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Relationship Between Coronary Artery Calcification and Plaque Vulnerability, a Qualitative and Quantitative Optical Coherence Tomography Study

Running Title: Coronary Artery Calcification and Plaque Vulnerability

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Abstract: Coronary artery calcification (CAC) is an established hallmark of advanced atherosclerosis. The detailed relationship between CAC and plaque vulnerability remains incompletely understood. We aim to investigate the relationship between calcification burden, calcification patterns, and plaque instability features. A total of 540 patients with established coronary artery disease who underwent optical coherence tomography imaging before percutaneous coronary intervention were included. Calcification plaques were investigated by qualitative (microcalcification, spotty calcification, and macrocalcification) and quantitative (non-calcified, low, intermediate, and high calcified plaque burden (CPB)) assessments across the culprit vessel. The vulnerable features were compared between the groups. The prevalence of thin-cap fibroatheroma (TCFA), layered plaque, cholesterol crystal, microchannel, macrophage cluster, plaque rupture, plaque erosion, and eruptive calcified nodules significantly differed in different CPB groups. Intermediate CPB but not spotty calcifications were associated with a significantly higher prevalence of TCFA and plaque rupture in these groups. The prevalence of TCFA, plaque rupture, layered plaque, and microchannel was lowest in the high CPB group. Macrophage clusters and cholesterol crystals were associated with a higher number of spotty calcifications, especially in young patients. Microchannels were associated with more microcalcifications. High CPB and macrocalcifications showed the lowest level of inflammation. In the patients with calcification, age, multivessel disease, macrophage cluster, and lipid index were independently associated with CPB. In conclusion, intermediate CPB was associated with a significantly higher prevalence of TCFA and plaque rupture, while spotty calcifications correlated with macrophage clusters and cholesterol crystals.

Keywords: Coronary artery calcification; thin-cap fibroatheroma; spotty calcification; plaque rupture; macrophage cluster

Introduction

Coronary artery calcification (CAC) is an established hallmark of advanced atherosclerosis and a predictor of future cardiovascular events independently from concomitant risk factors [1]. Both calcification burden and calcification patterns are closely related to plaque vulnerability. Patients without calcified plaque are accompanied by a lower prevalence of thin-cap fibroatheroma (TCFA) and are at particularly low risk of further cardiac events [2,3]. Previous studies have proposed that macrocalcification is associated with stable fibrotic plaques, whereas spotty calcification is more commonly a component of unstable plaque [4]. Though the CAC scoring detected by computed

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tomography (CT) reflects the overall coronary plaque burden, spotty calcifications are below the resolution of CT due to limited spatial resolution [5]. The detailed relationship between CAC and plaque vulnerability remains incompletely understood. Optical coherence tomography (OCT) enables the assessment of calcification plaques in detail, especially for detecting microcalcifications [6,7]. Recent OCT studies showed that the prevalence of TCFA did not correlate with the level of calcified plaque burden (CPB) [8,9], and another OCT study also reported that the prevalence of both plaque rupture and TCFA did not differ significantly between spotty calcification and macrocalcification [10]. Previous studies investigated calcification plaques only by qualitative or quantitative assessments; however, assessing calcification burden together with morphologic features may enable a more comprehensive understanding of CAC. In this current OCT study, we aim to conduct qualitative and quantitative assessments of calcification plaques, and investigate the relationship between calcification burden, calcification patterns, and plaque instability features.

Methods

Study Population

We retrospectively identified 736 patients with established coronary artery disease who underwent OCT imaging during a clinically indicated coronary angiogram at Tianjin Chest Hospital (Tianjin, China) between January 2020 and January 2023. Of those, 114 patients with OCT evaluation after balloon pre-dilation, 14 patients with vein grafts, 40 patients with in-stent restenosis, and 28 patients with suboptimal image quality were excluded. Finally, a total of 540 patients were included in the present study (88 patients presenting with ST-segment elevation myocardial infarction (STEMI), 74 patients presenting with non-ST-segment elevation myocardial infarction (NSTEMI), 344 patients presenting with unstable angina pectoris, and 34 patients presenting with stable angina pectoris). This retrospective study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Institute of Tianjin Chest Hospital. The Ethics Committee of the Institute of Tianjin Chest Hospital granted a waiver of consent for this retrospective study.

OCT Image Acquisition and Analysis

OCT imaging was performed using a commercially available OCT system (Dragonfly Duo or Dragonfly Optis, St Jude Medical, Inc., USA). Two blinded expert investigators (CWL and LW) independently performed qualitative and quantitative analyses of OCT imaging according to previously established criteria [11]. OCT analysis was conducted at the culprit and non-culprit lesions across the entire culprit vessel.

TCFA was described as a lipid-rich plaque with the thinnest fibrous cap of ≤65 μm and lipid arc of >90°. Microcalcification was defined as a maximal calcium angle < 22.5° and calcification length < 1 mm; spotty calcification was defined as a maximal calcium angle ranging from 22.5° to 90° and calcium length < 4 mm; macrocalcification was defined as a maximal calcium angle > 90°; superficial calcification was defined as the distance between the most superficial edge of calcification and the vessel lumen of ≤ 65μm, as previously described [12]. The calcium index was defined as the product of the mean calcium arc and the total calcium length. A macrophage cluster was defined as a large accumulation of confluent bright spots on plaque surfaces accompanied by high attenuation [13]. Inter-observer agreements were tested in 50 cases randomly selected from the entire cohort, with the Kappa values of 0.94, 0.96, and 0.95 for diagnosing microcalcification, spotty calcification, and macrocalcification.

Categorization of Patients

The patients were divided into four groups according to the level of CPB: one group without calcification and three groups based on the calcium index tertiles. In addition, based on morphologic features, patients were divided into three patterns: microcalcification, spotty calcification,

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and macrocalcification. Moreover, the above three calcification patterns were categorized into superficial (≤ 65μm) and deep (>65μm) calcification according to the minimal calcium depths. Two subgroup analyses were performed according to age and clinical presentation.

Statistical Analysis

Categorical variables were presented as counts and percentages and compared using the χ^2 test or the Fisher exact test. Continuous variables were shown as mean±SD or median (25th–75th percentiles) and compared using the Student t-test, Mann-Whitney U test, or Kruskal-Wallis test, as appropriate. Bonferroni correction following the tests was used in post hoc pairwise comparisons. A logistic regression was performed to identify the independent predictors for the presence of CAC. The variables exhibiting p< 0.05 in the univariate analysis were analyzed in the logistic model. Multiple linear regression analysis was performed to assess the risk factors for CPB in patients with calcification. A two-tailed P value of <0.05 was considered statistically significant. All data were analyzed using SPSS Statistics (IBM, version 26).

