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ORIGINAL RESEARCH

# Layered Plaque in Organic Lesions in Patients With Coronary Artery Spasm

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**BACKGROUND:** Coronary artery spasm plays a vital role in the pathogenesis of coronary plaques. We sought to investigate the plaque characteristics of co-existing organic lesions in patients with coronary artery spasm in comparison to those without coronary artery spasm by intracoronary optical coherence tomography (OCT).

**METHODS AND RESULTS:** We included 39 patients who presented with a symptom suspected of coronary spastic angina and had an organic lesion, defined as ≥plaque burden of 50% assessed by OCT. Coronary artery spasm was diagnosed by positive acetylcholine provocation test, or by spontaneous spasm detected during coronary angiography. A total of 51 vessels with an organic lesion were identified. Of these, coronary artery spasm was observed in 30 vessels (spasm), while not in 21 vessels (non-spasm). Organic lesions in the spasm vessels, compared with those in the non-spasm vessels, had a higher prevalence of layered plaque (93% versus 38%, $P<0.001$), macrophages (80% versus 43%, $P=0.016$), and intraplaque microchannels (73% versus 24%, $P<0.001$), and lower prevalence of macrocalcification (23% versus 62%, $P=0.009$) as assessed by OCT.

**CONCLUSIONS:** Layered plaque, macrophages, and intraplaque microchannels, were frequently observed in organic lesions in patients with coronary artery spasm. These findings suggest that coronary artery spasm induces local thrombus formation as well as active inflammatory response, therefore increasing the risk of rapid plaque progression and ischemic events in patients with coronary artery spasm.

**Key Words:** coronary artery spasm ■ healed plaque ■ layered plaque ■ optical coherence tomography

Coronary artery spasm plays a vital role in the pathogenesis of coronary artery disease (CAD). It often occurs in angiographically normal arteries but can be found in those with an atherosclerotic organic lesion. In patients with coronary spastic angina (CSA) and angiographically normal or minimally narrowed coronary artery, intravascular ultrasound (IVUS) analyses revealed the presence of subtle atherosclerotic organic lesions at the site of vasospasm which were prone to make rapid progression.[1–3] In addition, the presence of organic coronary artery lesions, even angiographically non-significant stenosis, is associated with a worse prognosis in patients with CSA.[4,5]

Intracoronary optical coherence tomography (OCT) is an imaging modality that generates high-resolution images, providing detailed qualitative information on structures of the coronary artery wall and plaque composition.[6] Several OCT-derived features of vulnerable coronary artery plaque were identified, such as lipid-rich plaques, thin-cap fibroatheroma, layered plaques, macrophage infiltration, and intraplaque microchannels, leading to subsequent rapid plaque

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## CLINICAL PERSPECTIVE

### What Is New?

- Layered plaque, macrophages, and intraplaque microchannels were frequently observed in organic lesions in patients with coronary artery spasm.
- These findings suggest that coronary artery spasm induces local thrombus formation as well as active inflammatory response, therefore increasing the risk of rapid plaque progression and ischemic events in patients with coronary artery spasm.

### What Are the Clinical Implications?

- The assessment of coronary spasm should be integrated into the workup of identifying vulnerable plaques and vulnerable patients at high risk of future coronary events.
- Layered plaques accompanied with macrophages and intraplaque microchannels can be a clue to suspect the involvement of coronary artery spasm in patients with coronary artery disease.

### Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **ACh** | acetylcholine |
| **CSA** | coronary spastic angina |
| **QCA** | quantitative coronary angiography |
| **TCFA** | thin cap fibroatheroma |

progression and future adverse cardiac outcomes.[6,7] Whether these vulnerable features are prevalent in organic lesions related to coronary artery spasm remains unknown.

We hypothesized that a detailed assessment of plaque morphology related to coronary artery spasm would give a clue to understanding the link between coronary artery spasm, rapid progression of coexistent atherosclerosis, and poor prognosis. Hence, we sought to investigate the morphological characteristics of significant organic lesions associated with coronary artery spasm, by using intracoronary OCT, in comparison to those without coronary artery spasm as reference.

## METHODS

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

### Study Design

The present study is a single-center, cross-sectional study at Kawasaki Medical School. We include patients who presented with a symptom suspected of CSA and had an organic coronary artery lesion, defined as ≥plaque burden of 50% assessed by OCT. Diagnosis of coronary artery spasm was made by positive or negative acetylcholine (ACh) provocation test, or by spontaneous spasm detected during coronary angiography. We excluded patients with no organic coronary lesion, inadequate OCT image quality, and angiographically significant left main coronary artery disease. We also excluded vessels where the ACh provocation test had not been performed for reasons other than spontaneous spasm leading to the diagnosis of CSA before the ACh provocation test. Based on coronary angiographic findings with the ACh provocation test or spontaneous spasm, patients and vessels were categorized into either a spasm group or a non-spasm group. The vessels in the spasm group were further divided into two subgroups according to the spasm sites in relation to the organic lesion sites, ie, spasm occurred at the site of the organic lesion (organic-lesion site spasm), or spasm occurred distal to the organic lesion (distal spasm). This study was approved by the institutional ethics committee (approval No. 5455) and complied with the Declaration of Helsinki on ethical principles for medical research involving human subjects. The study was exempted from the requirement for individual informed consent because of the opt-out policy, using their information from the database.

