment–elevation myocardial infarction.

Change in Calcification at 1-Year Follow-Up

To evaluate the predictors for rapid progression of calcification over a longer period, we analyzed a subgroup of patients who had 1-year follow-up. OCT images of 46 calcified plaques in 18 patients were available with a median follow-up period of 373 (365–383) days. Median calcification index at baseline was 105.8 (58.2–272.8) and median change in calcification index from baseline to 6-month follow-up was 44.4 (11.9–84.1) and that from baseline to 1-year follow-up was 68.1 (24.5–165.5). Calcified plaques were divided into 2 groups according to the change in calcification index from baseline to 1-year follow-up. One-year rapid progression of calcification was defined as an increase

Table 2. Serial Optical Coherence Tomography Analysis of Calcified Plaque

	Baseline	Follow-Up	Change	*P* Value
Quantitative analysis of calcification				
Maximal calcification arc, degree	61.0 (43.0–101.0)	68.0 (48.0–106.0)	4.0 (1.0–10.0)	<0.001
Mean calcification arc, degree	46.0 (35.0–70.0)	54.0 (38.0–80.0)	5.0 (1.0–12.0)	<0.001
Calcification length, mm	2.6 (1.6–4.4)	3.2 (2.0–5.5)	0.4 (0.1–1.0)	<0.001
Calcification index	132.0 (58.5–281.2)	178.2 (86.4–402.8)	40.6 (12.2–107.1)	<0.001
Minimal calcium depth, μm	90 (40–180)	70 (40–170)	–10 (–30 to 0)	<0.001
Calcium classification				<0.001
Microcalcification, n (%)	11 (4.7)	5 (2.1)	…	
Spotty calcification, n (%)	129 (54.9)	120 (51.1)	…	
Macrocalcification, n (%)	95 (40.4)	110 (46.8)	…	
Superficial–65, n (%)	90 (38.3)	105 (44.7)	…	0.007
Superficial–100, n (%)	122 (51.9)	136 (57.9)	…	0.008
Calcified plaque characteristics				
Lipid-rich plaque, n (%)	123 (52.3)	121 (51.5)	…	0.500
Maximal lipid arc, degree	147.0 (123.0–186.0)	141.0 (116.0–181.3)	–7.0 (–16.8 to –1.0)	<0.001
Mean lipid arc, degree	116.0 (98.0–135.8)	110.0 (95.0–154.0)	–6.0 (–10.8 to –0.3)	<0.001
Lipid length, mm	5.7 (4.2–7.1)	5.5 (3.6–7.1)	–0.4 (–0.9 to 0.0)	<0.001
Thinnest FCT, μm	80 (60–120)	115 (90–150)	30 (10–50)	<0.001
Lipid index	636.4 (475.7–918.3)	571.2 (393.2–854.9)	–90.4 (–133.9 to –13.1)	<0.001
TCFA, n (%)	32 (13.6)	16 (6.8)	…	<0.001
Macrophage, n (%)	109 (46.4)	95 (40.4)	…	0.022
Cholesterol crystal, n (%)	30 (12.8)	25 (10.6)	…	0.267
Microvessel, n (%)	76 (32.3)	76 (32.3)	…	1.000
Minimal lumen area, mm^2	4.42 (3.20–6.21)	4.30 (2.76–5.90)	–0.25 (–1.09 to 1.41)	0.001
Area stenosis, %	45.6 (38.2–56.2)	49.1 (38.6–60.7)	1.8 (–3.6 to 7.3)	0.002

Values are mean±SD, n (%), or median (interquartile range). FCT indicates fibrous cap thickness; and TCFA, thin-cap fibroatheroma.

in calcification index above the median value (median calcification index change was 68.1). Table S4 shows the results of univariable and multivariable analysis. Although male, DM, CKD, current smoker, and prior history of myocardial infarction were associated with 1-year rapid progression of calcification in univariable analysis, no factor was found to be an independent clinical predictor for 1-year rapid progression of calcification in the multivariable analysis. On the other hand, macrophages were found to be an independent morphologic predictor for 1-year rapid progression of coronary calcification in multivariable analysis.

De Novo Calcified Plaque and OCT Findings

At follow-up, 14 de novo calcified plaques were identified in 14 patients. The baseline characteristics of patients with or without de novo calcified plaque are shown in Table S5. Patients with de novo calcified plaque had significantly higher rates of prior PCI and DM. Six of 14 (42.9%) de novo calcified plaques were identified in the previously stented arteries. Table S6 shows the serial OCT findings of de novo calcified plaque and its precursor plaques. Among 14 de novo calcified plaques, microcalcifications were observed in 7 (50.0%) and spotty calcifications were observed in 7 (50.0%). Among precursor plaques of de novo calcified plaques, 85.7% were LRP and 71.4% had macrophage infiltration.

Univariable and multivariable analysis were performed to find the predictors for de novo calcified plaque (Table S7). Multivariable analysis showed that DM and prior history of PCI were the independent clinical predictors for de novo calcified plaque. There was no significant morphologic predictor for de novo calcification in the multivariable analysis, although LRP tended to be associated with de novo calcified plaque.

Progression of Calcification, De Novo Calcification, and High-Sensitivity C-Reactive Protein

The relationships between progression of calcification, de novo calcified plaque, and hs-CRP (high-sensitivity

