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Differences in intravascular ultrasound and histological findings in culprit coronary plaques between ST-segment elevation myocardial infarction and stable angina

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Abstract A comprehensive evaluation of culprit coronary lesions may help to understand vulnerable plaques responsible for ST-segment elevation myocardial infarction (STEMI). We compared intravascular ultrasound (IVUS) and histological findings in culprit coronary plaques from 94 patients with STEMI ($n = 54$) or stable angina ($n = 40$). Tissue specimens were obtained by directional coronary atherectomy and IVUS was performed before percutaneous coronary intervention. IVUS and histological data were analyzed. Clinical characteristics were largely similar between the two groups. Plaque rupture and thrombi were more frequently found in the STEMI group than in the stable angina group. There were no significant differences between plaque types or proximal and distal reference measurements in the two groups. However, the site of minimal lumen area had a greater vessel area, remodeling index, and plaque burden with lesser lumen area in the STEMI group than in the stable angina group. Plaque areas immunopositive for CD68 and CD31 were significantly larger in the STEMI group, while the area immunopositive for α-smooth muscle actin was larger in the stable angina group. In conclusion, culprit lesions in STEMI patients showed a greater plaque burden, remodeling index, and more frequent thrombi with increased inflammation and neovascularization compared to the stable angina group, supporting the current concept of vulnerable plaques being responsible for STEMI.

Keywords Histology · Intravascular ultrasound · Myocardial infarction

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Plaque rupture with thrombotic vessel occlusion is the major cause of ST-segment elevation myocardial infarction (STEMI). Lipid-rich plaques with a thin fibrous cap, infiltrating inflammatory cells, and neovascularization are considered vulnerable and at high risk of rupture [1–6]. Intravascular ultrasound (IVUS) studies [7, 8] have shown that plaque rupture with thrombosis is more common in patients with acute coronary syndrome than in patients with stable angina.

The concept of vulnerable plaques is primarily derived from autopsy studies but is subject to limitations because of incomplete ex vivo histology. A comprehensive in vivo evaluation of culprit lesions in STEMI patients may help understand the complex phenomenon of plaque instability. In the present study, we compared IVUS and histological findings in culprit coronary plaques in patients with STEMI or stable angina.

Methods

Study patients

Coronary atherosclerotic plaques were obtained from a local biobank that collected atherectomy-derived tissues from 94 consecutive patients with either STEMI ($n = 54$) or stable angina ($n = 40$). Patient demographics, clinical characteristics, and the procedures applied to each patient were prospectively recorded. Patients were considered suitable for directional coronary atherectomy if they had a significant stenotic lesion with a large plaque burden but lacked heavy thrombi in a non-tortuous epicardial coronary artery >3 mm in diameter [9, 10]. Each sample corresponded to a de novo lesion from a single patient. Directional coronary atherectomy was performed with a FlexiCut catheter (Abbott Laboratories/Guidant Vascular Interventions, Santa Clara, CA, USA) under IVUS guidance. The study protocol was approved by the local Institutional Review Committee and all patients provided written informed consent.

Intravascular ultrasound

IVUS examinations were performed before percutaneous coronary intervention and after intracoronary administration of 0.2 mg nitroglycerin using a motorized transducer pullback system (0.5 mm/s) and a commercial scanner (SCIMED/Boston Scientific, Natick, MA) consisting of a rotating 40 MHz transducer. IVUS measurements were taken by a colleague who was blind to the clinical data. A ruptured plaque was defined as one containing a cavity that communicated with the lumen and that showed an overlying residual fibrous cap fragment. A thrombus was defined as an intraluminal mass with a layered or lobulated appearance, showing evidence of blood flow within the mass, and demonstrating speckling or scintillation. Using computerized planimetry, the external elastic membrane (EEM) and lumen cross-sectional area (CSA) (mm^2) were measured in culprit and reference segments. A reference segment was defined as the most normal-appearing cross-section within a 5 mm region proximal and distal to the lesion but before any side branch. The remodeling index was calculated as the lesion EEM CSA divided by the mean reference EEM CSA, and the plaque burden was calculated as plaque + media CSA divided by EEM CSA.