Intracoronary ACh provocation tests were performed according to the Japanese Circulation Society guidelines for the diagnosis and treatment of patients with CSA.[8] Positive coronary artery spasm was defined as transient luminal narrowing (99% focal spasm or 90% severe diffuse vasoconstriction associated with usual chest pain/symptom or ischemic ECG findings induced by the ACh provocation test or due to spontaneous coronary artery spasm).[8,9]

After the ACh provocation test, we performed an OCT imaging using either an OPTIS (Abbott, Santa Clara, CA) or a LUNAWAVE (Terumo Corporation, Tokyo, Japan) system after the injection of nitrates and confirming the relief of the vasospasm and vasodilation. In the quantitative OCT analysis, maximum plaque burden and minimum lumen area within the organic lesions were evaluated. Qualitative OCT analyses were performed at significant coronary artery lesions, including stenotic lesion and reference. A layered plaque was defined as a region having one or more layers with different optical densities from underlying components and a clear border.[10–14] The other qualitative OCT analyses were based on previously established criteria.

For more details on the diagnosis of coronary artery spasm, quantitative coronary angiography (QCA), and OCT analysis, please see Data S1.

## Statistical Analysis

Categorical variables were expressed as numbers and percentages and compared using Fisher's exact test. Continuous data are expressed as mean±standard deviation or median (interquartile range) and compared using the Student *t* test or Mann-Whitney U test as appropriate for per-patient analysis. For analysis of multiple lesions per patient, generalized estimating equations were used to account for clustering effects. Multivariable analyses were performed to make adjustment for the following covariates included in the equations: age, sex, coronary risk factors (hypertension, diabetes, dyslipidemia, current smoker, prior percutaneous coronary intervention, and prior myocardial infarction), estimated glomerular filtration rate (eGFR) which significantly differs between the spasm and non-spasm groups. The normality of the continuous variables was confirmed with the Shapiro-Wilk test. SPSS version 24 (IBM SPSS Statistics, IBM Corporation, Armonk, NY) was used for statistical analyses. A value of $P<0.05$ was considered statistically significant.

# RESULTS

From March 2016 to September 2021, 39 patients (mean age 65±10 years, male 67%) underwent both OCT imaging and diagnosis of CSA or non-CSA based on the presence or absence of ACh-induced or spontaneous spasm. Of these, coronary artery spasm was observed in 23 patients, while not in 16. Patients in the spasm group were significantly younger and had higher eGFR levels than those in the non-spasm group. Other clinical characteristics were not significantly different between the 2 groups (Table 1).

A total of 51 vessels with significant organic lesions were identified in the 39 patients. Of these, spasm was observed in 30 vessels, including focal spasm in 9 and diffuse spasm in 21 vessels. The severity of organic lesions, and QCA measurements were not significantly different between the spasm and non-spasm groups (percentage diameter stenosis by QCA, 37%±11% versus 32%±13%) (Table 2).

In the quantitative OCT analysis, maximum plaque burden and minimum lumen area were not significantly different between the spasm and non-spasm groups (Table 3). The qualitative OCT assessment demonstrated that the prevalence of layered plaque (93% versus 38%, $P<0.001$), macrophages (80% versus 43%, $P=0.016$), and intraplaque microchannels (77% versus 24%, $P<0.001$) was higher in the organic lesions in the spasm group than those in the non-spasm group.

**Table 1. Patient Characteristics**

| | Spasm (n=23) | Non-spasm (n=16) | *P* value |
|---|---|---|---|
| Age, y | 62±10 | 72±9 | 0.005 |
| Male | 16 (70%) | 10 (63%) | 0.74 |
| Hypertension | 23 (83%) | 10 (63%) | 0.26 |
| Diabetes | 11 (48%) | 5 (31%) | 0.34 |
| Dyslipidemia | 17 (74%) | 10 (63%) | 0.50 |
| Current smoker | 6 (26%) | 3 (19%) | 0.71 |
| Prior PCI | 4 (17%) | 3 (19%) | >0.99 |
| Prior myocardial infarction | 1 (4%) | 1 (6%) | >0.99 |
| Diagnosis at presentation | | | 0.96 |
| Resuscitated cardiac arrest | 2 (9%) | 1 (6%) | |
| Myocardial infarction | 4 (17%) | 3 (19%) | |
| Unstable Angina | 17 (74%) | 12 (75%) | |
| Medication | | | |
| Aspirin | 10 (44%) | 3 (21%) | 0.17 |
| P2Y12 inhibitor | 4 (17%) | 1 (6%) | 0.39 |
| Oral anticoagulant | 1 (4%) | 0 (0%) | >0.99 |
| ACE-I or ARB | 10 (44%) | 5 (31%) | 0.52 |
| β-blocker | 3 (13%) | 0 (0%) | 0.26 |
| Calcium channel blocker | 11 (48%) | 7 (44%) | >0.99 |
| Long-acting nitrate | 3 (13%) | 0 (0%) | 0.26 |
| Nicorandil | 2 (9%) | 1 (6%) | >0.99 |
| Statin | 11 (48%) | 3 (19%) | 0.093 |
| Diuretics | 1 (4%) | 0 (0%) | >0.99 |
| Laboratory data | | | |
| HbA1c (%) | 6.2 (5.7, 6.9) | 6.0 (5.7, 6.4) | 0.20 |
| Triglyceride (mg/dL) | 126 (74, 150) | 126 (74, 164) | 0.64 |
| HDL-cholesterol (mg/dL) | 52 (40, 62) | 42 (35, 49) | >0.99 |
| LDL-cholesterol (mg/dL) | 94±39 | 106±32 | 0.30 |
| eGFR (mL/min per 1.73 $m^2$) | 75±14 | 65±15 | 0.030 |
| eGFR<60 mL/min per 1.73 $m^2$ | 5 (22%) | 5 (31%) | 0.71 |
| Troponin T elevation> URL | 6 (26%) | 3 (19%) | 0.71 |
| Identified spasm site | | | |
| LAD | 19 (83%) | | |
| LCx | 9 (39%) | | |
| RCA | 12 (52%) | | |

ACE-I indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate; HDL, high density protein; LAD, left anterior descending coronary artery; LCx, left circumflex; LDL, low density protein; RCA, right coronary artery; and URL, upper reference limit.