Results

Baseline Characteristics of CAC

This current study analyzed 540 patients (average age 61±11years, 75% male), including 106 patients without CAC and 434 patients with CAC. Patients with CAC were divided into three groups based on the calcium index tertiles: 144 individuals in the low CPB group (0<calcium index≤311), 146 individuals in the intermediate CPB group (311<calcium index≤917), and 144 individuals in the high CPB group (calcium index>917). Meanwhile, patients were categorized into three different calcification patterns: 9 in the microcalcification group, 230 in the spotty calcification group, and 195 in the macrocalcification group. Spearman's correlation showed a significant relationship between calcium index and the number of spotty calcifications (r=0.470, p<0.001) and macrocalcifications (r=0.699, p<0.001). The comparison of baseline characteristics and laboratory data between different CPB groups is shown in Table 1. Patients with high CPB were much older, more frequently had unstable angina pectoris, prior percutaneous coronary intervention (PCI) history, and multivessel disease. Moreover, similar results were found in the macrocalcification group when comparing different calcification patterns (Table 2).

Table 1. Baseline characteristics among different CPB groups.

	No Calcification (N=106)	**Low CPB (N=144)**	**Intermediate CPB (N=146)**	**High CPB (N=144)**	**p**
Age	57.4±12.4[a,d]	59.7±10.5[b]	61.6±9.83[c,d]	65.5±9.89[a,b,c]	<0.001
male	75 (71%)	110 (76%)	110 (75%)	108 (75%)	0.771
Prior MI	8 (8%)	12 (8%)	12 (8%)	24 (17%)	0.038
Prior PCI	6 (6%)[a]	16 (11%)	16 (11%)	31 (22%)[a]	0.001
Hypertension	61 (58%)	80 (56%)	93 (64%)	97 (67%)	0.157
Diabetes mellitus	28 (26%)	40 (28%)	42 (29%)	53 (37%)	0.236
Clinical Diagnosis					
SAP	11 (10%)	10 (7%)	9 (6%)	4 (3%)	<0.001
UAP	54 (51%)[a]	83 (58%)[b]	91 (62%)[c]	116 (81%)[a,b,c]	
STEMI	22 (21%)[a]	32 (22%)[b]	23 (16%)	11 (8%)[a,b]	
NSTEMI	19 (18%)	19 (13%)	23 (16%)	13 (9%)	
WBC (10^9/L)	7.39±2.53	7.22±2.33	7.36±2.27	6.74±1.98	0.067
neutrophils	4.91±2.23	4.76±2.09	4.91±2.12	4.32±1.67	0.046
lymphocytes	1.83±0.67	1.85±0.55	1.83±0.67	1.80±0.65	0.951
monocytes	0.471±0.174	0.433±0.160	0.446±0.191	0.433±0.148	0.263
platelets (10^9/L)	228±61	228±63	223±52	225±66	0.878
hemoglobin (g/L)	139±17	140±13	140±15	137±16	0.303

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hs-CRP (mg/l)	2.10 (0.98-5.05)[a]	1.31 (0.60-3.91)	1.49 (0.50-4.20)	0.99 (0.50-2.73)[a]	0.007
FBG (mmol/l)	5.40 (4.93-6.36)	5.54 (4.99-6.57)	5.59 (5.01-6.70)	5.55 (5.03-6.59)	0.647
HbA1c	6.00 (5.70-6.80)	6.10 (5.70-7.20)	6.10 (5.78-7.30)	6.37 (5.65-7.30)	0.464
TC (mmol/l)	4.17±0.99	4.25±1.17	4.17±1.08	3.93±1.06	0.080
TG (mmol/l)	1.54 (1.18-2.20)	1.49 (1.10-2.14)	1.56 (1.13-2.06)	1.31 (1.06-2.08)	0.086
LDLC (mmol/l)	2.66±0.91	2.71±0.95	2.65±0.93	2.48±1.00	0.187
HDLC (mmol/l)	1.03±0.24	1.01±0.22	1.04±0.24	1.05±0.22	0.541
NHR	5.07±2.67[a]	4.99±2.62[b]	4.99±2.38[c]	4.31±1.93[a,b,c]	0.029
MHR	0.457 (0.326-0.577)	0.411 (0.309-0.556)	0.406 (0.304-0.566)	0.399 (0.309-0.530)	0.285
LDL/HDL	2.67±0.96	2.79±1.07	2.67±1.07	2.45±1.10	0.051
TGs	1.52±0.65	1.58±0.72	1.55±0.63	1.39±0.61	0.065
eGFR (ml/min1.73m^2)	90.2±17.8	90.0±14.3	89.0±14.6	86.6±15.4	0.197
Uric acid (umol/L)	338 (277-405)	325 (273-393)	319 (268-369)	317 (272-396)	0.336
D-dimer (μg/ml)	0.23 (0.19-0.31)	0.24 (0.19-0.34)	0.24 (0.19-0.37)	0.26 (0.22-0.39)	0.076
BNP (pg/ml)	16.5 (0-71.3)	19 (0-57)	13 (0-65)	21 (0-51)	0.788
LVEF (%)	60 (55-61)	60 (56-62)	60 (56-62)	60 (57-62)	0.189
culprit vessel					
LAD	61 (58%)[a]	101 (70%)	112 (77%)[a]	109 (76%)	0.033
RCA	33 (31%)[a]	31 (21%)	23 (16%)[a]	26 (18%)	
LCX	12 (11%)	11 (8%)	11 (8%)	7 (5%)	
LM	0 (0%)	1 (1%)	0 (0%)	2 (1%)	
multivessel disease					
one vessel	55 (52%)[a]	51 (36%)[b]	35 (24%)[a]	19 (13%)[a,b]	<0.001
two vessels	25 (24%)	51 (35%)	46 (31%)	46 (32%)	
three vessels	26 (24%)[a]	42 (29%)[b]	65 (45%)[a,b]	79 (55%)[a,b]	
Medications					
Statins	35 (33%)	47 (33%)	52 (36%)	58 (40%)	0.525
β-blockers	41 (39%)	50 (35%)	52 (36%)	53 (37%)	0.928
ACEI/ARB	21 (20%)	36 (25%)	40 (27%)	37 (26%)	0.571
CCB	26 (25%)	38 (26%)	39 (27%)	41 (29%)	0.920
aspirin	91 (86%)	122 (85%)	127 (87%)	119 (83%)	0.765

Values are mean±SD, n (%), or median (25th–75th percentile). [a,b,c,d] The Same letter indicates a significant difference in the 2-group comparison with the Bonferroni correction. CPB = calcified plaque burden; MI = myocardial infarction; PCI = percutaneous coronary intervention; SAP = stable angina pectoris; UAP = unstable angina pectoris; NSTEMI = non-ST-segment elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction; WBC = white blood cells; hs-CRP = high-sensitivity C-reactive protein; FBG = fasting blood glucose; TC= total cholesterol; TG = triglyceride; LDLC = low-density lipoprotein cholesterol; HDLC = high-density lipoprotein cholesterol; NHR = neutrophil/HDL ratio; MHR = monocyte/HDL ratio; eGFR = estimated glomerular filtration rate; BNP = brain natriuretic peptide; LVEF = left ventricular ejection fraction; LAD = left anterior descending; LCX = left circumflex artery; RCA = right coronary artery; LM = left main; ACEI = angiotensin-converting enzyme inhibitors; ARB = angiotensin receptor blocker; CCB = calcium channel blockers.