Tissue preparation

Tissue specimens were formalin-fixed and embedded in donor paraffin blocks. Tissue microarrays were produced by re-embedding tissues from the pre-existing donor paraffin blocks into an array on a recipient paraffin block. Sections from the master block were cut with a microtome, mounted on microscope slides, and used for subsequent staining procedures.

Histological analysis

Standard hematoxylin and eosin staining was performed to determine cellularity and general morphologic features. The area of each plaque was measured with a microscopic image analysis system (Motic Images Advanced 3.2, Motic, Xiamen, China). Plaques were classified as atheromatous (i.e., with necrotic cores and cholesterol clefts, but without connective tissue matrix) or fibrocellular, and were graded as paucicellular (<30 spindle cells per high-power field), moderately cellular (30–100 spindle cells), or hypercellular (≥100 spindle cells). Sections of each tissue specimen were stained with monoclonal antibodies (mAbs) against α-smooth muscle actin (1:200, mouse anti-human macrophage antibody clone 1A4; DAKO, Carpinteria, CA, USA), CD31 (1:200, mouse anti-human endothelial cell antibody clone WM59; BD Biosciences, Franklin Lakes, NJ, USA), and CD68 (1:200, mouse anti-human macrophage antibody clone KP-1; DAKO). Staining was performed with the Envision-Plus Immunostaining Kit and 3,3-diaminobenzidine or 3-amino-9-ethylcarbazole as the chromogen, according to the manufacturer's instructions (DAKO). Briefly, samples were incubated with primary antibodies (diluted in antibody diluent, DAKO) for 1 h, washed twice (5 min each) with tris-buffered saline/Tween-20, incubated with secondary antibodies conjugated with horseradish peroxidase (HRP)-labeled polymer (DAKO) for 1 h, and washed again. As negative controls, adjacent sections were stained with species- and isotype-matched irrelevant antibodies, including normal rabbit IgG (Abcam). The immunopositive area was calculated as the ratio of the area of positively stained regions to the total plaque area.

Statistical analysis

Continuous variables are expressed as the mean ± standard deviation or medians with interquartile ranges. Categorical variables are expressed as frequencies. Continuous variables were compared using the Student's t test or the Mann–Whitney U-test, and categorical variables were analyzed using the Chi squared test. Statistical significance was defined as a two-sided p value < 0.05.

Table 1 Clinical characteristics

Characteristics	STEMI ($n = 54$)	Stable angina ($n = 40$)	p value
Age (years)	56.7 ± 10.3	60.5 ± 8.7	0.061
Male/female	45 (83.3 %)	30 (75 %)	0.437
Current smoker	26 (48.1 %)	13 (32.5 %)	0.144
Diabetes mellitus	10 (18.5 %)	10 (25.0 %)	0.458
Hypertension	27 (50.0 %)	20 (50.0 %)	1.000
Total cholesterol (mg/dl)	197.9 ± 53.9	144.2 ± 32.4	<0.001
Triglyceride (mg/dl)	208.6 ± 143.0	107.4 ± 44.3	<0.001
HDL cholesterol (mg/dl)	35.5 ± 8.3	42.1 ± 12.5	0.008
Hs-CRP (mg/dl)	2.5 ± 3.3	1.4 ± 1.4	0.164
Multivessel coronary disease	22 (40.7 %)	13 (32.5 %)	0.518
Culprit coronary artery			0.142
Left anterior descending	33 (61.1 %)	22 (55.0 %)	
Left circumflex	6 (11.1 %)	1 (2.5 %)	
Right	15 (27.8 %)	17 (42.5 %)	
TIMI flow grade 3 at baseline[a]	8 (14.8 %)	40 (100 %)	<0.001
Medications at the time of DCA			
Aspirin	54 (100 %)	40 (100 %)	1.000
Clopidogrel	54 (100 %)	40 (100 %)	1.000
ACEI/ARB	3 (5.6 %)	5 (12.5 %)	0.279
β-blockers	3 (5.6 %)	17 (42.5 %)	<0.001
Calcium antagonists	7 (13.0 %)	29 (72.5 %)	<0.001
Statins	12 (22.2 %)	29 (72.5 %)	<0.001