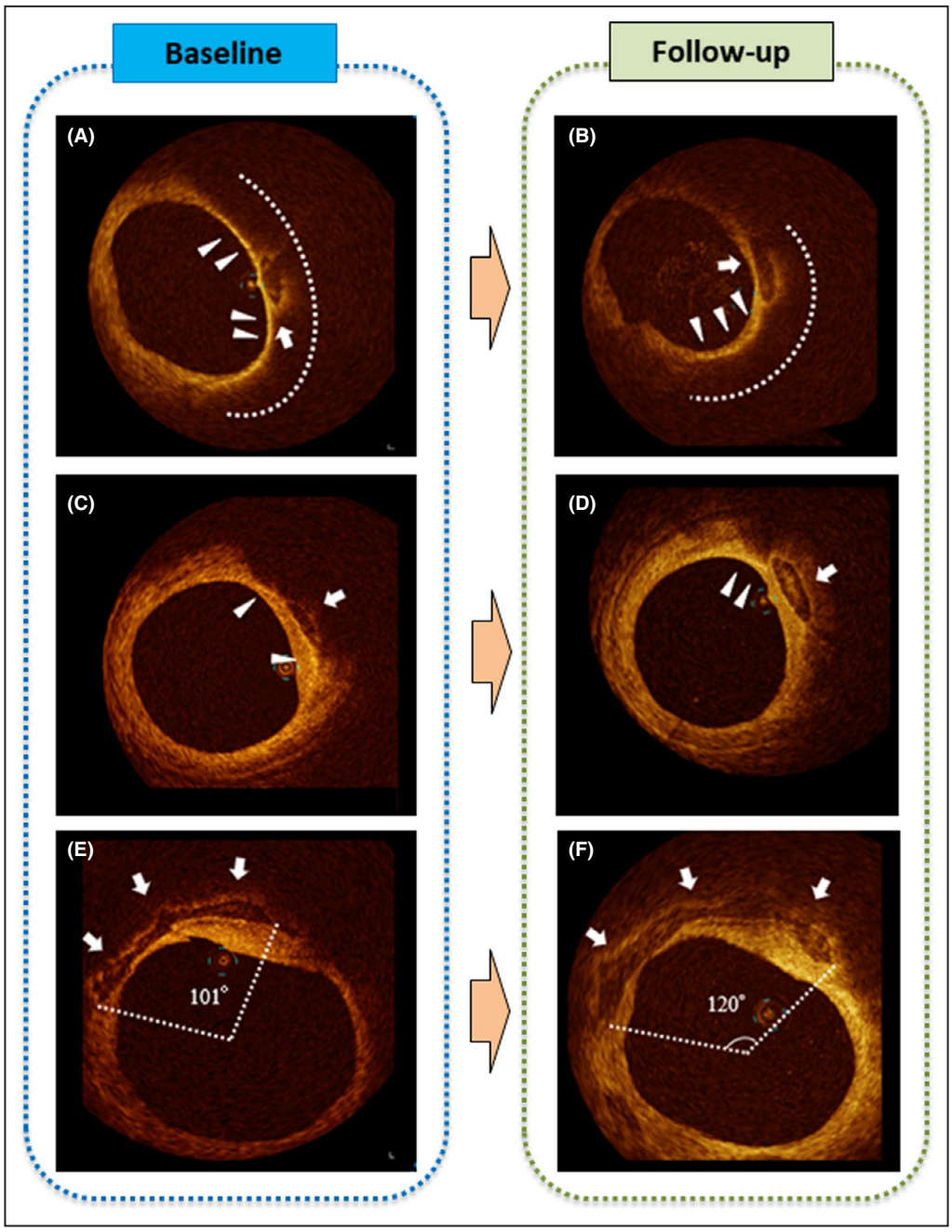
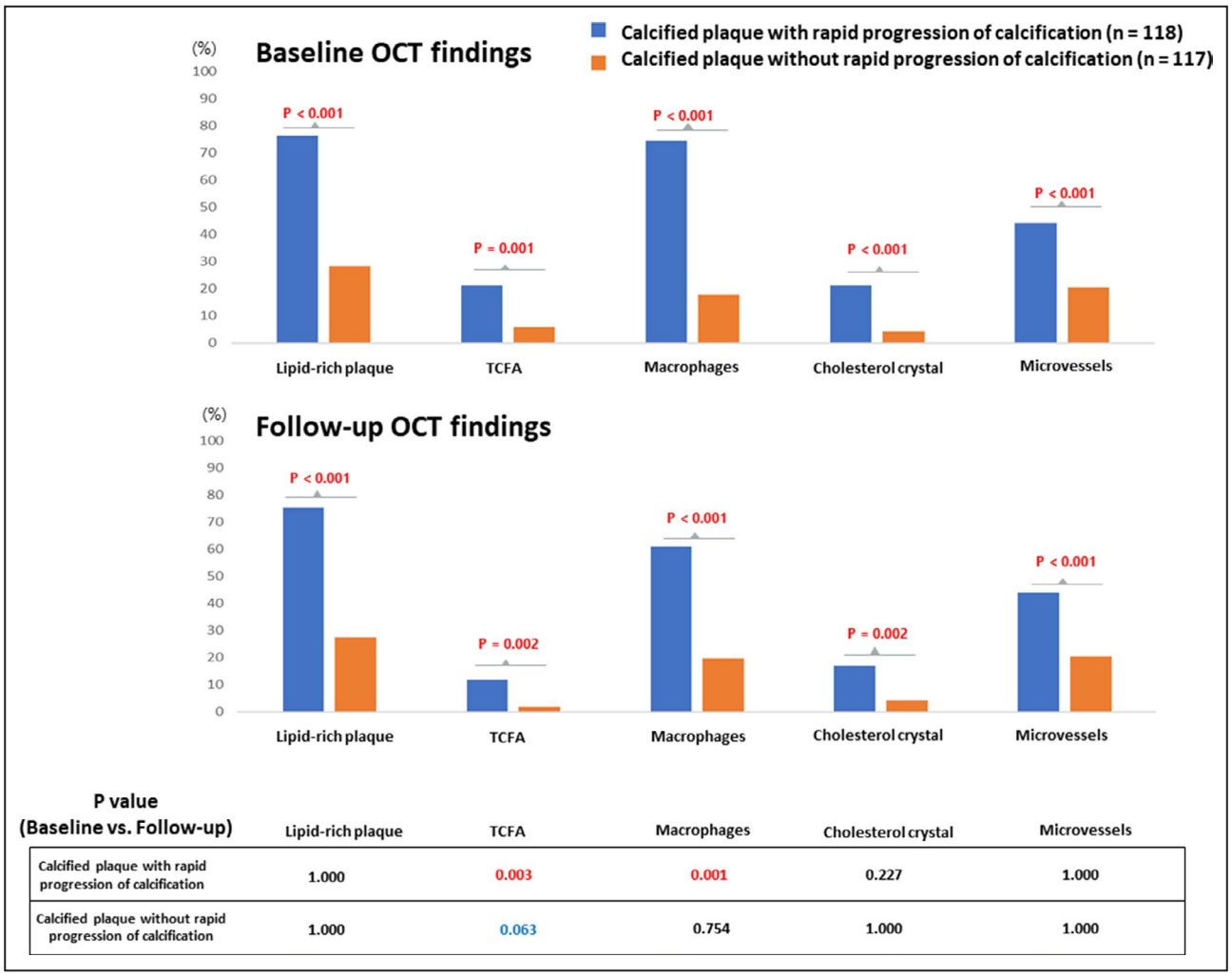


Figure 1. Representative images of calcified plaque.
Representative images of progression of coronary calcification with a reduction of inflammatory features. Calcification (white arrow) progressed from baseline (**A**, **C**, and **E**) to follow-up (**B**, **D,** and **F**). Images display a calcified plaque with lipid pool (dashed half circle) with macrophages (arrowhead) (**A** and **B**), calcified plaque with macrophages but without lipid pool (**C** and **D**), and the progression of macrocalcification (maximal calcification arc >90°) from baseline to 6-month follow-up (**E** and **F**).

C-reactive protein) are shown in Figure S2 and S3. Neither hs-CRP levels at baseline and follow-up, nor the change in hs-CRP levels were significantly different between patients with or without rapidly progressed calcified plaque or between patients with or without de novo calcified plaque.

DISCUSSION

To the best of our knowledge, this is the largest study to investigate predictors for progression of coronary calcification using serial OCT imaging. Our study demonstrated that (1) calcification was detected in 56.1%



P value (Baseline vs. Follow-up)	Lipid-rich plaque	TCFA	Macrophages	Cholesterol crystal	Microvessels
Calcified plaque with rapid progression of calcification	1.000	0.003	0.001	0.227	1.000
Calcified plaque without rapid progression of calcification	1.000	0.063	0.754	1.000	1.000

Figure 2. Optical coherence tomography findings of calcified plaque with or without rapid progression of calcification. Calcified plaque with rapid progression of calcification had significantly higher rates of lipid-rich plaque, thin-cap fibroatheroma, macrophages, cholesterol crystals and microvessels, both at baseline and follow-up. TCFA and macrophages significantly decreased in calcified plaques with rapid progression of calcification from baseline to follow-up. OCT indicates optical coherence tomography; and TCFA, thin-cap fibroatheroma.

of patients; (2) patients with calcified plaques had significantly higher prevalence of DM and prior PCI, and a trend toward higher prevalence of CKD; (3) the calcification index increased in 95.3% of the patients, while lipid burden and macrophage decreased over 6 months; (4) DM, CKD, LRP, and macrophages were independent predictors for rapid progression of calcification (Figure 3).

Natural History of Calcification

Previous pathology studies have reported the natural history of plaque calcification.[17,18] Early microcalcification which originates from vascular smooth muscle cell (VSMC) apoptosis and matrix vesicles is present in lipid pools. Microcalcifications coalesce into larger masses and involve both the necrotic core and surrounding collagen-rich extracellular matrix to form speckled and fragmented calcification. Further progressed calcified plaque forms calcified sheets or calcified plates. In some cases, calcified plates may fracture, resulting in calcified nodules. Hence, natural history studies show how the lipid component of plaques and formation of calcification inside plaques are intimately related during the progression of atherosclerosis.

DM and CKD for Progression of Coronary Calcification

Previous studies have reported the relationship between DM and CKD, and coronary calcification.[19–21] In patients with DM, insulin resistance and hyperglycemia increase oxidative stress by increasing glucose oxidation in the citric acid cycle. Oxidative stress upregulates Runt-related transcription factor-2