Table 2. Baseline characteristics among different calcification patterns.

	No Calcification (N=106)	**Microcalcificationspotty calcification (N=9)**	**(N=230)**	**Macrocalcification (N=195)**	**p**
Age	57.4±12.4[a,c]	55.6±10.6[c]	60.7±10.2[a,b,c]	64.3±10.1[a,b,c]	<0.001
male	75 (71%)	7 (78%)	179 (78%)	142 (73%)	0.481
Prior MI	8 (8%)	0 (0%)	21 (9%)	27 (14%)	0.182
Prior PCI	6 (6%)[a]	2 (22%)	28 (12%)	33 (17%)[a]	0.035
Hypertension	61 (58%)	4 (44%)	136 (59%)	130 (67%)	0.210
Diabetes mellitus	28 (26%)	1 (11%)	66 (29%)	68 (35%)	0.208
Clinical Diagnosis					
SAP	11 (10%)	0 (0%)	14 (6%)	9 (5%)	0.006

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UAP	54 (51%)[a]	5 (56%)	143 (62%)	142 (73%)[b]	
STEMI	22 (21%)	4 (44%)[a]	42 (18%)	20 (10%)[a]	
NSTEMI	19 (18%)	0 (0%)	31 (14%)	24 (12%)	
WBC (10^9/L)	7.39±2.53	7.39±3.86	7.19±2.19	6.99±2.15	0.523
neutrophils	4.91±2.23	5.01±3.33	4.72±2.01	4.58±1.87	0.556
lymphocytes	1.83±0.67	1.76±0.65	1.86±0.61	1.79±0.64	0.716
monocytes	0.471±0.174	0.424±0.155	0.436±0.170	0.440±0.165	0.315
platelets (10^9/L)	228±61	249±132	224±55	224±62	0.655
hemoglobin (g/L)	139±17	141±12	139±14	139±15	0.900
hs-CRP (mg/l)	2.10 (0.98-5.05)[a]	1.20 (0.41-2.72)	1.25 (0.65-4.94)	1.00 (0.48-3.12)[a]	0.004
FBG (mmol/l)	5.40 (4.93-6.36)	6.02 (4.69-7.21)	5.54 (4.94-6.57)	5.60 (5.04-6.65)	0.644
HbA1c	6.00 (5.70-6.80)	6.05 (5.75-6.58)	6.10 (5.60-7.10)	6.30 (5.70-7.40)	0.324
TC (mmol/l)	4.17±0.99	4.04±1.03	4.25±1.11	3.96±1.09	0.053
TG (mmol/l)	1.54 (1.18-2.20)	1.43 (1.26-2.66)	1.52 (1.08-2.15)	1.45 (1.09-2.10)	0.527
LDLC (mmol/l)	2.66±0.91	2.48±0.53	2.73±0.94	2.48±1.00	0.054
HDLC (mmol/l)	1.03±0.24	0.94±0.19	1.03±0.23	1.03±0.22	0.656
NHR	5.07±2.67	5.65±4.04	4.80±2.33	4.68±2.27	0.405
MHR	0.457 (0.326-0.577)	0.488 (0.279-0.632)	0.405 (0.314-0.537)	0.400 (0.304-0.558)	0.273
LDL/HDL	2.67±0.96	2.75±0.71	2.76±1.08	2.48±1.09	0.060
TGs	1.52±0.65	1.69±0.77	1.53±0.67	1.47±0.64	0.610
eGFR (ml/min1.73m^2)	90.2±17.8	91.7±16.4	89.4±14.1	87.2±15.5	0.312
Uric acid (umol/L)	338 (277-405)	350 (278-365)	318 (272-390)	323 (271-384)	0.610
D-dimer (μg/ml)	0.23 (0.19-0.31)	0.22 (0.18-0.32)	0.23 (0.19-0.32)	0.25 (0.21-0.39)	0.058
BNP (pg/ml)	16.5 (0-71.3)	10 (0-23)	19 (0-56)	21 (0-53)	0.765
LVEF (%)	60 (55-61)	60 (58-63)	60 (57-62)	60 (57-62)	0.206
culprit vessel					
LAD	61 (58%)[a]	6 (67%)	166 (72%)[a]	150 (77%)[a]	0.027
RCA	33 (31%)[a]	3 (33%)	41 (18%)[a]	36 (19%)	
LCX	12 (11%)	0 (0%)	21 (9%)	8 (4%)	
LM	0 (0%)	0 (0%)	2 (1%)	1 (1%)	
multivessel disease					
one vessel	55 C[a]	3(33%)	73 (32%)[a,b]	29 (15%)[a,b]	<0.001
two vessels	25 (24%)	4 (44%)	72 (31%)	67 (34%)	
three vessels	26 (24%)[a]	2 (22%)	85 (37%)[b]	99 (51%)[a,b]	
Medications					
Statins	35 (33%)	3 (33%)	74 (32%)	80 (41%)	0.260
β-blockers	41 (39%)	4 (44%)	81 (35%)	70 (36%)	0.885
ACEI/ARB	21 (20%)	2 (22%)	60 (26%)	51 (26%)	0.606
CCB	26 (25%)	2 (22%)	61 (27%)	55 (28%)	0.902
Aspirin	91 (86%)	9 (100%)	192 (84%)	1671 (86%)	0.546

Values are mean±SD, n (%), or median (25th–75th percentile). [a,b,c,d] The Same letter indicates a significant difference in the 2-group comparison with the Bonferroni correction. Abbreviations are as in Table 1.