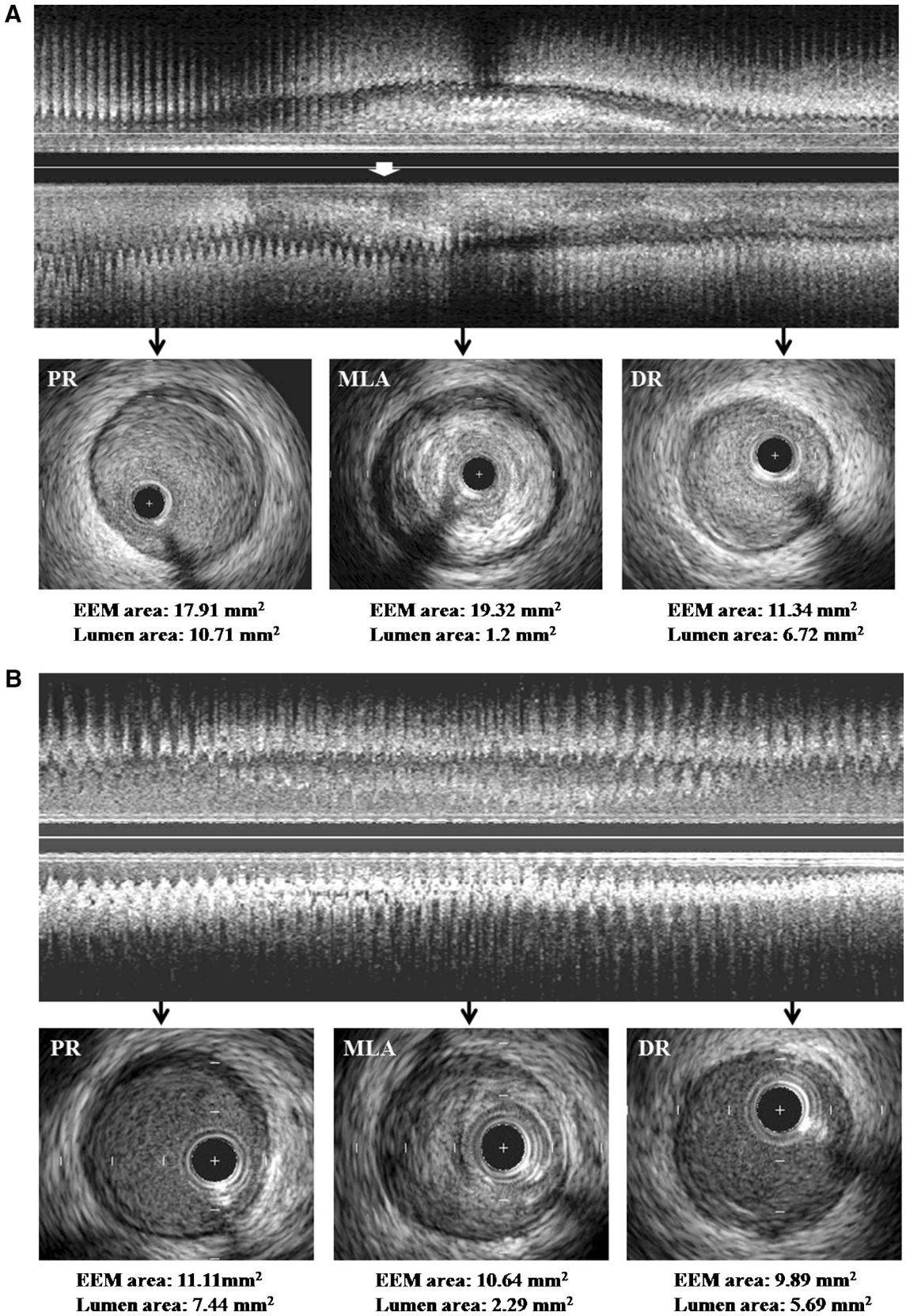
hs-CRP high-sensitivity C-reactive protein, *ACEI* angiotensin-converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *DCA* directional coronary atherectomy, *TIMI* thrombolysis in myocardial infarction