Table 3. Predictors for Rapid Progression of Calcification

	Univariable		Multivariable	
	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value
Patient clinical characteristics				
Follow-up duration, d	1.003 (0.983–1.024)	0.755		
Age, y	1.031 (0.999–1.064)	0.055	1.022 (0.991–1.055)	0.173
Men	0.654 (0.317–1.348)	0.250		
ACS	0.748 (0.394–1.422)	0.376		
Hypertension	0.943 (0.480–1.851)	0.864		
Dyslipidemia	1.400 (0.681–2.877)	0.360		
Diabetes mellitus	4.217 (2.314–7.685)	<0.001	3.911 (2.177–7.072)	<0.001
Chronic kidney disease	3.560 (1.550–8.176)	0.003	2.432 (1.054–5.615)	0.037
Family history of CAD	0.701 (0.196–2.501)	0.584		
Current smoker	0.764 (0.368–1.586)	0.470		
Prior MI	1.697 (0.902–3.192)	0.101		
Prior PCI	1.436 (0.743–2.774)	0.282		
Aspirin at discharge	…	1.000		
Clopidogrel at discharge	1.272 (0.181–8.924)	0.809		
Statin at discharge	3.135 (0.304–32.285)	0.335		
ACEI/ARB at discharge	0.745 (0.389–1.427)	0.375		
Plaque characteristics				
TCFA	4.224 (1.852–9.634)	0.001	0.843 (0.295–2.413)	0.751
Lipid-rich plaque	8.182 (4.098–16.334)	<0.001	2.698 (1.076–6.762)	0.034
Macrophage	13.410 (6.843–26.312)	<0.001	6.782 (3.142–14.637)	<0.001
Cholesterol crystal	6.022 (1.902–19.012)	0.002	2.890 (0.881–9.477)	0.080
Microvessel	3.053 (1.777–5.245)	<0.001	1.571 (0.760–3.249)	0.223
Minimal lumen area, mm^2	0.909 (0.824–1.003)	0.058	0.993 (0.867–1.138)	0.993
Area stenosis, %	1.015 (0.993–1.038)	0.186		
Baseline calcium index	1.005 (1.002–1.009)	0.006	1.004 (1.000–1.007)	0.074
Minimal calcium depth, μm	0.994 (0.991–0.998)	0.001	0.998 (0.994–1.001)	0.229

ACEI/ARB indicates angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; ACS, acute coronary syndrome; CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; and TCFA, thin-cap fibroatheroma.

and promotes VSMC calcification. Furthermore, driven by hyperglycemia and oxidative stress, apoptosis of endothelial cells and their overall dysfunction promote endothelial permeability, exposing VSMCs to hyperglycemia and other inflammatory circulation factors known to promote calcification such as tumor necrosis factor-α and receptor activator of the nuclear factor-κB ligand.[20] Moreover, the production of tumor necrosis factor-α from both endothelial and VSMCs induces the production of bone morphogenetic protein-2, a potent osteoblastic differentiation factor, which promotes osteogenesis and calcification of VSMCs.[22] One previous study reported that type 2 DM did not have an impact on localization, size, shape, or extension of calcification using OCT.[16] However, this study evaluated only culprit lesions before coronary intervention at one time point. Thus, the prevalence of calcified plaque including non-culprit plaques and the progression of calcification over time could not be evaluated. In addition, patients with ACS or CKD were excluded in this study. Moreover, the rate of statin therapy was relatively low in this study. Other studies showed the association between statin therapy and calcification.[6,7] It is important to study the relationship between DM and calcification in patients treated with statin because most patients with coronary artery disease are treated with statin. Furthermore, the number of plaques evaluated in this study was small (n=105). In contrast, our study evaluated changes in calcification over time using serial OCT imaging. In addition, our study included the patients with ACS or CKD.

In patients with CKD, dysregulation of calcium and phosphate metabolism, and inflammation are the most common factors causing vascular calcification.[23] Elevated calcium and phosphate have direct effects

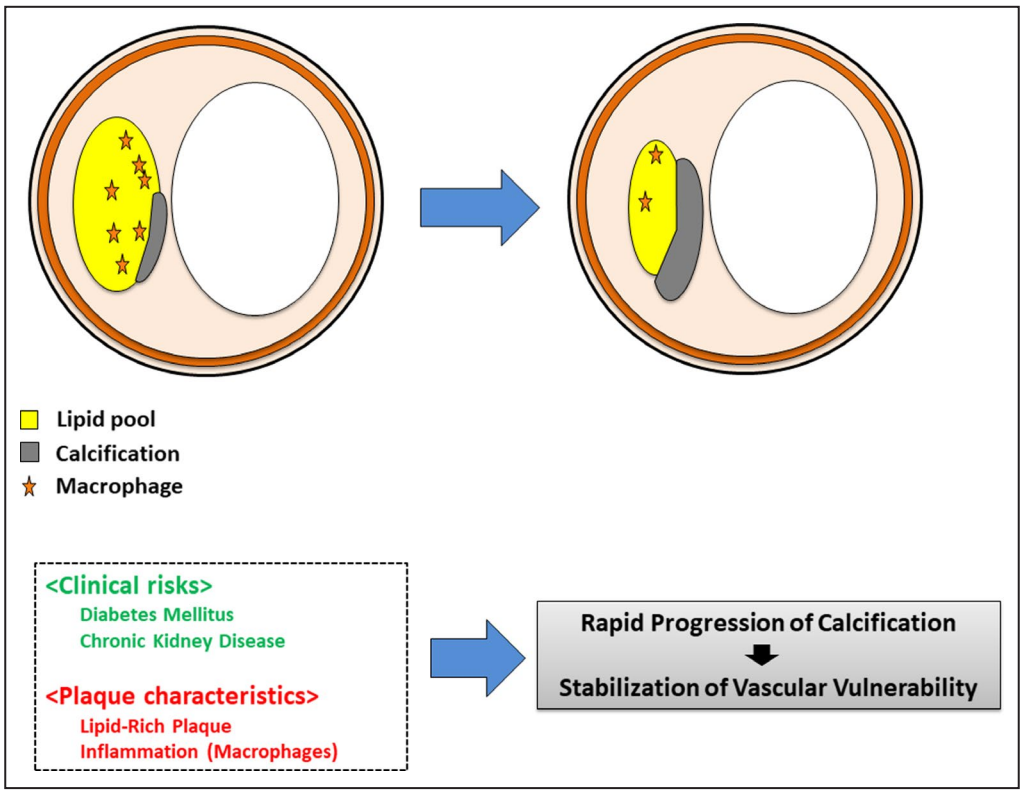


Figure 3. Predictors for rapid progression of coronary calcification.
Diabetes mellitus, chronic kidney disease, baseline lipid-rich plaque, and macrophages were the independent predictors for rapid progression of calcification. Those plaques with rapid progression of calcification showed a significant reduction of inflammatory features such as thin-cap fibroatheroma and macrophages. Baseline vascular inflammation and subsequent stabilization of vascular inflammation may be related to rapid progression of calcification.

on VSMCs that promote vascular calcification, including stimulation of osteogenic/chondrogenic differentiation, vesicle release, apoptosis, loss of inhibitors (such as matrix Gla protein and osteopontin), and extracellular matrix degeneration.[23] Furthermore, increased systemic and local inflammation and oxidative stress cause the development of calcifying particles by multiple mechanisms.[24,25] Inflammation triggers and precedes osteogenic conversion of VSMC through an endoplasmic reticulum stress pathway activation, and release of calcifying extracellular vesicles, which promotes the calcification process.[26] Moreover, activated macrophages produce high levels of metalloproteinases, cysteine endoproteases, and cytokines. They will lead to elastin and collagen degeneration, and remodeling of structural changes of extracellular matrix, which will contribute to create a nidus for calcium-phosphate (CaP) crystal growth.[26]

Prevalence of Calcified Plaque in ACS and SAP

ACS is usually associated with higher inflammation, compared with SAP. Some studies reported that the number of calcium deposits was higher in ACS than in patients with SAP.[4,14] However, prevalence of calcified plaque was not different between ACS and SAP in our study. In addition, the rate of ACS was not significantly different between those with or without rapid progression of calcification (43.3% versus 44.7%, P=0.885). These results might be attributable to a small number of ST-segment–elevation myocardial infarction (STEMI) in the ACS cohort (STEMI is usually associated with high inflammation).[27] To better understand the relationship between the progression of calcification and ACS, large studies are warranted.