Representative images of OCT-derived layered plaque were demonstrated in Figure 1 through 4. The prevalence of lipid-rich plaque was not significantly different between the 2 groups (67% versus 52%, $P=0.37$). In comparison, the prevalence of macrocalcification,

**Table 2. Visual Assessment and Quantitative Analyses of Coronary Angiography**

| | Spasm (n=30) | Non-spasm (n=21) | *P* value |
|---|---|---|---|
| Visual stenosis | 40±18% | 42±16 | 0.73 |
| Visual stenosis category | | | 0.61 |
| ≤25% | 9 (43%) | 16 (53%) | |
| >25%, ≤50% | 10 (48%) | 10 (33%) | |
| ≥75% | 2 (10%) | 4 (13%) | |
| QCA data | | | |
| MLD | 1.76±0.49 | 1.99±0.65 | 0.15 |
| Reference diameter at MLD site | 2.82±0.65 | 2.88±0.71 | 0.73 |
| Proximal reference diameter | 2.86±0.70 | 2.96±0.70 | 0.60 |
| Distal reference diameter | 2.62±0.59 | 2.74±0.69 | 0.48 |
| Percentage diameter stenosis | 37±11% | 32±13% | 0.12 |
| Lesion length | 11.6±10.2 | 9.3±4.0 | 0.27 |

MLD indicates minimum luminal diameter; and QCA, quantitative coronary angiography.

especially calcification arc >180°, was significantly lower in the spasm group (23% versus 62%, *P*=0.009) (Table 3). In the multivariable analyses with adjustment for age, sex, coronary risk factors and eGFR, the differences in the prevalence of layered plaque, macrophages, and intraplaque microchannels between the 2 groups remained statistically significant (*P*=0.018, 0.027, and 0.006, respectively), while macrocalcification did not (*P*=0.26).

To explore whether these findings are consistent across the spasm site in relation to organic lesions, we compared the prevalence of the identified spasm-related OCT findings between the organic-lesion site spasm and the distal spasm subgroups, and the non-spasm group. Both spasm subgroups showed a trend towered a higher prevalence of layered plaque, macrophage accumulation, and intraplaque microchannel than the non-spam group although the difference in the prevalence of macrophage accumulation between the non-spasm group and the distal spasm subgroup did not achieve statistically significant level (Table 4). Macrocalcification was less frequently observed in the organic-lesion site spasm group than the non-spasm group (Table 4).

**Table 3. OCT Findings of Organic Lesions in Vessels With and Without Spasm**

| | Spasm (n=30) | Non-spasm (n=21) | *P* value |
|---|---|---|---|
| Maximum plaque burden | 67% (57%, 72%) | 61% (55%, 74%) | 0.23 |
| Minimum lumen area, $mm^2$ | 3.6 (2.1, 4.6) | 5.1 (3.0, 6.6) | 0.077 |
| Layered plaque | 28 (93%) | 8 (38%) | <0.001 |
| Macrophages | 24 (80%) | 9 (43%) | 0.016 |
| Microchannels | 23 (77%) | 5 (24%) | <0.001 |
| Cavity | 4 (13%) | 0 (0%) | 0.13* |
| TCFA | 1 (3%) | 1 (5%) | 0.80 |
| Lipid-rich plaque | 20 (67%) | 11 (52%) | 0.37 |
| Maximum lipid arc | 132 (0, 204) | 103 (0, 164) | 0.49 |
| Macrocalcification | 7 (23%) | 13 (62%) | 0.009 |
| Calcification arc 90 –179° | 6 (20%) | 4 (19%) | |
| Calcification arc >180° | 1 (3%) | 9 (43%) | |

*P* values were calculated by generalized estimating equations when applicable; otherwise calculated by Fisher's exact test for categorical variables (*). OCT indicates optical coherence tomography; and TCFA, thin cap fibroatheroma.

## DISCUSSION

The present study evaluated plaque characteristics of organic lesions assessed by OCT in patients with and without coronary artery spasm. The main findings were as followings: (1) Organic lesion related to coronary artery spasm was frequently accompanied with layered plaques and ongoing inflammatory characteristics such as macrophages infiltration and intraplaque microchannels; (2) these findings were consistent across the coronary spasm sites in relation to the organic lesion sites, ie, spasm occurred at the site of the organic lesion and spasm occurred distal to the organic lesion; and (3) coronary calcification, especially macrocalcification, was less frequently observed compared with patient without ACh-induced spasm.

Layered plaque, also known as healed plaque, has been considered the result of one or more silent episodes of plaque rupture or erosion with nonocclusive thrombus formation.[15,16] As the initial healing process after silent thrombotic episodes, the thrombus is organized and gradually replaced by proteoglycan-rich granulation tissue and type III collagen. The type III collagen is gradually replaced by type I collagen, forming a new fibrous layer,