[a] Antegrade TIMI flow grade 3 through the coronary culprit lesion before coronary angioplasty

Table 2 Intravascular ultrasound findings

Variable	STEMI ($n = 54$)	Stable angina ($n = 40$)	p value
Lesion length (mm)	25.2 ± 11.8	23.6 ± 13.3	0.548
Plaque morphology			0.774
Hyperechoic	14 (25.9 %)	12 (30.0 %)	
Mixed	11 (20.4 %)	6 (15.0 %)	
Hypoechoic	29 (53.7 %)	22 (55.0 %)	
Attenuated plaque	28 (51.9 %)	24 (60.0 %)	0.530
Calcium, deep	27 (50 %)	17 (42.5 %)	0.534
Calcium, superficial	34 (63.0 %)	28 (70.0 %)	0.516
Thrombus (%)	48 (88.9 %)	5 (12.5 %)	<0.001
Plaque rupture	45 (83.3 %)	10 (25 %)	<0.001
Proximal reference			
EEM CSA (mm^2)	17.5 ± 5.1	16.4 ± 3.9	0.285
Lumen CSA (mm^2)	9.8 ± 3.0	9.6 ± 3.2	0.771
Distal reference segment			
EEM CSA (mm^2)	10.4 ± 3.7	11.6 ± 5.1	0.184
Lumen CSA (mm^2)	6.1 ± 2.3	7.2 ± 3.2	0.088
Minimal lumen site			
EEM CSA (mm^2)	14.4 ± 4.3	12.7 ± 3.6	0.047
Lumen CSA (mm^2)	1.1 ± 0.4	1.5 ± 0.6	<0.001
Plaque burden (%)	91.4 ± 3.7	87.1 ± 6.1	<0.001
Remodeling index	1.1 ± 0.3	1.0 ± 0.2	0.005

EEM external elastic membrane, *CSA* cross-sectional area


A
PR
MLA
DR
EEM area: 17.91 mm²
Lumen area: 10.71 mm²
EEM area: 19.32 mm²
Lumen area: 1.2 mm²
EEM area: 11.34 mm²
Lumen area: 6.72 mm²
B
PR
MLA
DR
EEM area: 11.11mm²
Lumen area: 7.44 mm²
EEM area: 10.64 mm²
Lumen area: 2.29 mm²
EEM area: 9.89 mm²
Lumen area: 5.69 mm²

◀**Fig. 1** Intravascular ultrasound images (**a**, **b**) and corresponding atherectomy histology (**c**–**h**) in patients with STEMI (**a**, **c**, **d**, **e**) or stable angina (**b**, **f**, **g**, **h**). The *culprit lesion* (**a**, **b**) shows a greater plaque burden and positive remodeling (MLA site, cross-sectional image) with plaque rupture and thrombus (*arrow*, *longitudinal image*) in a patient with STEMI compared to a patient with stable angina. Immunohistochemical staining reveals a larger positive area of CD68 (**c**, **f**) or CD31 (**d**, **g**), and a smaller positive area of α–smooth muscle actin (**e**, **h**) in a patient with STEMI. Magnification × 200. *DR* distal reference, *EEM* external elastic membrane, *MLA* minimal lumen area, *PR* proximal reference

Results

Clinical characteristics

Clinical characteristics were similar between the two groups, except lipid profiles and medications (Table 1). The mean age of the patients was 58.3 ± 9.8 years, 79.8 % were men, 50 % had a history of hypertension, 21.3 % had diabetes, and 41.5 % were current smokers. The median time from onset of chest pain to angioplasty was 3.5 h (range 1–12 h) for STEMI. At the time of the index procedure, β-blockers, calcium antagonists, and statins were less frequently used in patients with STEMI than in those with stable angina.