LRP and Macrophages for Progression of Coronary Calcification

In our study, LRP and macrophages were associated with progression of vascular calcification. Previous studies reported that there are 2 phases of calcium progression: early-stage inflammation-related calcification (microcalcification and spotty calcification) and later-stage stabilized calcification (macrocalcification).[28,29] Histological studies have suggested that

early microcalcification occurs within the lipid pool and that VSMCs and macrophages play important roles in the progression of calcification.[17,18,30,31] Macrophages are classified into 2 groups according to their role: the inflammatory macrophage and resolving macrophage.[28,32–35] In plaques with necrotic lipid cores, inflammatory macrophages promote the initiation of the early phase of calcification through vesicle-mediated mineralization as the result of the apoptosis of macrophages and VSMCs.[28] Furthermore, the progression of early-stage calcification proceeds through osteogenic trans-differentiation of VSMCs by the action of proinflammatory cytokines such as tumor necrosis factor-α and oncostatin M produced from inflammatory macrophages (tumor necrosis factor-α and oncostatin M increase the activity of alkaline phosphate, which is an ectoenzyme indispensable for bone mineralization in vascular smooth muscle cells).[28,36] On the other hand, calcification is also regarded as a healing and stabilizing process in the late stage. After the initial inflammation process, the main component of macrophages switch from inflammatory macrophages to resolving macrophages. This is fundamental for the resolution of inflammation and the healing response.[28,34,35] In this phase, the progression of calcification from early-stage inflammation-related calcification (microcalcification and spotty calcification) proceeds to later-stage stabilized calcification (macrocalcification) through the resolving macrophage facilitation of osteogenic differentiation and maturation of VSMCs.[28] Thus, macrophages play an important role in calcium progression in both the early inflammatory phase and the late stabilizing phase. In our study, baseline LRP and macrophages were independent predictors for rapid progression of calcification. On the other hand, significant improvement of inflammatory features (TCFA and macrophages) over time was observed, as well as improvement of the lipid index in the calcified plaque with rapid progression of calcification. Progression and healing of atherosclerotic plaque are dynamic processes. Thus, there is a possibility that both increased macrophage apoptosis in an active inflammation phase and reduced macrophage infiltration in a resolving phase may be associated with a decrease in macrophages. This study demonstrated that not only macrophages, but also the lipid index decreased from baseline to 6-month follow-up. This result may imply that reduced macrophage infiltration (with resolving inflammation and regression of lipid content) is more strongly associated with rapid progression of calcification. Hence, baseline LRP and high inflammatory status in the early calcification stage and late-stage recovery from inflammation may both be important for the rapid progression of calcification. On the other hand, although macrophages significantly decreased from baseline to follow-up, they were still frequently observed in calcified plaque with rapid progression of calcification at follow-up. This may not only reflect the ongoing recovery from inflammation, but also the fact that main component of macrophages switched from inflammatory macrophages to resolving macrophages and facilitated plaque stabilization and further progression of calcification in the late stabilizing phase. Thus, the major content of macrophages in rapidly progressed calcified plaques at baseline might be inflammatory macrophages which are usually abundant in active inflammatory plaques and promote the progression of calcification through inflammatory cytokines. On the other hand, major content of macrophages which were still observed at follow-up might be resolving macrophages which also promote the calcification through proliferation and osteogenic differentiation of VSMCs.[28,37] Unfortunately, OCT cannot differentiate macrophage content, including the distinction between inflammatory macrophages and resolving macrophages.

To better understand the association between calcification and macrophages, we analyzed the relationship between change in calcification index and change in macrophages (Figure S4). Calcified plaques were divided into 4 groups based on changes in macrophage; Group 1: no macrophage both at baseline and at follow-up; Group 2: macrophages detected only at follow-up, but not present at baseline; Group 3: macrophages present at baseline but not detected at follow-up; Group 4: macrophages detected both at baseline and at follow-up. Groups 3 and 4 (groups with macrophages present at baseline), compared with Group 1, showed significant increases in the calcification index. This analysis suggests that baseline vascular inflammation (evidenced by macrophage infiltration) is particularly important for rapid progression of calcification.

Almost all patients were prescribed statin at discharge. Previous studies reported that statin facilitates the healing process against plaque inflammation by enhancing the resolving macrophages, resulting in progression of calcification.[28,38,39] A previous study reported the possibility of a favorable response to statin in baseline inflammatory and vulnerable features.[40] Thus, in our study, statins may have facilitated the rapid progression of calcification under baseline high inflammatory status.

Change in Calcification Over a Longer Period

A consistent result on macrophages was obtained at 6-month and 1-year follow-ups. On the other hand, no clinical factor was found to be an independent predictor for 1-year rapid progression of calcification in the multivariable analysis. However, these results should be

interpreted with caution because of the small sample size.

Change in Laboratory Data and Progression of Calcification

We also evaluated the relationship between the change in laboratory data and progression of calcification. Laboratory data at follow-up and changes are shown in Table S8. The levels of low-density lipoprotein, triglyceride, and hs-CRP significantly improved only in patients with rapid progression of calcification. In addition, the low-density lipoprotein level at follow-up was significantly lower in patients with rapid progression of coronary calcification, compared with those without. Furthermore, we analyzed the correlation between changes in each laboratory parameters with changes in calcification index. However, changes in lipid profiles or in inflammatory marker did not correlate with changes in calcification index (low-density lipoprotein; r=−0.090, P=0.202, high-density lipoprotein; r=0.028, P=0.687, triglyceride; r=0.105, P=0.134, hs-CRP; r=−0.038, P=0.695). The lack of direct correlation may be related to the small sample size.

De Novo Calcified Plaque

In our study, patients with de novo calcified plaque at follow-up had significantly higher rates of DM and prior history of PCI. Multivariable analysis also showed that DM and prior history of PCI were the independent predictors for de novo calcified plaque, although this analysis was limited by the small number of cases with de novo calcification. It has been previously described that DM is associated with progression of calcification, and patients with a prior history of PCI usually have more extensive atherosclerotic features than patients without a history of PCI. Our result showed that 78.6% of de novo calcified plaques were LRP. Furthermore, TCFA and macrophages were frequently observed both in precursor plaques at baseline and de novo calcified plaques at follow-up. From these results, early-stage calcium deposition may occur in a continuously active inflammatory state. This is consistent with previous pathological studies.[17,18] In fact, all de novo calcified plaques presented as microcalcification or spotty calcification, which is usually observed in the early-stage inflammatory calcification phase.[28]

Clinical Implications

Current study reveals that baseline high vascular inflammation, evidenced by lipid-rich plaque and macrophage accumulation, and subsequent stabilization of vascular inflammation, evidenced by reduction of TCFA and macrophage accumulation, may be related to coronary calcification. Thus, the data from this study support the notion that coronary calcification represents an advanced, but stable stage of atherosclerosis.

Study Limitations

This study has several limitations. First, the Massachusetts General Hospital OCT Registry is a multicenter, prospective registry for all comers, which does not have strict inclusion criteria or guidelines for OCT image acquisition. Follow-up OCT was not mandated in this registry. Patients who had a follow-up OCT were retrospectively selected in this study. Therefore, selection bias cannot be excluded. Second, we did not have detailed information on medications, such as type, dose, and duration of medications including statin. Third, the number of de novo calcified plaque was small. Although we were able to evaluate characteristics of de novo calcified plaque to some extent, larger studies are warranted to analyze de novo calcified plaques in more detail. Fourth, we did not collect the data of serum calcium and serum blood urea nitrogen, although they might affect the progression of calcification, especially in patients with CKD. Finally, although this is the largest study on this topic, the sample size is still small for evaluating clinical significance.

CONCLUSIONS

This study demonstrated that DM, CKD, LRP, and macrophages were independent predictors for rapid progression of coronary calcification. Moreover, rapid progression of calcification was associated with a significant reduction of inflammatory features of plaques (TCFA and macrophages). Baseline vascular inflammation and subsequent stabilization of vascular inflammation may stimulate rapid progression of calcification.

ARTICLE INFORMATION

Received September 7, 2020; accepted December 21, 2020.

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Sources of Funding

Dr Jang's research was supported by Mr and Mrs Allan and Gill Gray, and Mr and Mrs Michael and Kathryn Park. They had no role in the design or conduct of this research. Dr Malhotra was supported by the National Heart, Lung, and Blood Institute (NHLBI) (R01 HL 142809).

Disclosures

Dr Jang has received educational grant support from Abbott Vascular and a consulting fee from Svelte Medical Systems. The remaining authors have no disclosures to report.

Supplementary Material

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Definitions

The diagnosis of acute coronary syndrome (ACS) included ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation acute coronary syndrome (NSTE-ACS) [41, 42]. STEMI was defined as continuous chest pain lasting >30 min, arrival at the hospital within 12 h from the onset of symptoms, ST-segment elevation >0.1 mV in ≥2 contiguous leads or new left bundle-branch block on 12-lead electrocardiography, and elevated cardiac marker levels (creatine kinase-MB or troponin). NSTE-ACS included non-ST-segment elevation myocardial infarction (NSTEMI) and unstable angina pectoris (UAP). NSTEMI was defined as ischemic symptoms in the absence of ST-segment elevation on electrocardiogram with elevated cardiac marker levels. UAP was defined as presence of newly developed/accelerating chest symptoms on exertion or rest angina within 2 weeks of presentation without biomarker release. Stable angina pectoris (SAP) was defined as chest pain on exertion, without changes in the frequency, intensity, and duration of symptoms and/or positive stress test. Patients with diabetes mellitus (DM) were defined as patients who were receiving an hypoglycemic agent or insulin, patients with a known fasting plasma glucose value of ≥ 126 mg/dl or 2-h plasma glucose value of ≥ 200 mg/dl by oral glucose tolerance test, classic symptoms with random

plasma glucose level, or hemoglobin A1c level ≥ 6.5% [43]. Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate (eGFR) of ≤60 mL/min per 1.73 m^2. The eGFR was calculated using the Modification of Diet in Renal Disease equation: eGFR (mL/min per 1.73 m^2) = 175 × (serum creatinine [mg/dL])$^{-1.154}$ × (age)$^{-0.203}$ × 0.742 (if female) × 1.210 (if black) [44].