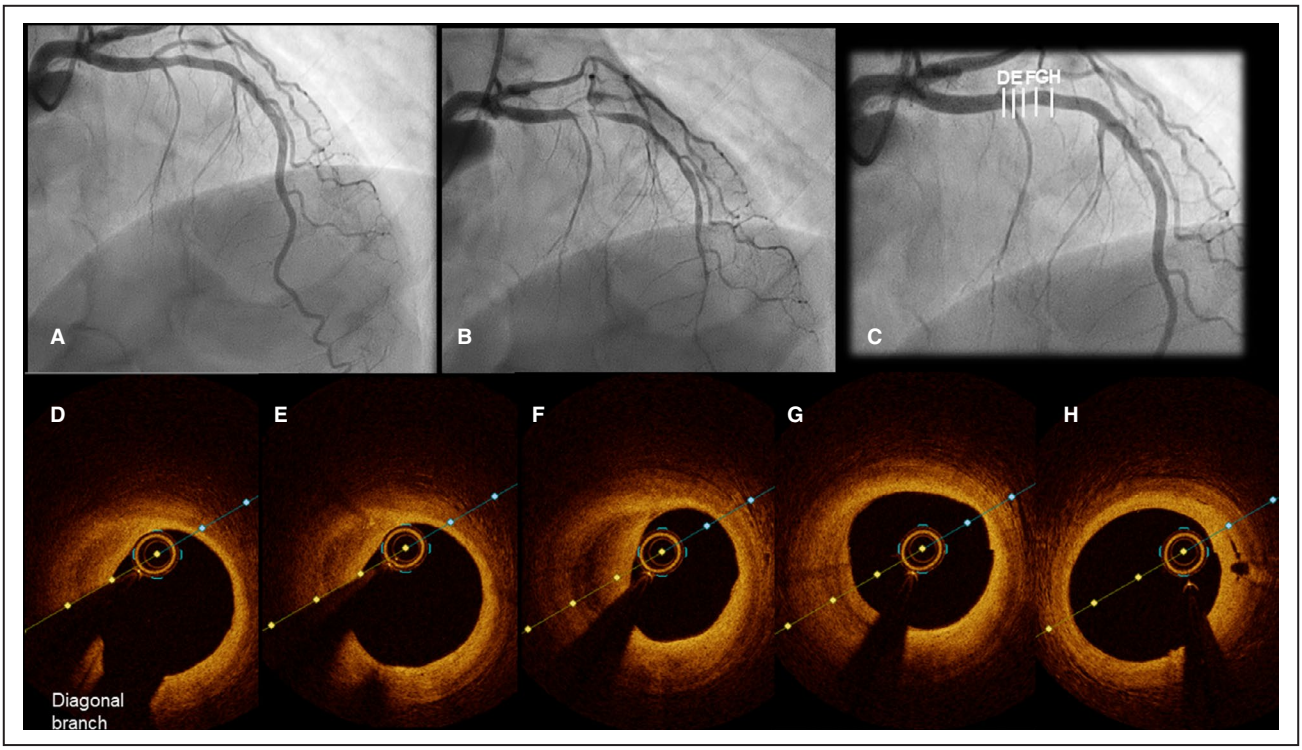


**Figure 1. An example case who presented with chest pain, inverted T waves on the ECG, and serum troponin elevation.** **A**, Coronary angiography shows a mild lesion in the mid-left anterior descending coronary artery (LAD). **B**, Intracoronary acetylcholine injection provoked a significant focal spasm in the mid LAD and a diffuse spasm in the distal LAD. **C**, Angiography after intracoronary nitrate injection shows mild residual organic stenosis in the LAD. The white lines on the angiogram indicate the sites where the optical coherence tomography (OCT) images of (**D** through **H**) were acquired. **D** through **H**, OCT shows layered plaques (**D** through **G**, at 6 to 12 o'clock) and intraplaque microchannels (**H**, at 3 o'clock).

which is later completely re-endothelialized.[10,17] The different optical properties of the thrombus and fibrous tissue of the healed plaque can be visualized as a layered pattern by OCT, therefore called layered plaque. A histopathological study assessed plaques excised by directional coronary atherectomy in stable CAD and showed that intramural thrombus and fibrous tissue are the main components of layered plaques detected by OCT, supporting the capability of OCT to identify healed plaques containing intramural thrombus as layered plaques.[11] A postmortem *ex vivo* study demonstrated that layered plaque detected by OCT showed high positive and negative predictive values for predicting histologically defined healed plaque.[12] According to previous OCT studies, layered plaque was not rare, with the prevalence ranging from 17.8 to 57.8 in a wide variety of ischemic heart diseases.[10,11,14,18] The prevalence of layered plaque in the non-spasm vessels in the present study (38%) is in line with that observed in these previous studies, while that in the spasm vessels was exceedingly higher (93%) in the present study.

The highly frequent prevalence of layered plaque in those with coronary artery spasm suggests that coronary artery spasm is one of the underlying etiologies of layered plaque formation. This is biologically plausible in that coronary artery spasm-related local thrombus formation can be triggered by reduced blood flow as well as endothelial or intimal injury secondary to critical vascular constriction and later result in layered plaque which reflects healing after the thrombosis. Previous biomarker studies have shown that plasma levels of fibrinopeptide A, a marker of thrombin generation, and plasminogen activator inhibitor 1 are increased after attacks of coronary spasm and show a circadian variation in parallel with the attacks of CSA.[19–21] Platelets are also shown to be activated after attacks of coronary spasm but not after those of stable effort angina.[22] In addition to such indirect evidence of thrombosis, coronary thrombosis at the spasm site was indeed demonstrated by angiography and angioscopy and at postmortem examination in previous case series.[1,23,24] Furthermore, a recent OCT study by Shin et al evaluated morphological characteristics of coronary artery spasm sites in 69 CSA patients and demonstrated that thrombus was frequently detected by OCT at coronary artery spasm sites in patients with CSA (28.8% of total spasm sites); and OCT-defined erosion was common in patients with CSA (26%).[25] It is conceivable that the