IVUS findings

IVUS data are summarized in Table 2. Thrombus and plaque rupture was more frequently observed in STEMI patients than in patients with stable angina. However, there were no significant differences in attenuated plaques, calcification, or plaque morphology between the two groups. Likewise, proximal and distal reference measurements were similar between the two groups. By contrast, the site of the minimal lumen area had a greater EEM area, remodeling index, and plaque burden with a smaller lumen area in the STEMI group than in the stable angina group. Representative IVUS images and corresponding histopathology are shown in Fig. 1.

Histological findings

Histological findings are summarized in Table 3. The total plaque area was similar between the two groups. Plaque types tended to be more cellular in the STEMI group than in the stable angina group. Thrombi were observed in 74.1 and 10.0 % of specimens from patients with STEMI and stable angina, respectively ($p < 0.001$). The relative plaque areas immunopositive for CD68 and CD31 were significantly larger in the STEMI group, whereas the relative area immunopositive for α-smooth muscle actin was larger in the stable angina group.

Discussion

In the present study, culprit lesions in patients with STEMI had a smaller lumen area, a greater plaque burden and remodeling index, and more frequent thrombi than those with stable angina. In addition, CD68-and CD31-immunopositive areas were larger in culprit plaques of STEMI patients, whereas the α-smooth muscle actin immunopositive area was larger in the plaques of stable angina patients. These findings are consistent with previous reports, supporting the concept of vulnerable plaques responsible for STEMI [1–6].