OCT imaging analysis

Lipid was defined as a low-signal region with a diffuse border [45]. The degree of lipid arc was measured at 1-mm intervals. Lipid length was measured on the longitudinal view, and lipid index was obtained as the product of mean lipid arc and lipid length [46]. Lipid-rich plaque (LRP) was defined as a plaque with a maximal lipid arc greater than 90° [47]. In lipid plaques, fibrous cap thickness was measured 3 times at the thinnest part, and the average value was calculated. Thin-cap fibroatheroma (TCFA) was defined as a plaque with a maximal lipid arc greater than 90° and thinnest FCT less than 65 μm [47, 48]. Macrophages were recognized as granular structures with strong backscatter [49]. The presence or absence of macrophages were evaluated, as we intended to find predictors for rapid progression of calcification. Microvessels were identified as signal-poor structures with vesicular or tubular shapes [12, 46]. Cholesterol crystals were identified as thin and linear regions of high signal intensity with high backscattering within a plaque [12, 46].

Table S1. Variance inflation factors of each candidate factor.

	VIF
Age	1.104
Diabetes mellitus	1.030
Chronic kidney disease	1.134
TCFA	1.247
Lipid rich plaque	1.664
Macrophage	1.641
Cholesterol crystal	1.226
Microvessel	1.162
Minimal lumen area	1.060
Baseline calcium index	1.334
Minimal calcium depth	1.165

TCFA = thin-cap fibroatheroma; VIF = variance inflation factor.

Table S2. Predictors for Rapid Progression of Calcification (rigorous selection: variables with p < 0.05 in the univariable analysis were included in the multivariable analysis model).

	Univariable		Multivariable	
	Odds ratio [95%CI]	**P value**	**Odds ratio [95%CI]**	**P value**
Patient Clinical Characteristics				
Follow-up duration, days	1.003 [0.983, 1.024]	0.755		
Age, y	1.031 [0.999, 1.064]	0.055		
Male	0.654 [0.317, 1.348]	0.250		
ACS	0.748 [0.394, 1.422]	0.376		
Hypertension	0.943 [0.480, 1.851]	0.864		
Dyslipidemia	1.400 [0.681, 2.877]	0.360		
Diabetes mellitus	4.217 [2.314, 7.685]	< 0.001	3.863 [2.141, 6.968]	< 0.001
Chronic kidney disease	3.560 [1.550, 8.176]	0.003	2.850 [1.253, 6.484]	0.013
Family history of CAD	0.701 [0.196, 2.501]	0.584		
Current smoker	0.764 [0.368, 1.586]	0.470		
Prior MI	1.697 [0.902, 3.192]	0.101		
Prior PCI	1.436 [0.743, 2.774]	0.282		
Aspirin at discharge	-	1.000		
Clopidogrel at discharge	1.272 [0.181, 8.924]	0.809		
Statin at discharge	3.135 [0.304, 32.285]	0.335		
ACEI/ARB at discharge	0.745 [0.389, 1.427]	0.375		
Plaque Characteristics				
TCFA	4.224 [1.852, 9.634]	0.001	0.847 [0.301, 2.285]	0.754
Lipid rich plaque	8.182 [4.098, 16.334]	< 0.001	2.681 [1.089, 6.598]	0.032
Macrophage	13.410 [6.843, 26.312]	< 0.001	6.846 [3.189, 14.696]	< 0.001
Cholesterol crystal	6.022 [1.902, 19.012]	0.002	2.886 [0.888, 9376]	0.078
Microvessel	3.053 [1.777, 5.245]	< 0.001	1.572 [0.765, 3.232]	0.218
Minimal lumen area, mm^2	0.909 [0.824, 1.003]	0.058		
Area stenosis, %	1.015 [0.993, 1.038]	0.186		

Baseline calcium index	1.005 [1.002, 1.009]	0.006	1.004 [1.000, 1.007]	0.069
Minimal calcium depth, μm	0.994 [0.991, 0.998]	0.001	0.998 [0.994, 1.001]	0.231

ACEI/ARB = angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; ACS = acute coronary syndrome; CAD = coronary artery disease; CI = confidence interval; MI = myocardial infarction; PCI = percutaneous coronary intervention; TCFA = thin-cap fibroatheroma.

Table S3. Clinical Predictors for Rapid Progression of Calcification (model included both age and sex).

	Univariable		Multivariable	
	Odds ratio [95%CI]	**P value**	**Odds ratio [95%CI]**	**P value**
Patient Clinical Characteristics				
Follow-up duration, days	1.003 [0.983, 1.024]	0.755		
Age, y	1.031 [0.999, 1.064]	0.055	1.020 [0.987, 1.054]	0.232
Male	0.654 [0.317, 1.348]	0.250	0.893[0.448, 1.783]	0.749
ACS	0.748 [0.394, 1.422]	0.376		
Hypertension	0.943 [0.480, 1.851]	0.864		
Dyslipidemia	1.400 [0.681, 2.877]	0.360		
Diabetes mellitus	4.217 [2.314, 7.685]	< 0.001	3.846 [2.091, 7.073]	< 0.001
Chronic kidney disease	3.560 [1.550, 8.176]	0.003	2.465 [1.091, 5.571]	0.030
Family history of CAD	0.701 [0.196, 2.501]	0.584		
Current smoker	0.764 [0.368, 1.586]	0.470		
Prior MI	1.697 [0.902, 3.192]	0.101		
Prior PCI	1.436 [0.743, 2.774]	0.282		
Aspirin at discharge	-	1.000		
Clopidogrel at discharge	1.272 [0.181, 8.924]	0.809		
Statin at discharge	3.135 [0.304, 32.285]	0.335		
ACEI/ARB at discharge	0.745 [0.389, 1.427]	0.375		

ACEI/ARB = angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; ACS = acute coronary syndrome; CAD = coronary artery disease; CI = confidence interval; MI = myocardial infarction; PCI = percutaneous coronary intervention.

Table S4. Predictors for One-year Rapid Progression of Calcification.

	Univariable		Multivariable	
	Odds ratio [95%CI]	**P value**	**Odds ratio [95%CI]**	**P value**
Patient Clinical Characteristics				
Follow-up duration, days	1.003 [0.997, 1.008]	0.365		
Age, y	0.971 [0.921, 1.024]	0.277		
Male	2.533 [1.420, 4.521]	0.002	1.574 [0.616, 4.022]	0.344
ACS	0.549 [0.224, 1.344]	0.189		
Hypertension	0.459 [0.168, 1.251]	0.128		
Dyslipidemia	0.758 [0.284, 2.025]	0.580		
Diabetes mellitus	2.917 [1.050, 8.103]	0.040	2.144 [0.801, 5.739]	0.129
Chronic kidney disease	4.632 [1.049, 20.440]	0.043	5.034 [0.627, 40.406]	0.128
Family history of CAD	-	1.000		
Current smoker	2.917 [1.166, 7.297]	0.022	1.630 [0.376, 7.061]	0.513
Prior MI	2.917 [1.166, 7.297]	0.022	1.630 [0.376, 7.061]	0.513
Prior PCI	0.840 [0.294, 2.400]	0.745		
Aspirin at discharge	-	1.000		
Clopidogrel at discharge	-	1.000		
Statin at discharge	-	1.000		
ACEI/ARB at discharge	0.820 [0.304, 2.217]	0.696		
Plaque Characteristics				
TCFA	2.353 [0.417, 13.289]	0.333		
Lipid rich plaque	16.333 [3.999, 66.715]	< 0.001	4.315 [0.698, 26.686]	0.116
Macrophage	18.889 [4.494, 79.385]	< 0.001	11.432 [2.268, 57.627]	0.003
Cholesterol crystal	3.300 [0.169, 64.298]	0.431		
Microvessel	3.300 [1.189, 9.160]	0.022	4.632 [0.651, 32.937]	0.126
Minimal lumen area, mm^2	1.092 [0.920, 1.296]	0.313		
Area stenosis, %	0.984 [0.963, 1.006]	0.147		
Baseline calcium index	1.002 [0.999, 1.006]	0.215		
Minimal calcium depth, μm	0.994 [0.987, 1.000]	0.069		

ACEI/ARB = angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; ACS = acute

coronary syndrome; CAD = coronary artery disease; CI = confidence interval; MI = myocardial infarction; PCI = percutaneous coronary intervention; TCFA = thin-cap fibroatheroma.