Plaque Vulnerability and CPB

The relationship between plaque vulnerability and the level of CPB is shown in Table 3. The prevalence of TCFA, layered plaque, cholesterol crystal, microchannel, macrophage cluster, plaque rupture, plaque erosion, and eruptive calcified nodules significantly differed in different CPB groups. The prevalence of TCFA, plaque rupture, and cholesterol crystals was highest in the intermediate CPB group. High CPB showed the lowest prevalence of layered plaque and microchannel. Plaque erosion had the least calcification, while eruptive calcified nodules were predominantly seen in the high CPB group. Bonferroni post hoc test demonstrated a significantly higher prevalence of TCFA in the intermediate CPB group compared with the no-calcification and high CPB groups (36% vs 20%, 11%, $p<0.05$, Figure 1). Similarly, the prevalence of plaque rupture, layered plaque, and microchannel was significantly higher in the intermediate CPB group compared with the high CPB group (23% vs.

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11%, 38% vs. 17%, 52% vs. 31%, respectively, p<0.05). Moreover, a significantly higher prevalence of cholesterol crystals was observed in the intermediate CPB group compared with the no-calcification group (32% vs 12%, p<0.05). In the subgroup analysis stratified by clinical presentation, a higher prevalence of plaque instability features was observed in the intermediate CPB group in patients with angina pectoris. Still, this trend did not reach statistical significance in patients with acute myocardial infarction (AMI) (Supplemental Table S1 and S2). The results of the subgroup analysis according to age were consistent with those of the entire cohort. Notably, a higher prevalence of TCFA and plaque rupture was found in the low CPB group in patients older than 70 (Supplemental Tables S3–S5).

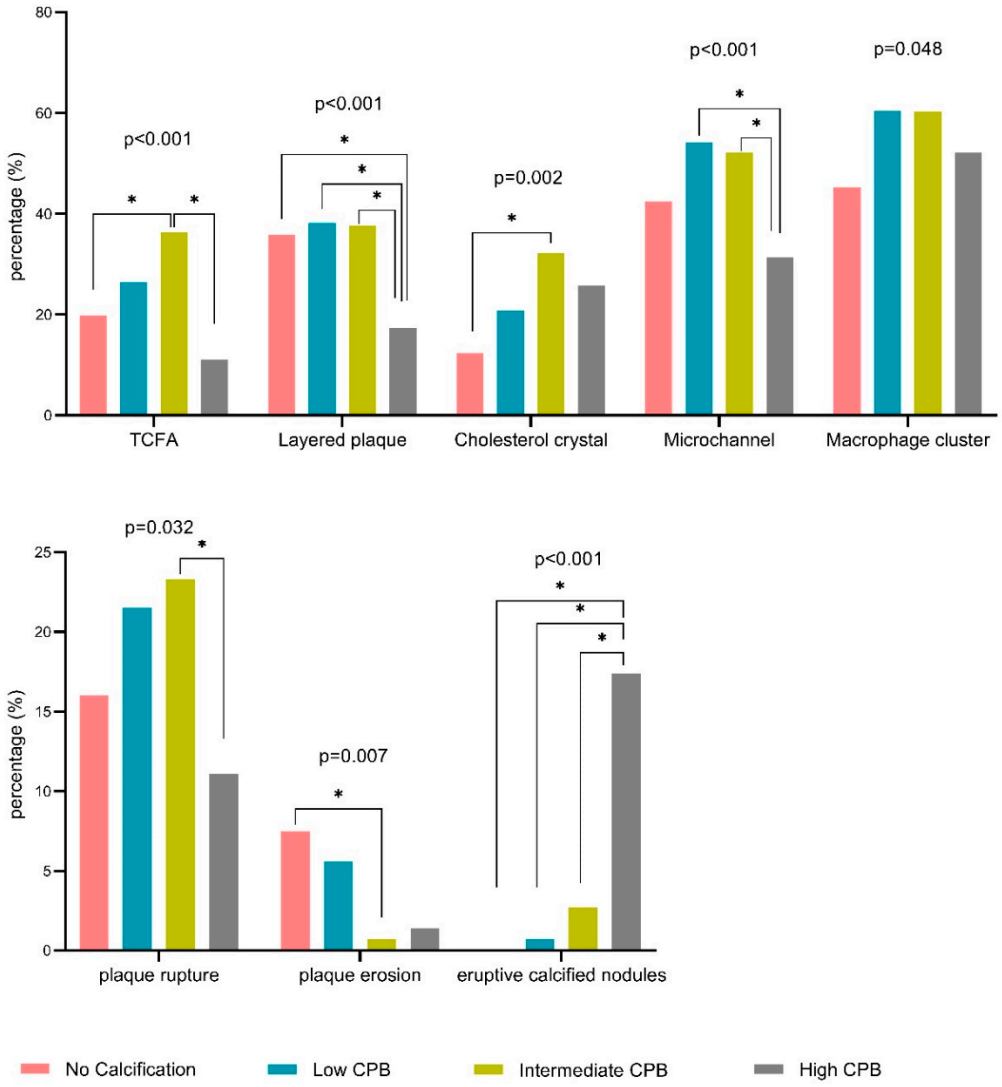

Figure 1. Comparison of plaque vulnerability among different CPB groups. * indicated a significant difference in Bonferroni post hoc analysis between the two groups.

Table 3. OCT findings among different CPB groups.

OCT	No Calcification (N=106)	Low CPB (N=144)	Intermediate CPB (N=146)	High CPB (N=144)	p
MLA	2.07 (1.45-2.89)[a]	1.93 (1.37-2.72)[b]	1.95 (1.31-2.62)	1.69 (1.31-2.31)[a,b]	0.002
FCT(um)	126 (81-188)	113 (71-171)	99 (61-168)	116 (88-160)	0.069
Max lipid arc °	273 (240-360)[a]	290 (245-360)[b]	311 (256-360)	360 (280-360)[a,b]	<0.001
Lipid length	28.56±14.4[a,b]	30.5±12.9[c,d]	33.8±12.9[b,c]	34.8±11.5[a,d]	<0.001
Lipid index	5768±3351[a,b]	6201±2986[c,d]	7034±3038[b,c]	7449±2735[a,d]	<0.001
Total plaque index	5773±3343[a,b]	6421±3007[c,d]	7675±3109[a,c,e]	9215±2939[b,d,e]	<0.001
TCFA	21 (20%)[a]	38 (26%)[b]	53 (36%)[a,c]	16 (11%)[b,c]	<0.001
Layered plaque	38 (36%)[a]	55 (38%)[b]	55 (38%)[c]	25 (17%)[a,b,c]	<0.001
Cholesterol crystal	13 (12%)[a]	30 (21%)	47 (32%)[a]	37 (26%)	0.002