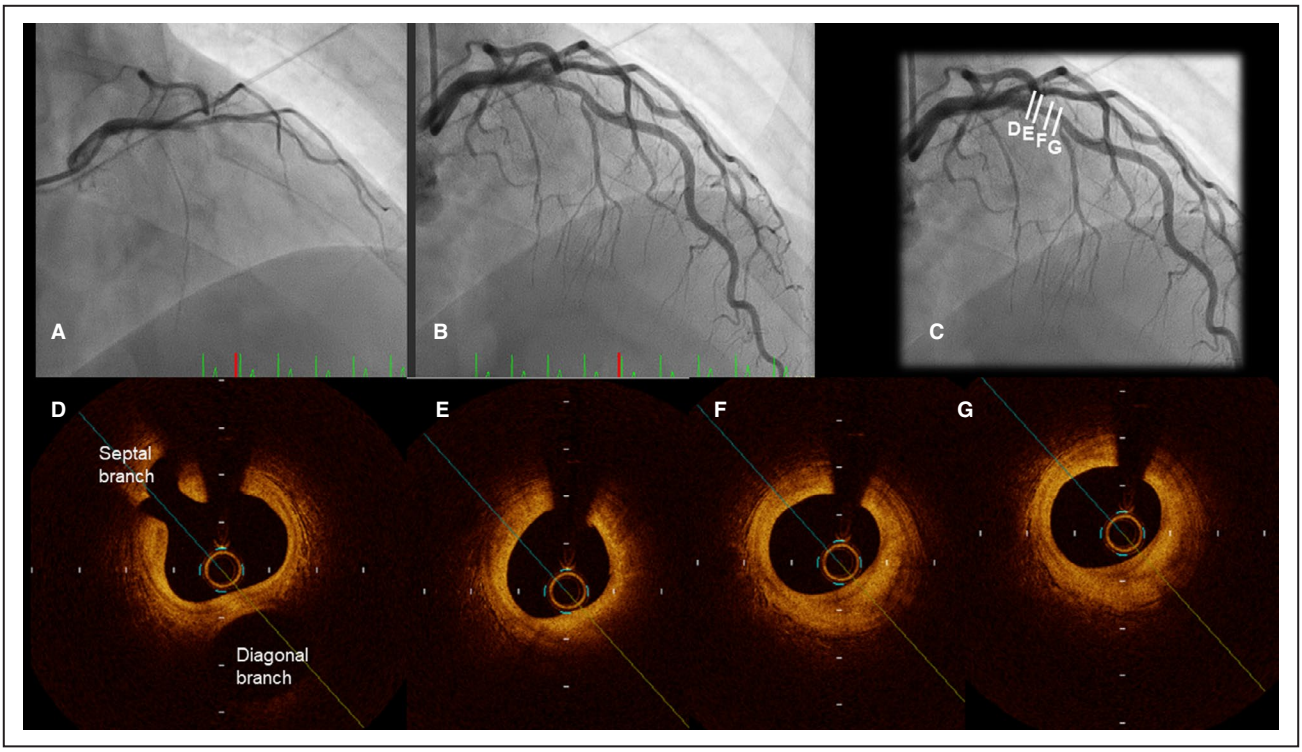


**Figure 2. An example case who presented with intermittent resting angina and transient ST-segment elevation on ECG.** **A**, The mid left anterior descending artery (LAD) occluded spontaneously during coronary angiography (CAG). **B**, CAG after intracoronary nitrate injection shows no significant stenosis in the LAD. **C**, The white lines on the angiogram indicate the sites where the optical coherence tomography (OCT) images of (**D**) through (**G**) were acquired. **D** through **G**, OCT showed macrophage accumulation (**E**, at 1 to 3 o'clock), and layered plaque (**F** and **G**, at 3 to 7 o'clock).

layered plaques observed by OCT in the present study would be later-stage findings of coronary thrombosis caused by coronary artery spasm. Interestingly, in the present study, layered plaques were observed not only in the organic lesions at the spasm site but also in those located proximal to the spasm site (ie, in the distal spasm group). An animal study using electron microscopy demonstrated that a 40%–60% reduction in luminal diameter of the coronary arteries resulted in endothelial denudation, platelet deposition, and microthrombi formation in areas proximal to the point of maximal constriction.[26] In the previous OCT study, among 21 patients presenting with CSA and OCT-defined erosion, thrombus was found proximal to spasm sites in 4 patients (19%) although at spasm sites in 17 (81%).[25] Distal flow disturbance due to vasospasm might promote endothelial dysfunction, plaque disruption, thrombi formation, and subsequent layered plaque in the proximal aspect of spasm sites as well as at spasm sites.

Previous intracoronary imaging studies indicated that coronary plaque at the site of spasm represented early stages of atherosclerosis. A previous study showed that CSA patients had diffusely thickened fibrous-dominant coronary plaque compared with non-CSA patients, and plaque components assessed by virtual histology IVUS were similar between patients with CSA and those without.[27] Another study employed OCT and showed that non-stenotic coronary segments affected by CSA had homogeneous and diffuse thickening of the intimal layer without lipid and calcium accumulation in both intimal and medial layers of the arterial wall.[28] These imaging studies assessed CSA patients essentially with no obstructive CAD with minimal atherosclerosis and therefore did not unveil plaque features of more advanced organic lesions associated with vasospasm. An animal experiment showed that repeated coronary spasm causes intraplaque hemorrhage, luminal occlusion, and endothelial damage without plaque rupture in pigs with diet-induced atherosclerosis.[29] Another animal study showed that in coronary lesions of myocardial infarction–prone strain of heritable hyperlipidemic rabbits with provoked coronary spasm, intimal injury was observed in 60.9% in the form of endothelial cell protrusions (39.1%), denudation (30.4%), and macrophage extravasation (56.5%) were also observed; plaque disruption with luminal thrombus was seen in 8.7%.[30] A recent clinical study assessed the left anterior descending by OCT and functional testing in patients