Table S5. Comparison between Patients with and without *de novo* Calcified plaque.

	Patients with *de novo* calcified plaque (n = 14)	**Patients without *de novo* calcified plaque (n = 173)**	**P value**
Follow-up duration, days	182 (171 – 195)	184 (175 - 194)	0.932
Length of OCT observation, mm	50.0 (41.7 – 56.3)	53.2 (31.5 – 93.1)	0.529
Number of observed vessels, n	2.0 (1.0 – 2.3)	2.0 (1.0 – 2.0)	0.434
Age, years	58.4 ± 8.9	59.3 ± 10.5	0.759
Male, n (%)	8 (57.1)	124 (71.7)	0.197
Clinical Presentation			0.568
STEMI, n (%)	0 (0.0)	13 (7.5)	
NSTE-ACS, n (%)	5 (35.7)	57 (32.9)	
SAP, n (%)	9 (64.3)	103 (59.5)	
Prior MI, n (%)	7 (50.0)	57 (32.9)	0.158
Prior PCI, n (%)	13 (92.9)	89 (51.4)	0.003
Hypertension, n (%)	11 (78.6)	104 (60.1)	0.172
Dyslipidemia, n (%)	12 (85.7)	122 (70.5)	0.185
Diabetes mellitus, n (%)	11 (78.6)	65 (37.6)	0.003
Insulin user, n (%)	11 (100.0)	36 (55.4)	0.003
CKD, n (%)	3 (21.4)	14 (8.1)	0.121
Family history of CAD, n (%)	1 (7.1)	9 (5.2)	0.550
Smoking			0.143
Current smoker, n (%)	1 (7.1)	44 (25.4)	
Past smoker, n (%)	2 (14.3)	39 (22.5)	
Never smoker, n (%)	11 (78.6)	90 (52.0)	
Creatinine, mg/dl	0.84 (0.70 – 1.07)	0.89 (0.79 – 1.02)	0.884
eGFR, mL/min per 1.73m^2	86.9 (61.5 – 103.6)	84.1 (74.1 – 100.3)	0.986
LDL-C, mg/dl	76.9 ± 21.5	93.3 ± 34.5	0.093

HDL-C, mg/dl	48.3 ± 18.5	42.4 ± 10.9	0.266
Triglyceride, mg/dl	123.0 ± 55.1	145.1 ± 114.6	0.493
HbA1c, %	6.0 (6.0 – 6.0)	6.0 (6.0 – 7.1)	0.572
Medication at discharge			
Aspirin, n (%)	14 (100.0)	169 (97.7)	0.731
Clopidogrel, n (%)	13 (92.9)	160 (92.5)	0.718
Statin, n (%)	14 (100.0)	168 (97.1)	0.675
ACEI/ARB, n (%)	8 (57.1)	58 (33.5)	0.071

Values are mean ± SD, n (%), or median (interquartile range).

ACEI/ARB = angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; CAD = coronary artery disease; eGFR = estimated glomerular filtration rate; HbA1c = glycosylated hemoglobin; HDL-C = High-density lipoprotein cholesterol; LDL-C = Low-density lipoprotein cholesterol; MI = myocardial infarction; NSTE-ACS = non-ST-segment elevation acute coronary syndrome; PCI = percutaneous coronary intervention; OCT = optical coherence tomography; SAP = stable angina pectoris; STEMI = ST-segment elevation myocardial infarction.

Table S6. Serial Optical Coherence Tomography analysis of *De novo* Calcified plaque and its Precursor plaques.

	Baseline	Follow-up	Change	p value
Lipid-rich plaque, n (%)	12 (85.7)	11 (78.6)	-	1.000
Maximal lipid arc, degree	157.1 ± 43.4	151.8 ± 44.4	-5.3 ± 8.0	0.043
Mean lipid arc, degree	124.6 ± 25.2	118.5 ± 29.5	-6.1 ± 9.0	0.040
Lipid length, mm	7.3 ± 2.2	6.9 ± 1.8	-0.4 ± 1.1	0.239
Thinnest FCT, μm	68.3 ± 22.9	75.8 ± 40.1	7.5 ± 26.0	0.339
Lipid index	885.2 ± 249.4	799.3 ± 238.6	-85.9 ± 134.0	0.048
TCFA, n (%)	5 (35.7)	5 (35.7)	-	1.000
Macrophage, n (%)	10 (71.4)	12 (85.7)	-	0.500
Cholesterol crystal, n (%)	3 (21.4)	4 (21.4)	-	1.000
Microvessel, n (%)	5 (35.7)	5 (35.7)	-	1.000
Minimal lumen area, mm^2	4.70 ± 1.83	4.79 ± 1.90	0.10 ± 0.26	0.196
Area stenosis, %	50.8 ± 9.6	50.6 ± 9.9	-0.2 ± 2.0	0.721
Quantitative analysis of calcification				
Maximal calcification arc, degree	-	22.0 ± 12.2	-	-
Mean calcification arc, degree	-	18.9 ± 8.9	-	-
Calcification length, mm	-	0.9 ± 0.3	-	-
Calcification index	-	17.7 ± 11.5	-	-
Minimal calcium depth, μm	-	214 ± 154	-	-
Calcium classification				
Microcalcification, n (%)	-	7 (50.0)	-	-
Spotty calcification, n (%)	-	7 (50.0)	-	-
Macrocalcification, n (%)	-	0 (0.0)	-	-

Values are mean ± SD, n (%), or median (interquartile range).

FCT = fibrous cap thickness; LAD = left anterior descending artery; LCX = left circumflex artery;

RCA = right coronary artery; TCFA = thin-cap fibroatheroma.

Table S7. Predictors for *De novo* Calcified plaque.

	Univariable		Multivariable	
	Odds ratio [95%CI]	**P value**	**Odds ratio [95%CI]**	**P value**
Patient Clinical Characteristics				
Follow-up duration, days	1.000 [0.971, 1.029]	0.993		
Age, y	0.992 [0.949, 1.037]	0.715		
Male	0.527 [0.174, 1.597]	0.257		
ACS	0.817 [0.263, 2.542]	0.728		
Hypertension	2.433 [0.655, 9.037]	0.184		
Dyslipidemia	2.508 [0.542, 11.609]	0.239		
Diabetes mellitus	6.092 [1.639, 22.651]	0.007	4.800 [1.244, 18.524]	0.023
Chronic kidney disease	3.097 [0.773, 12.418]	0.111		
Family history of CAD	1.402 [0.165, 11.935]	0.757		
Current smoker	0.226 [0.029, 1.774]	0.157		
Prior MI	2.035 [0.681, 6.081]	0.203		
Prior PCI	12.270 [1.571, 95.853]	0.017	9.773 [1.212, 78.816]	0.032
Aspirin at discharge	-	1.000		
Clopidogrel at discharge	1.056 [0.128, 8.721]	0.959		
Statin at discharge	-	1.000		
ACEI/ARB at discharge	2.644 [0.876, 7.978]	0.085		
Plaque Characteristics				
TCFA	3.450 [1.055, 11.307]	0.040	1.863 [0.531, 6.535]	0.331
Lipid rich plaque	6.000 [1.276, 28.213]	0.023	4.783 [0.932, 24.555]	0.061
Macrophage	1.842 [0.549, 6.186]	0.323		
Cholesterol crystal	1.527 [0.389, 5.995]	0.544		
Microvessel	0.923 [0.296, 2.876]	0.890		
Minimal lumen area, mm^2	1.099 [0.856, 1.410]	0.460		

Area stenosis, %	1.038 [0.981, 1.098]	0.198

ACEI/ARB = angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; ACS = acute coronary syndrome; CAD = coronary artery disease; CI = confidence interval; MI = myocardial infarction; PCI = percutaneous coronary intervention; TCFA = thin-cap fibroatheroma.