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Microchannel	45 (43%)	78 (54%)[a]	76 (52%)[b]	45 (31%)[a,b]	<0.001
Macrophage cluster	48 (45%)	87 (60%)	88 (60%)	75 (52%)	0.048
Plaque rupture	17 (16%)	31 (22%)	34 (23%)[a]	16 (11%)[a]	0.032
Plaque erosion	8 (8%)[a]	8 (6%)	1 (1%)[a]	2 (1%)	0.007
Eruptive calcified nodules	0 (0%)[a]	1 (1%)[b]	4 (3%)[c]	25 (17%)[a,b,c]	<0.001
Superficial microcalficialtions (n)	NA	0.076±0.292	0.158±0.560	0.118±0.450	0.455
Deep microcalficialtions (n)	NA	0.167±0.443	0.158±0.435	0.174±0.448	0.926
Superficial spotty calcifications (n)	NA	0.347±0.651[a,b]	1.01±1.25[a,c]	2.26±2.17[b,c]	<0.001
Deep spotty calcifications (n)	NA	1.17±1.01[a,b]	2.55±1.88[a]	3.10±2.53[b]	<0.001
Superficial macrocalcifications (n)	NA	0.028±0.165[a,b]	0.377±0.656[a,c]	2.27±1.90[b,c]	<0.001
Deep macrocalcifications (n)	NA	0.056±0.230[a,b]	0.178±0.450[a]	0.299±0.739[b]	<0.001

Values are mean±SD, n (%), or median (25th–75th percentile). [a,b,c,d] The Same letter indicates a significant difference in the 2-group comparison with the Bonferroni correction. CPB = calcified plaque burden; MLA = minimal lumen area; FCT = fibrous cap thickness; TCFA = thin cap fibroatheroma;.

Plaque Vulnerability and Calcification Patterns

The prevalence of TCFA, plaque rupture, and layered plaque did not differ significantly between different calcification patterns (Figure 2). The Bonferroni post hoc test revealed a significantly higher prevalence of cholesterol crystals in the spotty calcification group compared with the no calcification group (27% vs 12%, p=0.015). Interestingly, a significantly higher prevalence of microchannels and plaque erosion was found in the microcalcification group (Table 4). Moreover, the macrocalcification group presented with the highest prevalence of eruptive calcified nodules.

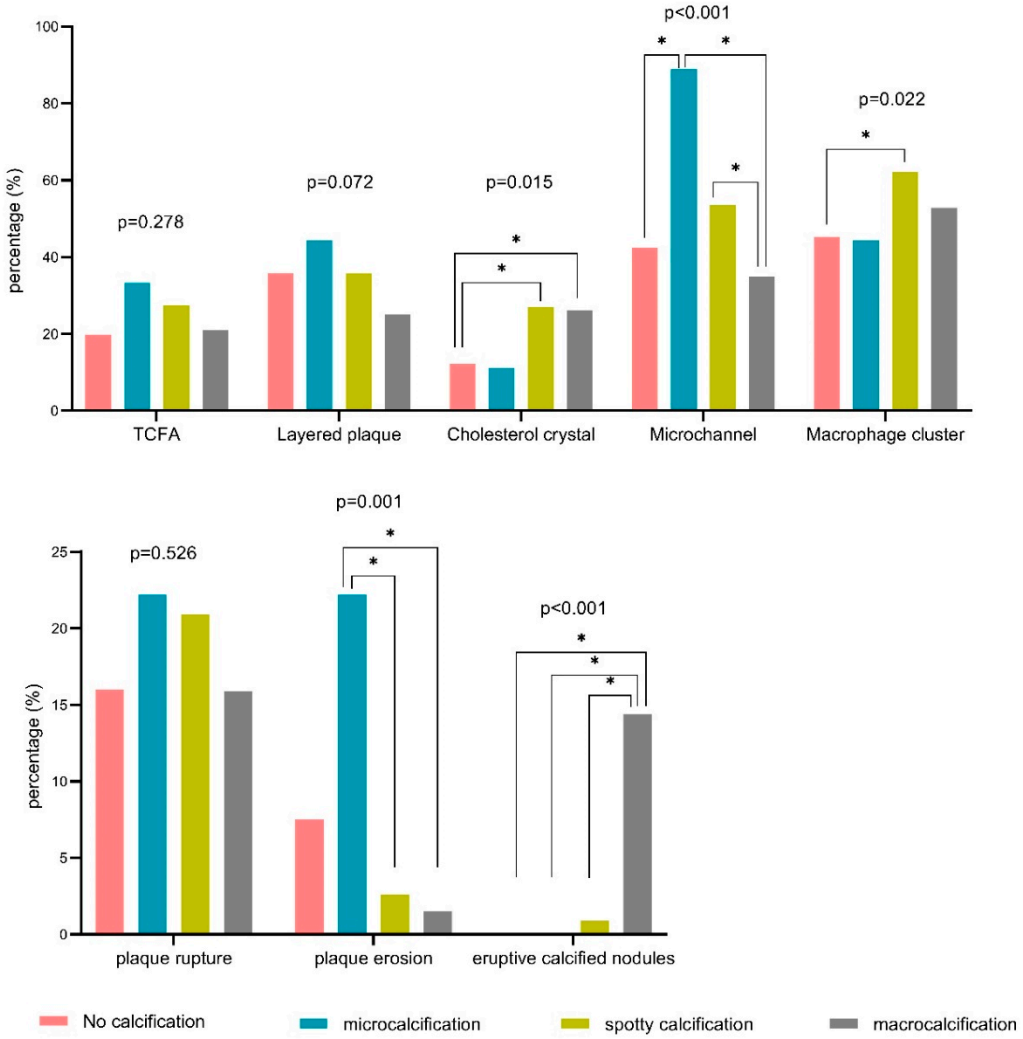

Figure 2. Comparison of plaque vulnerability among different calcification patterns. * indicated a significant difference in Bonferroni post hoc analysis between the two groups.

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The relationship between plaque instability characteristics and the number of calcium deposits with different depths is presented in Supplemental Tables S6–S8. TCFA, plaque rupture, and layered plaque were associated with fewer superficial macrocalcifications (0.406±0.917 vs. 0.812±1.520, p=0.026; 0.337±0.688 vs. 0.803±1.510, p=0.033; 0.405±0.964 vs. 0.867±1.55, p=0.001, respectively); cholesterol crystals and macrophage clusters were associated with more spotty calcifications (3.43±2.72 vs. 2.61±2.91, p<0.001; 3.09±2.98 vs. 2.43±2.72, p=0.003, respectively, figure 3); microchannels were associated with more microcalcifications (0.328±0.708 vs. 0.145±0.390, p=0.002) and fewer macrocalcifications (0.639±1.25 vs. 1.05±1.66, p=0.003). However, the number of spotty calcifications did not show a significant relationship with TCFA, plaque rupture, and microchannels.

Table 4. OCT findings among different calcification patterns.