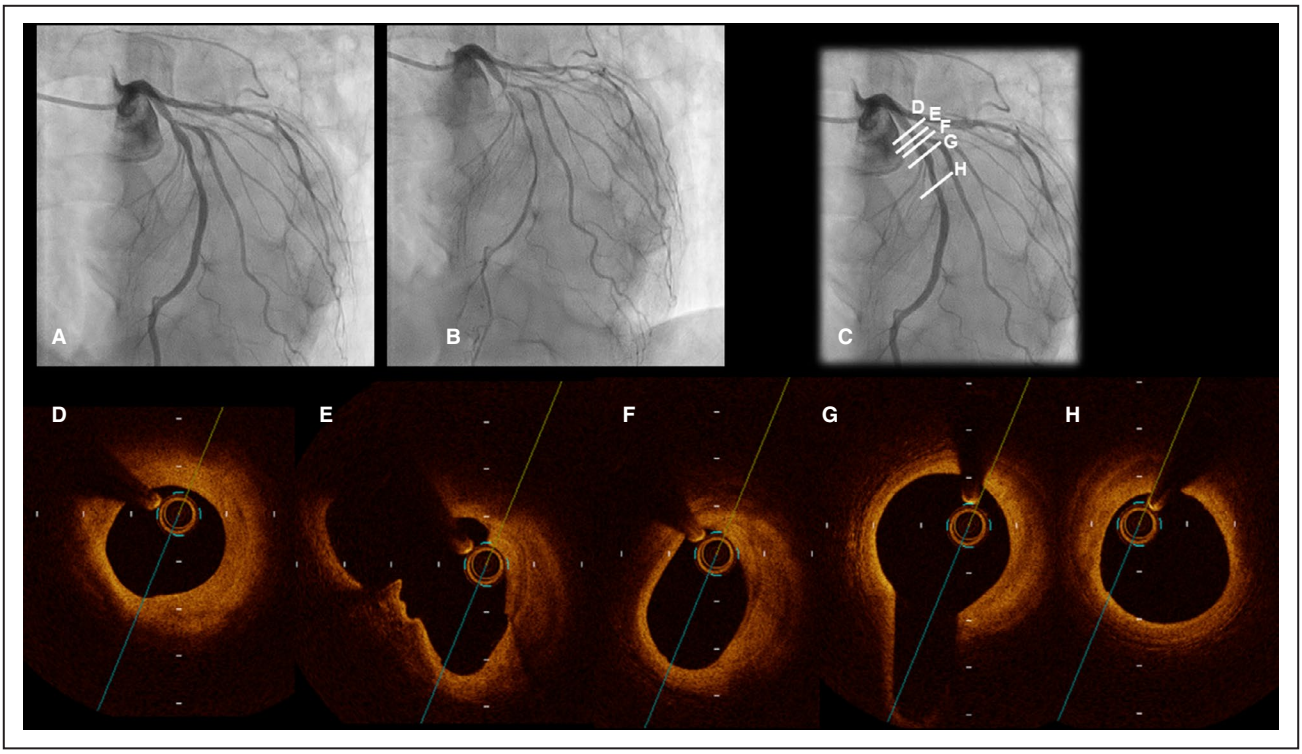


**Figure 3. An example case who had episodes of resting chest pain with transient ST-segment elevation and inverted T waves on ECG.**

**A**, Coronary angiography shows mild lesions in the proximal left anterior descending coronary artery (LAD). **B**, Intracoronary acetylcholine injection provoked significant diffuse spasm in the proximal to distal LAD. **C**, The white lines on the angiogram indicate the sites where the optical coherence tomography (OCT) images of **D** through **H** were acquired. **D** through **H**, OCT identified layered plaques.

with ischemia and no obstructive CAD and found that density of adventitial vasa vasorum was higher in patients with vasospasm than controls and that patients with focal spasm showed a higher prevalence of advanced atherosclerotic features such as fibroatheroma, macrophages, intraplaque microchannels, and cholesterol crystals.[31] Our findings that macrophages and intraplaque microchannels were frequently observed in vessels with coronary artery spasm are in line with the previous studies. In addition, previous studies have reported that layered plaques are frequently accompanied by macrophages and intraplaque microchannels, as observed in the present study, and associated with rapid plaque progression and higher adverse cardiac events.[7,14,18] Macrophage accumulation and intraplaque microchannels reflect active and ongoing inflammation. There is known to be the interplay between inflammation and thrombosis; thrombosis enhances inflammation, in turn, inflammation can trigger thrombosis.[32] From the present study, coronary spasm is likely relevant to the interplay in coronary atherosclerotic lesion. Taken together, our findings, along with the previous ones, indicate the pathophysiological link between coronary spasm, layered plaque, and active inflammatory state observed as macrophage accumulation and microchannel proliferation, synergetically enhancing rapid plaque progression and increasing the risk for future adverse cardiac events.

The present study also showed that calcification was more frequently observed in vessels without inducible coronary artery spasm than those with spasm. The amount of coronary calcium is considered as a surrogate for total atherosclerotic burden. However, a previous study suggested that dense sheets of calcification as observed by histopathology may define a more stable type of plaque and donate less risk for rupture or erosion.[33] In addition, other studies have shown that statin therapy and exercise enhance the progression of coronary artery calcification, which can be considered stabilization of plaque.[34–36] Our findings support and extend such notion, suggesting that calcification may serve as a protective factor against coronary artery spasm and subsequent plaque erosion related to spasm. In the present study, the distal spasm subgroup had numerically, albeit not statistically significantly, higher prevalence of macrocalcification compared with the organic-lesion site spasm group. We speculate that a coronary lesion that had been previously susceptible to vasospasm became not vasospastic with a progression of calcification of the lesion