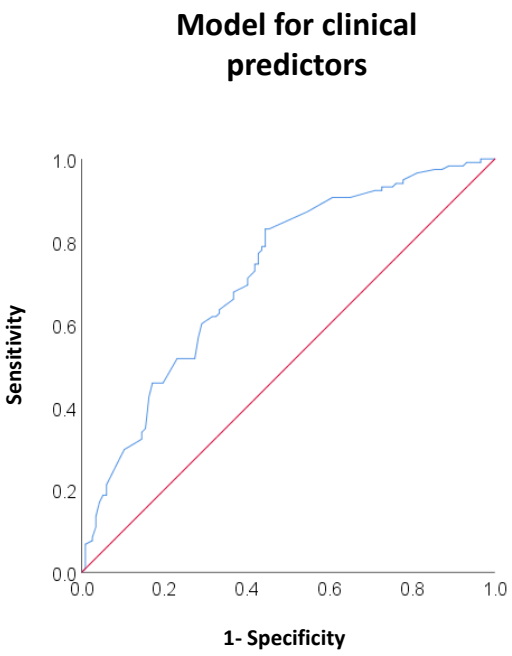
Table S8. Change in Laboratory data.

	Patients with rapid Progression of calcification (n = 67)	Patients without rapid Progression of calcification (n = 38)	P value
LDL cholesterol (Baseline), mg/dl	92.5 ± 36.5	99.0 ± 37.1	0.440
LDL cholesterol (Follow-up), mg/dl	68.5 ± 22.6	84.1 ±34.6	0.036
LDL cholesterol change, mg/dl	-23.9 ± 39.3	-14.9 ± 38.8	0.318
P value	< 0.001	0.052	
HDL cholesterol (Baseline), mg/dl	48.3 ± 16.3	50.4 ± 17.4	0.564
HDL cholesterol (Follow-up), mg/dl	47.3 ± 15.3	47.7 ± 19.9	0.916
HDL cholesterol change, mg/dl	-1.0 ± 12.8	-2.8 ± 16.3	0.577
P value	0.541	0.361	
Triglyceride (Baseline), mg/dl	145.4 ± 132.9	132.4 ± 60.5	0.619
Triglyceride (Follow-up), mg/dl	123.1 ± 83.0	130.8 ± 49.0	0.646
Triglyceride change, mg/dl	-22.3 ± 80.9	-1.6 ± 41.1	0.116
P value	0.038	0.831	
hs-CRP (Baseline), mg/dl	1.0 (1.0 – 3.5)	1.5 (1.0 – 3.0)	0.886
hs-CRP (Follow-up), mg/dl	0.8 (0.3 – 1.7)	0.9 (0.3 – 1.2)	0.735
hs-CRP change, mg/dl	-0.4 (-1.1 to 0.2)	-0.8 (-2.5 to 0.0)	0.972
P value	0.011	0.106	

Values are median (interquartile range).

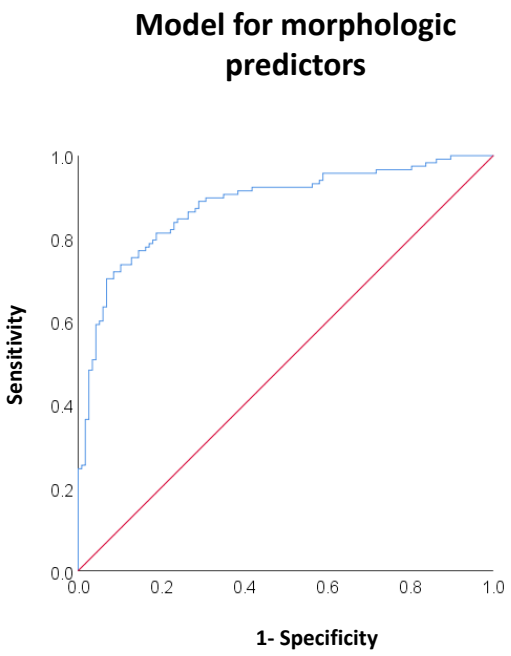
HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; LDL = low-density lipoprotein.

Figure S1. Analyses of area under the curve (AUC) and Hosmer-Lemeshow goodness-of-fit.