OCT	No Calcification (N=106)	Microcalcification (N=9)	spotty calcification (N=230)	Macrocalcification (N=195)	p
MLA	2.07 (1.45-2.89)	2.31 (1.92-3.51)	1.96 (1.39-2.81)	1.79 (1.31-2.46)	0.010
FCT(um)	126 (81-188)	86 (60-205)	112 (64-171)	110 (71-155)	0.372
Max lipid arc °	273 (240-360)[a]	280 (225-327)	292 (250-360)[b]	320 (280-360)[a,b]	<0.001
Lipid length	28.6±14.4[a,b]	21.1±11.7[c,d]	32.8±12.7[a,c]	33.9±12.2[b,d]	<0.001
Lipid index	5768±3351[a,b]	4132±2521[c,d]	6740±2946[a,c]	7205±2931[b,d]	<0.001
TCFA	21 (20%)	3 (33%)	63 (27%)	41 (21%)	0.278
Layered plaque	38 (36%)[a]	4 (44%)[b]	82 (36%)[c]	49 (25%)[a,b,c]	0.072
Cholesterol crystal	13 (12%)[a,b]	1 (11%)	62 (27%)[a]	51 (26%)[b]	0.015
Microchannel	45 (43%)[a]	8 (89%)[a,c]	123 (54%)[b]	68 (35%)[b,c]	<0.001
Macrophage cluster	48 (45%)[a]	4 (44%)	143 (62%)[a]	103 (53%)	0.022
Plaque rupture	17 (16%)	2 (22%)	48 (21%)[a]	31 (16%)[a]	0.526
Plaque erosion	8 (8%)[a]	2 (22%)[b,c]	6 (3%)[c]	3 (2%)[a,b]	0.001
Eruptive calcified nodules	0 (0%)[a]	0 (0%)	2 (1%)[b]	28 (14%)[a,b]	<0.001

Values are mean±SD, n (%), or median (25th–75th percentile). [a,b,c,d] The Same letter indicates a significant difference in the 2-group comparison with the Bonferroni correction. Abbreviations as in table 3.

Inflammation Index and Calcification

Laboratory findings showed markedly lower levels of systemic inflammation in the high CPB group (Table 1), including neutrophils, neutrophil/HDL ratio (NHR), and high-sensitivity C-reactive protein (hs-CRP). Meanwhile, a comparable trend of lower levels of hs-CRP was noted in the macrocalcification group (Table 2). The result of the subgroup analysis demonstrated a similar trend of hs-CRP level in different calcification groups in patients with angina pectoris (Supplement Table 1,9). However, these differences did not reach statistical significance in patients with AMI (Supplement Tables S2 and S10).

The prevalence of macrophage clusters was significantly higher in the intermediate CPB and spotty calcification groups than in the no calcification group (60%, 62% vs. 45%, p<0.05). A significantly higher number of deep and total spotty calcifications was found in patients with macrophage clusters (Supplement Table S5). In the subgroup analysis, more spotty calcifications were observed in patients with macrophage clusters in the angina pectoris group (Supplement Table S11). Interestingly, in patients less than 50 years old, a higher prevalence of macrophage cluster and cholesterol crystal was present in the intermediate CPB group; meanwhile, a significantly higher number of spotty calcifications was noted in the young patients present with macrophage clusters (2.80±2.74 vs.1.15±2.60, p<0.001) and cholesterol crystal (4.39±3.29 vs. 1.55±2.32, p<0.001). However, this relationship between spotty calcifications and inflammation levels was obviated by advanced age (Supplemental Tables S3–S5).

Risk Factors for the Presence of Calcification Deposits and CPB

Patients with calcium deposits had significantly higher age and lipid index, lower minimal lumen area (MLA), higher prevalence of multivessel disease, culprit lesion in LAD, prior history of

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PCI, plaque erosion, macrophage cluster, and cholesterol crystal. No significant differences were observed in statin use, diabetes mellitus, hypertension, AMI, TCFA, plaque rupture, microchannel, and layered plaque between these two groups. A multivariate logistic regression analysis demonstrated that culprit lesion in LAD (odds ratio [OR]: 0.336; 95% CI: 0.200-0.562, p<0.001), multivessel disease (OR:1.740; 95% CI: 1.258-2.407, p=0.001), and age (OR: 1.030; 95% CI: 1.009-1.052, p=0.005) were independently associated with the presence of calcium deposits (Supplement Table S12).

Multiple linear regression analysis in patients with calcium deposits demonstrated that age (standardized beta coefficient =0.255, p<0.001), multivessel disease (standardized beta coefficients =0.160, p=0.001), macrophage cluster (standardized beta coefficients =-0.165, p=0.001), and lipid index (standardized beta coefficients = 0.185, p<0.001) were independently associated with CPB (Supplement Table S13).

Discussion

The current study demonstrated that:1) intermediate CPB but not spotty calcifications were associated with a significantly higher level of plaque vulnerability, including TCFA and plaque rupture; 2) macrophage cluster and cholesterol crystal were associated with more spotty calcifications, especially in the young patients; 3) microchannels were associated with more microcalcifications; 4) high CPB and macrocalcifications showed the lowest level of inflammation.

CAC has long been considered a surrogate of atherosclerosis. Many imaging modalities have proposed spotty calcification to be a predictor of vulnerable plaque [14,15]. Intravascular ultrasound studies have demonstrated that patients with acute coronary syndrome (ACS) have more spotty calcifications [16], while those with stable angina pectoris have more extensive calcifications [17]. Spotty calcifications identified by coronary computed tomography angiography in the culprit lesions were associated with TCFA and macrophages validated in OCT [18]. High vulnerability with active inflammation may occur in early to intermediate calcification; with the accumulation of extensive calcification, plaques change to be more stable, and vascular inflammation subsides [4].

Based on our study, the relationship between CAC and plaque instability is complex. This current study first demonstrated that intermediate CPB, but not the number of spotty calcifications, at the culprit vessel level, was associated with a higher prevalence of TCFA and plaque rupture. The discrepancy may be explained by the increase in spotty calcifications in parallel with extensive calcifications across the culprit vessel. Consequently, a high number of spotty calcifications may be present in patients with extensive calcifications and a low level of plaque vulnerability. Therefore, CPB may serve as a more appropriate indicator of plaque instability for these patients. Similarly, a previous study reported that the number of calcified plaques did not add risk stratification beyond the CPB score in patients with high CPB [19]. Meanwhile, CAC is strongly and positively associated with age; the area of calcified plaques for patients in their seventh decade is almost twice as large as that for patients in their fifth decade [20]. The higher CPB in advanced ages may account for the highest prevalence of plaque vulnerability in the low CPB group in patients older than 70. In addition, our study found plaque erosion had the least calcification, with eruptive calcified nodules predominantly seen in the extensive calcification, which were in line with the pathophysiological mechanism of these two causes of ACS [21,22].