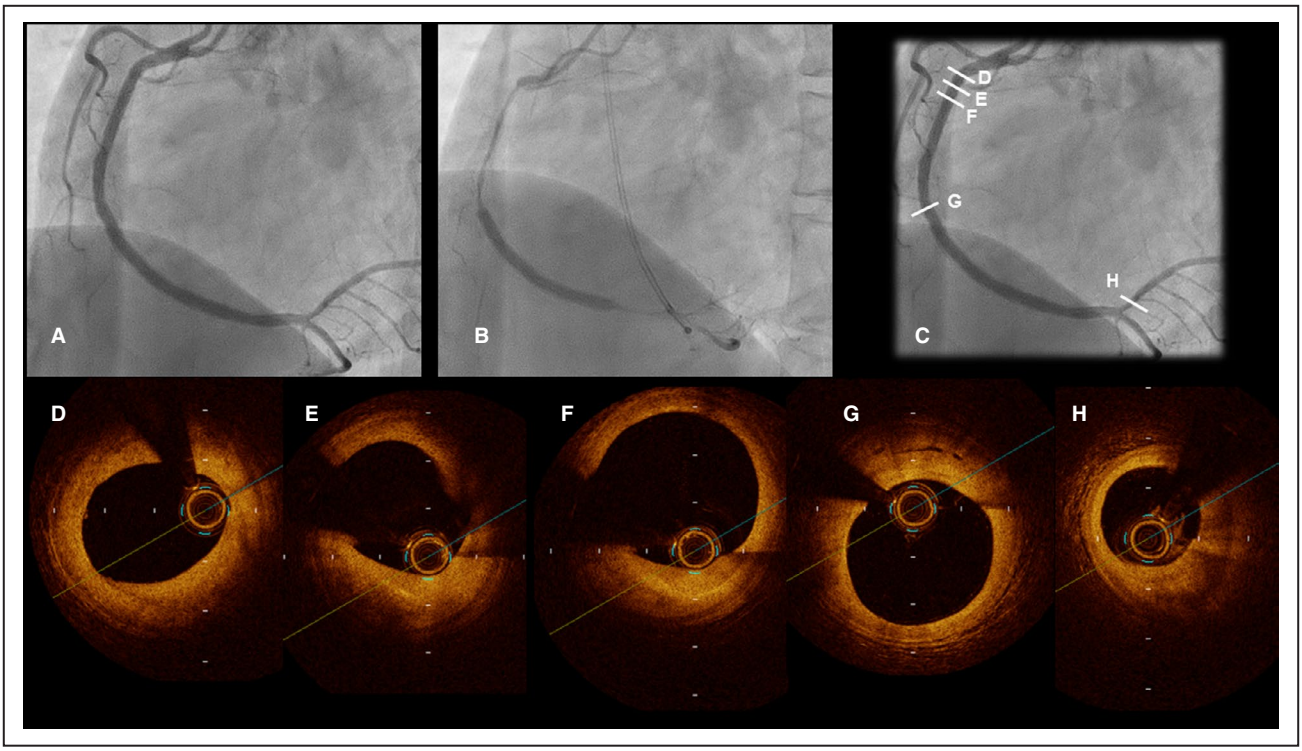


**Figure 4. An example case with a dug-eluting stent implanted in the mid right coronary artery (RCA), presenting with resent episodes of chest pain with minimal exertion.**
**A**, Coronary angiography shows no significant lesions in the RCA. **B**, Intracoronary acetylcholine injection provoked significant diffuse spasm throughout the RCA except previously stented segment. **C**, The white lines on the angiogram indicate the sites where the optical coherence tomography (OCT) images of (**D**) through (**H**) were acquired. **D** through **H**, OCT identified layered plaques proximal (**D** through **H**) and distal (**H**) to the stent and intraplaque microchannels (**G**, observed at 11 to 1 o'clock).

over time in some patients of the distal spasm group. This hypothesis needs to be investigated in the larger population.

## Clinical Implications

Conventionally, the assessment of plaque vulnerability has been predominantly focused on plaque morphology, namely, detection of a lipid-rich atherosclerotic plaque with the thin fibrous-cap, however, from our study and previous evidence, the assessment of coronary spasm should be integrated into the workup of identifying vulnerable plaques and vulnerable patients at high risk of future coronary events. Layered plaques accompanied with macrophages and intraplaque microchannels can be a clue to suspect the involvement of coronary artery spasm in patients with coronary artery disease. In view of the pathogenesis of plaque formation, combined medical therapy including calcium channel blocker, statin, and antiplatelet drugs would be beneficial for patients with coronary spasm and significant plaque with layered pattern, macrophage, and intraplaque microchannels to prevent plaque progression and improve their clinical outcomes.

**Table 4. OCT-Defined Layered Plaque, Microchannels, Macrophages and Macrocalcification in Vessels With Spasm and Without Spasm: A Subgroup Analysis Based on the Site of Spasm**

| | Distal spasm (n=9) | Organic-lesion site spasm (n=21) | Non-spasm (n=21) | *P* value: distal spasm vs non-spasm | *P* value: site spasm vs non-spasm | *P* value: distal spasm vs site spasm |
|---|---|---|---|---|---|---|
| Layered plaque | 9 (100%) | 19 (91%) | 8 (38%) | 0.003* | 0.001 | 0.57* |
| Microchannels | 8 (89%) | 15 (71%) | 5 (24%) | 0.049 | 0.005 | 0.33 |
| Macrophages | 7 (78%) | 17 (81%) | 9 (43%) | 0.10 | 0.022 | 0.84 |
| Macrocalcification | 4 (44%) | 3 (14%) | 13 (62%) | 0.16 | 0.004 | 0.15 |

The distal spasm subgroup includes vessels where spasm occurred distal to organic lesion, while the organic-lesion site spasm subgroup includes vessels where spasm occurred at the site of organic lesion. *P* values were calculated by generalized estimating equations when applicable; otherwise calculated by Fisher's exact test for categorical variables (*). OCT indicates optical coherence tomography.