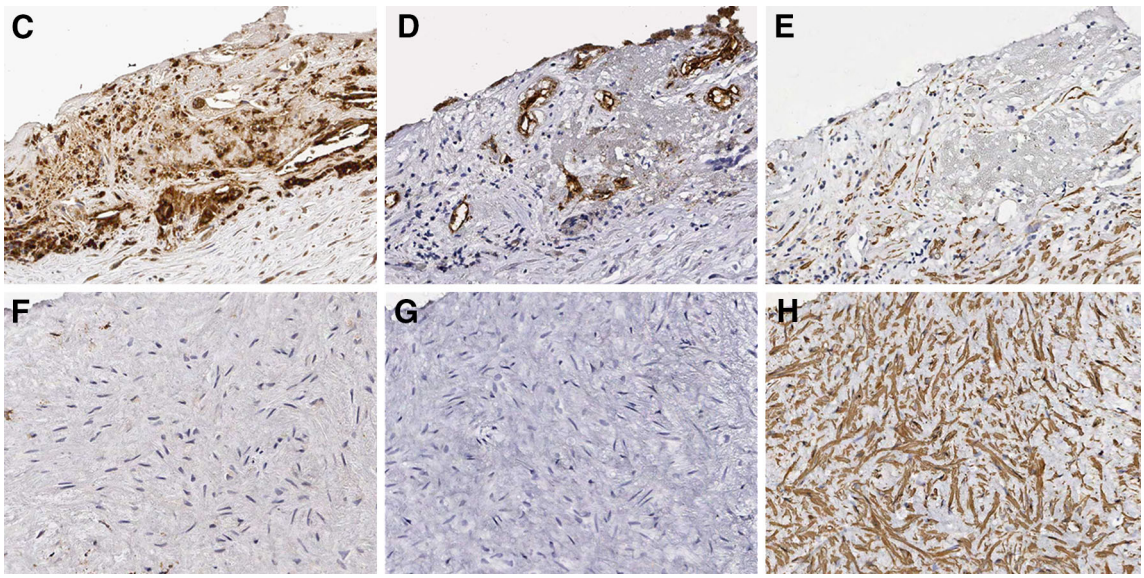


Fig. 1 continued

Table 3 Histological findings

Variable	STEMI ($n = 54$)	Stable angina ($n = 40$)	p-value
Histology			
Total plaque area (mm^2)	345.4 (241.0–504.6)	252.6 (197.4–453.6)	0.076
Atheroma	0.6 (0.1–49.4)	0.4 (0.1–21.2)	0.183
Fibrocellular area			
Paucicellular	39.2 (23.6–61.4)	62.3 (31.7–80.5)	0.023
Moderately cellular	0.3 (0–7.4)	0 (0–15.1)	0.493
Hypercellular	0 (0–1.0)	0 (0–0)	0.294
Calcium	0 (0–0.1)	0 (0–0)	0.810
Thrombus	1.7 (0–12.9)	0 (0–0)	<0.001
Immunohistochemistry			
CD68	9.2 (1.3–22.1)	1.3 (0.3–7.0)	0.002
CD31	0.9 (0.4–3.2)	0.3 (0.1–0.9)	<0.001
α-Smooth muscle actin	2.9 (1.8–5.6)	7.2 (3.1–15.5)	0.002

Data are expressed as percent-positive areas (immunostained area/total plaque area × 100), and as median values with interquartile ranges

Myocardial infarction is a major cause of death worldwide and often the first manifestation of coronary artery disease [11]. Early detection and treatment of vulnerable plaques before rupture and thrombosis is essential. Survival after myocardial infarction has improved with the progressive use of evidence-based care [12]. Pathologically, ruptured plaques are characterized by a necrotic core with an overlying thin-ruptured fibrous cap infiltrated by macrophages. In our study, the CD68 (macrophage marker)-immunostaining area in coronary culprit plaques was significantly larger in STEMI patients than instable angina patients, supporting the idea that inflammation drives the rupture of coronary atherosclerotic plaques. In addition, the CD31 (endothelial cell marker) immunostaining area was also significantly larger in culprit plaques of STEMI patients, indicating more active neovascularization in these patients. The presence of fragile neovessels would result in a lower threshold for plaque rupture and a greater likelihood of intraplaque hemorrhage leading to acute myocardial infarction [5, 6]. A thin fibrous cap is another hallmark of vulnerable plaques. In an autopsy study, the thickness of the cap near the rupture site measured 23 ± 19 μm, with 95 % of the caps measuring <65 μm [13]. In our study, atherosclerotic plaques were obtained from culprit lesions using directional coronary atherectomy; therefore, it was not possible to measure the fibrous cap thickness. Nevertheless, the relative area for vascular smooth muscle cells was smaller in culprit plaques of STEMI patients, which is compatible with autopsy findings.

IVUS has been used to investigate plaque characteristics, providing valuable information about vulnerable plaques. In our study, the culprit lesions in STEMI patients showed more positive remodeling, larger EEM, and plaque burden than the culprit lesions in stable angina patients. There have been several reports on arterial remodeling in relation to plaque instability [14–16]. Positive remodeling may minimize luminal encroachment despite a large plaque size. However, larger lumens create greater circumferential stress on the fibrous caps, thereby increasing their likelihood of plaque rupture [17]. Plaque rupture is the main causal mechanism of STEMI, but not all cases of plaque rupture cause STEMI. As shown in our study, some patients with plaque rupture present with stable angina, supporting the idea that both plaque rupture and the contents of the ruptured plaque play important roles in initiating the onset of STEMI [2]. A high content of tissue factor is more likely to cause occlusive thrombus formation after plaque rupture [18, 19], leading to STEMI. Plaque erosion also causes thrombus formation, but there are no ideal imaging tools to visualize it in STEMI patients. Finally, plaque types including attenuation, calcification and morphology may be related to plaque instability. In our study, however, no significant differences were observed between the two groups in terms of plaque types.

Potential limitations need to be addressed. First, the relatively low resolution of IVUS precludes a detailed assessment of the ruptured plaque, including fibrous cap thickness and plaque composition. Second, atherectomy tissues were extracted from selected lesions in large vessels because calcified, tortuous, or small vessels are not suitable for directional coronary atherectomy. Thus, it may not be possible to generalize our findings to lesions at sites other than large vessels. Nevertheless, our results support the concept that vulnerable plaques are characterized by a large lipid core and a thin fibrous cap with infiltrating inflammatory cells and neovascularization.

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Conflict of interest None.

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