AUC	95% confidential interval	P value
0.720	0.655 – 0.785	< 0.001

Hosmer - Lemeshow goodness - of - fit: p = 0.166

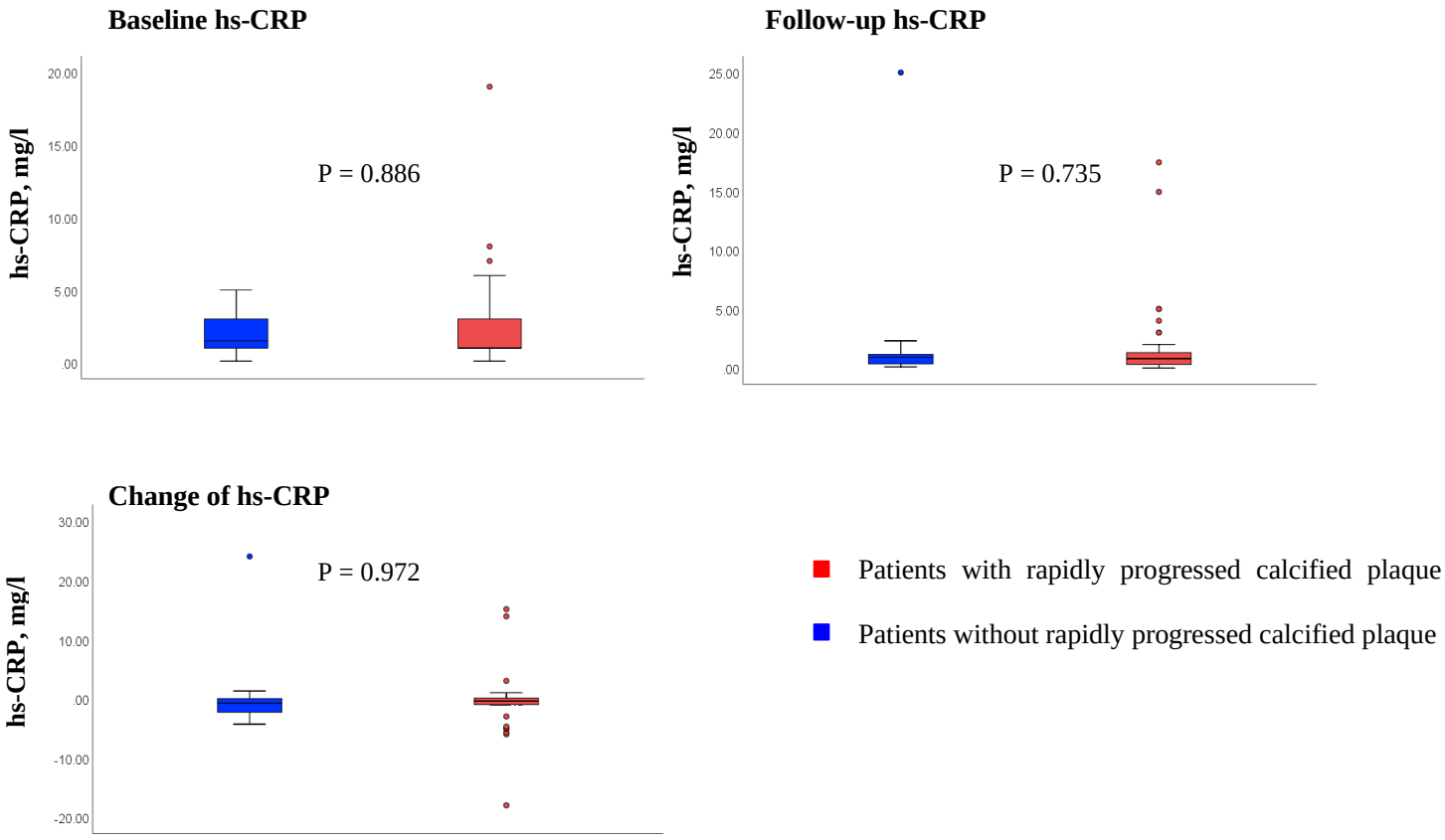


AUC	95% confidential interval	P value
0.881	0.837 – 0.926	< 0.001

Hosmer - Lemeshow goodness - of - fit: p = 0.594

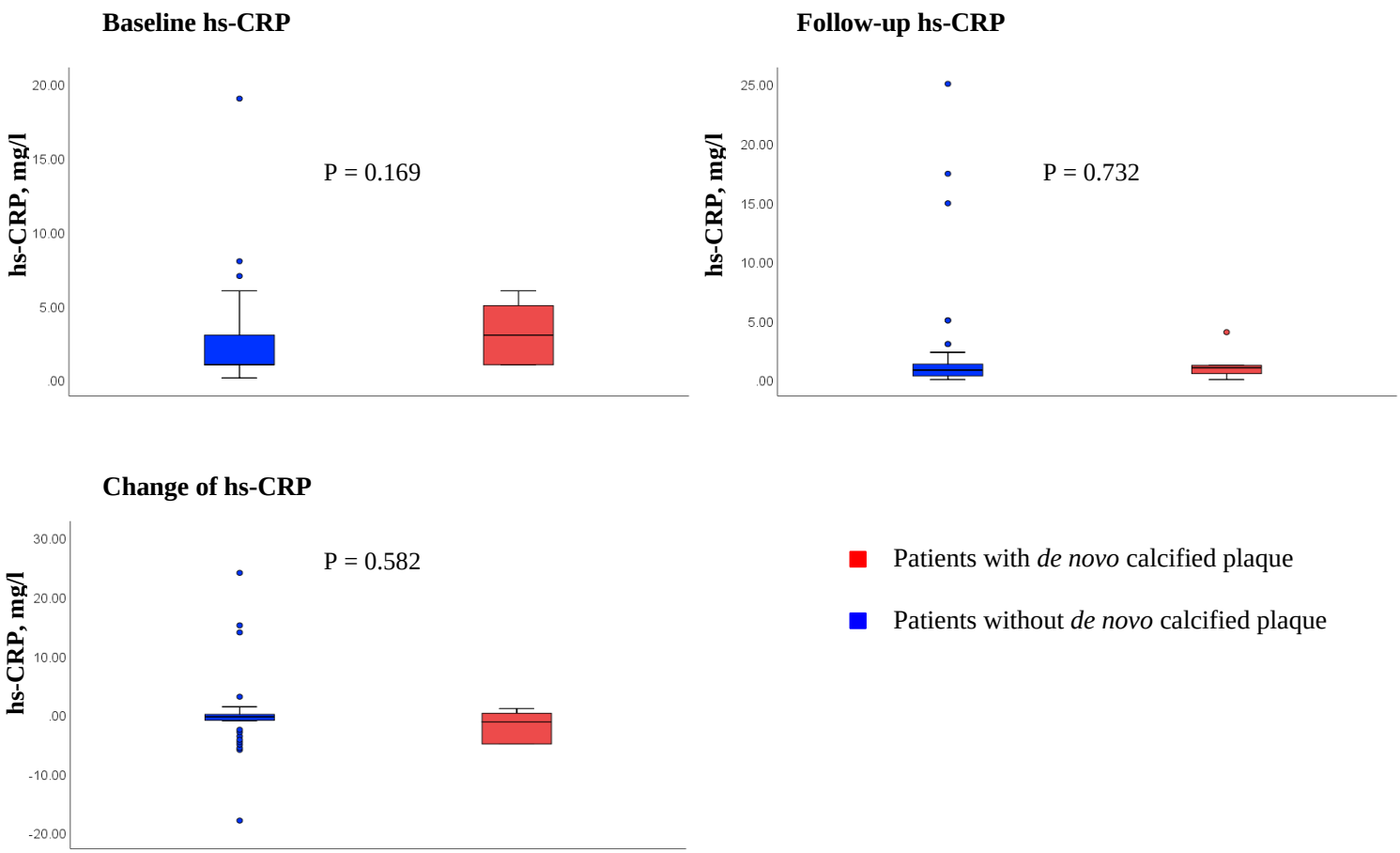
Both models (the model for clinical predictors and the model for morphological predictors) had good results for area under the curve (AUC) and Hosmer-Lemeshow goodness-of-fit testing.

Figure S2. High-sensitivity C-reactive protein in patients with or without rapidly progressed calcified plaque.



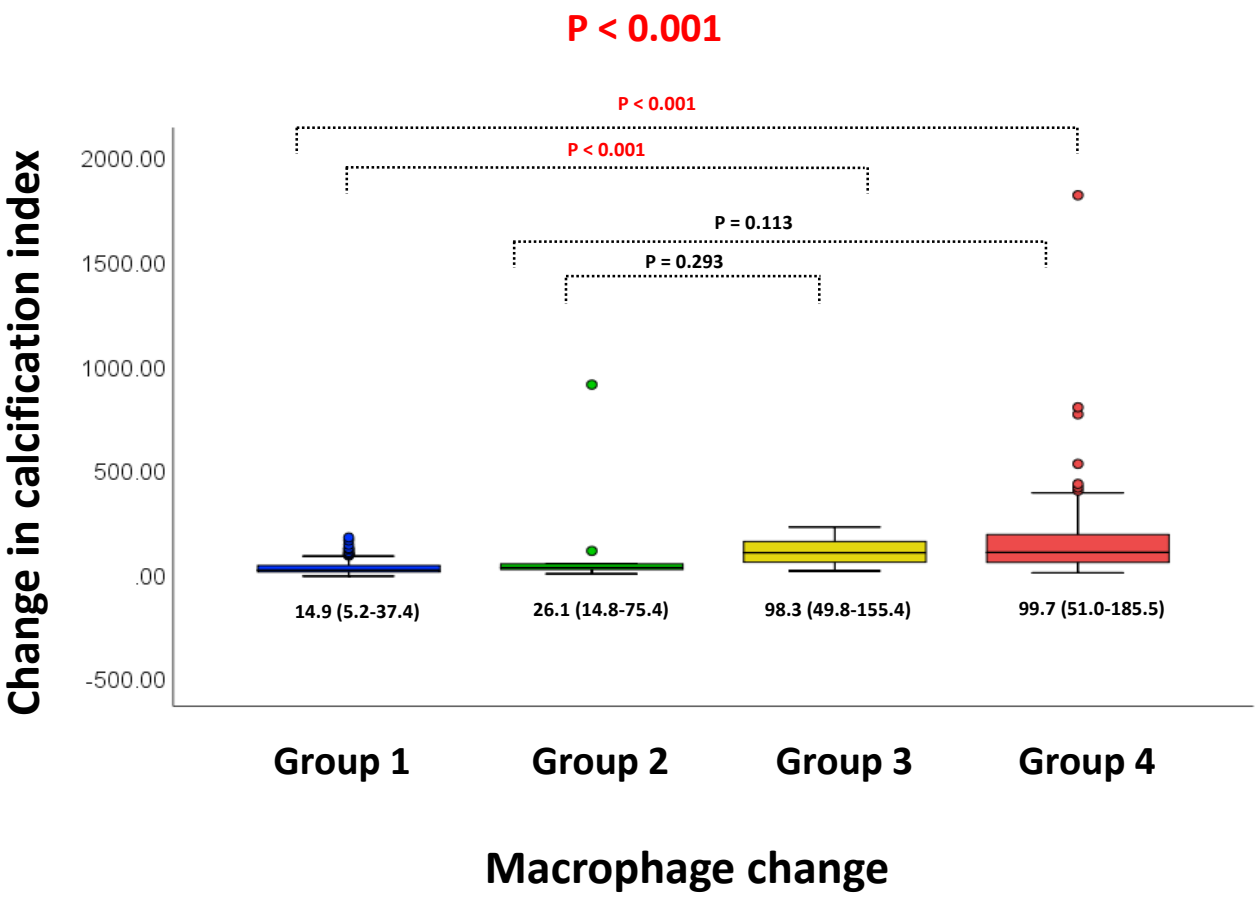
Neither high-sensitivity C-reactive protein (hs-CRP) levels at baseline and follow-up, nor the change in hs-CRP levels from baseline to follow-up, were significantly different between patients who had calcified plaque with rapid progression of calcification and patients who did not have calcified plaque with rapid progression of calcification.

Figure S3. High-sensitivity C-reactive protein in patients with or without *de novo* calcified plaque.



Neither hs-CRP levels at baseline and follow-up, nor the change in hs-CRP levels from baseline to follow-up, were significantly different between patients with or without *de novo* calcified plaque.

Figure S4. Change in calcification index and change in macrophage.



Calcified plaques were divided into four groups based on changes in macrophage; Group 1: no macrophage both at baseline and at follow-up; Group 2: macrophages detected only at follow-up, but not present at baseline; Group 3: macrophages present at baseline but not detected at follow-up; Group 4: macrophages detected both at baseline and at follow-up. Groups 3 and 4 (groups with macrophages present at baseline), compared to Group 1, showed significant increases in the calcification index.