## Limitations

First, this study was a single-center, cross-sectional study with a relatively small sample size. We could not conclude the causal relationship between coronary spasm and layered plaques with macrophage and intraplaque microchannels. Second, we included only Japanese patients. Previous studies suggested that the Asian populations has a higher prevalence of angina related to coronary spasm compared with the European populations although it is not conclusive because spasm provocation protocol and diagnostic definition varies between these studies.[37–39] Nonetheless, there may be a racial difference in the pathophysiology of coronary spasm, which needs to be further investigated. Third, we did not perform OCT and ACh provocation tests in all the coronary arteries of the included patients, which may cause selection bias. Fourth, we could not differentiate recanalized channels in thrombus from proliferated intraplaque neo blood vessels by OCT, both of which can be observed as intraplaque microchannels. Fifth, the present study does not include clinical and imaging follow-up to investigate the natural history of plaques in patients with CSA. Follow-up studies will provide definitive answers about the long-term prognosis of CSA patients with layered plaques.

# CONCLUSIONS

Layered plaque, macrophages, and intraplaque microchannels were frequently observed in organic lesions in patients with coronary artery spasm. These findings suggest that coronary artery spasm induces local thrombus formation as well as active inflammatory response, therefore increasing the risk of rapid plaque progression and ischemic events in patients with coronary artery spasm.

## ARTICLE INFORMATION

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### Disclosures

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### Supplemental Material

Data S1
References 40–43

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# SUPPLEMENTAL MATERIAL

## DATA S1. SUPPLEMENTARY METHODS

### Diagnosis of coronary artery spasm

Intracoronary ACh provocation tests were performed according to the Japanese Circulation Society guidelines for the diagnosis and treatment of patients with CSA[8]. In brief, after insertion of a temporary pacing electrode in the right ventricle via basilic, cephalic, or internal jugular vein, ACh was injected in incremental doses of 50, and 100 μg into the left coronary artery (LCA), and 20 and 50 μg into the right coronary artery (RCA) over a period of 20 s. After the ACh provocation test, 1 mg of isosorbide dinitrate was administered into the RCA and LCA. Coronary angiography was performed before and 1 min after each ACh injection, after isosorbide dinitrate injection, and when chest pain or ischemic ECG changes were observed. All vasodilators, such as calcium channel blocker, long-acting nitrate, and nicorandil were discontinued at least 48 h before the examination in the setting of non-emergency ACh provocation.

Positive coronary artery spasm was defined as transient luminal narrowing (99 % focal spasm or 90 % severe diffuse vasoconstriction associated with usual chest pain/symptom or ischemic ECG findings induced by the ACh provocation test or due to spontaneous coronary artery spasm[8,9]. Ischemic ECG changes include transient ST elevation ≥0.1 mV, ST depression ≥0.1 mV, or new appearance of negative U waves, recorded in at least two contiguous leads on the 12-lead ECG. Focal spasm was defined as ACh-induced or spontaneous coronary artery spasm <20 mm in length in the major epicardial arteries assessed by angiography, while diffuse spasm was ≥20 mm.

### Quantitative coronary angiography analysis

Quantitative coronary angiography (QCA) analysis was performed using standard commercial software (CAAS QCA, Pie Medical Imaging BV, Maastricht, the Netherlands). The QCA software provided automatic contour detection and automated identification of the sites of maximal luminal obstruction and the start and end of the stenosis. The following QCA parameters were obtained in the target lesion: minimum luminal diameter (MLD), reference diameter at the MLD site, proximal and distal reference diameter, percentage diameter stenosis, and lesion length.

### OCT Image Acquisition and Analysis

After the ACh provocation test, we performed an OCT imaging using either an OPTIS (Abbott, Santa Clara, CA, USA) or a LUNAWAVE (Terumo Corporation, Tokyo, Japan) system. After the injection of nitrates and confirming the relief of the vasospasm and vasodilation, the OCT imaging catheter (Dragonfly OPTIS / OpSter, Abbott; or FastView, Terumo Corporation) was advanced distally in the target vessel. OCT images were acquired during continuous injection of contrast medium at a pullback rate of 36 mm/s (180 frames/s) or 40 mm/s (160 frames/s) in the OPTIS and LUNAWAVE systems, respectively.

Quantitative analysis was performed using a validated OCT analysis software (echoPlaque, Indec Systems, Santa Clara, CA, USA). A significant organic lesion was defined as a stenotic lesion due to plaque accumulation where a 50% or greater plaque burden exists. External elastic membrane (EEM) and lumen area were manually traced at the leading edge of boundaries at 1-mm intervals from proximal to distal 5-mm reference segments throughout the organic lesion segment with automated interpolated measurements of the remaining frames. Plaque (intima + media) areas were defined as EEM area minus lumen area. When the EEM was not visible >90 degrees or grater at a certain frame, manual tracing of the EEM was not performed at the frame, but the automated interpolation based on the EEM boundaries in the neighboring frames was applied. Maximum plaque burden and minimum lumen area within the organic lesions were evaluated.

Qualitative OCT analyses were analyzed at significant coronary artery lesions, including stenotic lesion and reference segments by two experienced investigators blinded to clinical and angiographic information. A layered plaque was defined as a region having one or more layers with different optical densities from underlying components and a clear border[10-14]. The other qualitative OCT analyses were based on previously established criteria[40-43]. Lipid was defined as a signal-poor region with a poorly defined or diffuse border. Calcification was defined as a signal-poor or heterogeneous region with a sharply delineated border. The calcification arc and the lipid arc degrees were measured. Macrocalcification was defined as maximal calcium arc >90 degrees[43]. Lipid-rich plaques were defined as having lipid arcs >90 degrees, and thin-cap fibroatheroma (TCFA) were defined as lipid-rich plaques with a fibrous cap thickness <65 μm. Plaque cavity was defined as an intimal discontinuity with an empty space within a plaque. Macrophage images were defined as signal-rich, distinct or confluent punctuate regions with shadowing. A fresh thrombus

was defined as a mass attached to the luminal surface or floating within the lumen. Microchannels were defined as no-signal tubuloluminal structures without a connection to the vessel lumen recognized on 3 consecutive cross-sectional OCT images.