Coronary artery calcification is an inflammation-dependent process. Molecular imaging has demonstrated that the secretion of pro-osteogenic cytokines from macrophages promotes the osteogenic transformation of vascular cells and produces spotty calcifications in the earlier calcification stages [23]. OPTICO-ACS study revealed that spotty calcifications within ACS were associated with local congregation of innate immune cells and inflammatory cytokines [24]. Moreover, a strong negative relationship between the extent of calcification and macrophage infiltration was observed in carotid plaque [25]. Our results indicated that the relationship between spotty calcification and macrophage cluster differed according to age: macrophage cluster was associated with more spotty calcifications in young patients who have low CPB, while in patients

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with advanced age, higher CPB and more macrocalcifications resulted in a lower level of vascular inflammation, and attenuated the interrelationship between macrophage cluster and spotty calcifications.

Cholesterol crystallization originates from the accumulation of free cholesterol in macrophages and smooth muscle cells. Cholesterol crystals destabilize plaque via NLRP3 inflammasome, leading to interleukin-1β and CRP production [26,27]. Consistent with our study, a higher number of spotty calcifications was also observed in patients with cholesterol crystals in the culprit and non-culprit vessels in previous OCT studies [28–30]. These data suggest that spotty calcifications are associated with a higher level of vascular inflammation. Moreover, a higher prevalence of microchannel was associated with an increased number of spotty calcifications within non-culprit lesions in stable coronary artery disease [31]. In contrast, our research demonstrated a significant positive relationship between microchannels and microcalcifications, but not spotty calcifications. Different study populations may cause this discrepancy.

Study Limitations

There were some limitations in this study. First, this retrospective study analyzed the OCT imaging only in the culprit vessel but not three vessels, so the total CAC was not evaluated. Second, a previous study had indicated that total CAC burden was a marker of ACS risk, but segmental CAC was not associated with culprit lesions [32], so a plaque-based comparison of CAC was not performed. Third, selection bias cannot be excluded due to the relatively limited number of patients with AMI and stable angina pectoris in this retrospective, single-center study. Fourth, the detailed relationship between inflammation and CAC was not assessed since only a few proinflammatory biomarkers were available. Fifth, OCT image analysis was not performed in an independent core laboratory, and long-term follow-up of our patients was unavailable at this time.

Conclusions

The relationship between CAC and plaque vulnerability is complex: intermediate CPB was associated with a significantly higher prevalence of TCFA and plaque rupture, while spotty calcifications correlated with vascular inflammation, including macrophage clusters and cholesterol crystals.

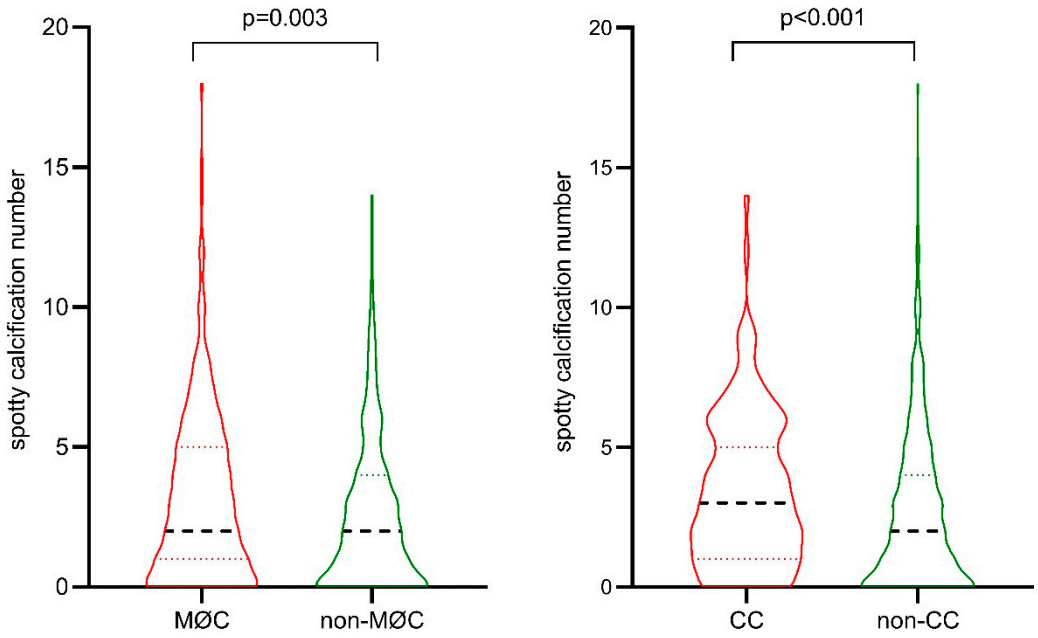

Figure 3. The relationship between macrophage cluster, cholesterol crystal, and spotty calcification. MØC = macrophage cluster; CC = cholesterol crystal.

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Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval: The study was conducted by the Declaration of Helsinki, and the Ethics Committee of the Institute of Tianjin Chest Hospital approved the protocol (2025LW-08).

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

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Abbreviations

CAC = Coronary artery calcification;
TCFA = Thin cap fibroatheromas;
OCT = Optical coherence tomography;
CPB = calcified plaque burden;
PCI = percutaneous coronary intervention;
UAP = unstable angina pectoris;
NSTEMI = non-ST-segment elevation myocardial infarction;
STEMI = ST-segment elevation myocardial infarction;
AMI = acute myocardial infarction
hs-CRP = high-sensitivity C-reactive protein;
LDLC = low-density lipoprotein cholesterol;
HDLC = high-density lipoprotein cholesterol;
NHR = neutrophil/HDL ratio;
MLA = minimal lumen area;
FCT = fibrous cap thickness;
ACS = acute coronary syndrome

References

1. Onnis, C, Virmani, R, Kawai, K, et al., Coronary Artery Calcification: Current Concepts and Clinical Implications, Circulation, 2024;149:251-266.
2. Razavi, AC, Agatston, AS, Shaw, LJ, et al., Evolving Role of Calcium Density in Coronary Artery Calcium Scoring and Atherosclerotic Cardiovascular Disease Risk, JACC Cardiovasc Imaging, 2022;15:1648-1662.
3. Osborne-Grinter, M, Kwiecinski, J, Doris, M, et al., Association of coronary artery calcium score with qualitatively and quantitatively assessed adverse plaque on coronary CT angiography in the SCOT-HEART trial, Eur Heart J Cardiovasc Imaging, 2022;23:1210-1221.
4. Mori, H, Torii, S, Kutyna, M, et al., Coronary Artery Calcification and its Progression: What Does it Really Mean?, JACC Cardiovasc Imaging, 2018;11:127-142.
5. Vancheri, F, Longo, G, Vancheri, S, et al., Coronary Artery Microcalcification: Imaging and Clinical Implications, Diagnostics (Basel), 2019;9.
6. Nakahara, T, Dweck, MR, Narula, N, et al., Coronary Artery Calcification: From Mechanism to Molecular Imaging, JACC Cardiovasc Imaging, 2017;10:582-593.
7. Onea, HL, Olinic, M, Lazar, FL, et al., A Review Paper on Optical Coherence Tomography Evaluation of Coronary Calcification Pattern: Is It Relevant Today?, J Cardiovasc Dev Dis, 2024;11.

 doi:10.20944/preprints202504.2113.v1

8. Fujimoto, D, Usui, E, Vergallo, R, et al., Relationship Between Coronary Artery Calcium Score and Vulnerability of Culprit Plaque Assessed by OCT in Patients With Established Coronary Artery Disease, Circ Cardiovasc Imaging, 2025;18:e017099.
9. Fujimoto, D, Kinoshita, D, Suzuki, K, et al., Relationship Between Calcified Plaque Burden, Vascular Inflammation, and Plaque Vulnerability in Patients With Coronary Atherosclerosis, JACC Cardiovasc Imaging, 2024;17:1214-1224.
10. Qin, Z, Yu, L, Zhang, Y, et al., Coronary artery calcification and plaque stability： an optical coherence tomography study, Heliyon, 2023;9:e23191.
11. Araki, M, Park, SJ, Dauerman, HL, et al., Optical coherence tomography in coronary atherosclerosis assessment and intervention, Nat Rev Cardiol, 2022;19:684-703.
12. Milzi, A, Burgmaier, M, Burgmaier, K, et al., Type 2 diabetes mellitus is associated with a lower fibrous cap thickness but has no impact on calcification morphology: an intracoronary optical coherence tomography study, Cardiovasc Diabetol, 2017;16:152.
13. Tearney, GJ, OCT imaging of macrophages: a bright spot in the study of inflammation in human atherosclerosis, JACC Cardiovasc Imaging, 2015;8:73-75.
14. Shi, X, Gao, J, Lv, Q, et al., Calcification in Atherosclerotic Plaque Vulnerability: Friend or Foe?, Front Physiol, 2020;11:56.
15. Gallone, G, Bellettini, M, Gatti, M, et al., Coronary Plaque Characteristics Associated With Major Adverse Cardiovascular Events in Atherosclerotic Patients and Lesions: A Systematic Review and Meta-Analysis, JACC Cardiovasc Imaging, 2023;16:1584-1604.
16. Sakaguchi, M, Hasegawa, T, Ehara, S, et al., New insights into spotty calcification and plaque rupture in acute coronary syndrome: an optical coherence tomography study, Heart Vessels, 2016;31:1915-1922.
17. Ehara, S, Kobayashi, Y, Yoshiyama, M, et al., Spotty calcification typifies the culprit plaque in patients with acute myocardial infarction: an intravascular ultrasound study, Circulation, 2004;110:3424-3429.
18. Kinoshita, D, Suzuki, K, Usui, E, et al., High-Risk Plaques on Coronary Computed Tomography Angiography: Correlation With Optical Coherence Tomography, JACC Cardiovasc Imaging, 2024;17:382-391.
19. Arnson, Y, Rozanski, A, Gransar, H, et al., Comparison of the Coronary Artery Calcium Score and Number of Calcified Coronary Plaques for Predicting Patient Mortality Risk, Am J Cardiol, 2017;120:2154-2159.
20. Otsuka, F, Sakakura, K, Yahagi, K, et al., Has our understanding of calcification in human coronary atherosclerosis progressed?, Arterioscler Thromb Vasc Biol, 2014;34:724-736.
21. Kolte, D, Yonetsu, T, Ye, JC, et al., Optical Coherence Tomography of Plaque Erosion: JACC Focus Seminar Part 2/3, J Am Coll Cardiol, 2021;78:1266-1274.
22. Torii, S, Sato, Y, Otsuka, F, et al., Eruptive Calcified Nodules as a Potential Mechanism of Acute Coronary Thrombosis and Sudden Death, J Am Coll Cardiol, 2021;77:1599-1611.
23. New, SE and Aikawa, E, Molecular imaging insights into early inflammatory stages of arterial and aortic valve calcification, Circ Res, 2011;108:1381-1391.
24. Nelles, G, Abdelwahed, YS, Alyaqoob, A, et al., Spotty calcium deposits within acute coronary syndrome (ACS)-causing culprit lesions impact inflammatory vessel-wall interactions and are associated with higher cardiovascular event rates at one year follow-up: Results from the prospective translational OPTICO-ACS study program, Atherosclerosis, 2023;385:117284.
25. Shaalan, WE, Cheng, H, Gewertz, B, et al., Degree of carotid plaque calcification in relation to symptomatic outcome and plaque inflammation, J Vasc Surg, 2004;40:262-269.
26. Niyonzima, N, Bakke, SS, Gregersen, I, et al., Cholesterol crystals use complement to increase NLRP3 signaling pathways in coronary and carotid atherosclerosis, EBioMedicine, 2020;60:102985.
27. Janoudi, A, Shamoun, FE, Kalavakunta, JK, et al., Cholesterol crystal induced arterial inflammation and destabilization of atherosclerotic plaque, Eur Heart J, 2016;37:1959-1967.
28. Qin, Z, Cao, M, Xi, X, et al., Cholesterol crystals in non-culprit plaques of STEMI patients: A 3-vessel OCT study, Int J Cardiol, 2022;364:162-168.

 doi:10.20944/preprints202504.2113.v1

29. Xue, C, Chen, Q, Bian, L, et al., The relationships between cholesterol crystals, NLRP3 inflammasome, and coronary atherosclerotic plaque vulnerability in acute coronary syndrome: An optical coherence tomography study, Front Cardiovasc Med, 2022;9:905363.
30. Dai, J, Tian, J, Hou, J, et al., Association between cholesterol crystals and culprit lesion vulnerability in patients with acute coronary syndrome: An optical coherence tomography study, Atherosclerosis, 2016;247:111-117.
31. Kataoka, Y, Puri, R, Hammadah, M, et al., Spotty calcification and plaque vulnerability in vivo: frequency-domain optical coherence tomography analysis, Cardiovasc Diagn Ther, 2014;4:460-469.
32. Puchner, SB, Mayrhofer, T, Park, J, et al., Differences in the association of total versus local coronary artery calcium with acute coronary syndrome and culprit lesions in patients with acute chest pain: The coronary calcium paradox, Atherosclerosis, 2018;274:251-257.