Atherosclerotic Plaque Component as a Risk Factor for Distal Embolization During Percutaneous Coronary Intervention
— Pathology of Tissue Obtained by Distal Protection Device —

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**Background:** Embolism during percutaneous coronary intervention (PCI) causes microcirculation impairment. The aim of this study was to clarify the relationship between the pathological characteristics of tissue captured by distal protection device (DPD) and amount of tissue accumulated in DPD.

**Methods and Results:** A total of 671 consecutive lesions in PCI using DPD were examined. The amount of necrotic debris, fibrous tissue, calcified particle, platelet thrombus and organized thrombus in the DPD baskets was histologically evaluated. The DPD tissue amount was assessed semi-quantitatively, and the relationship between the captured DPD tissue characteristics and tissue amount was investigated. On pathology, 40.7% of the lesions had necrotic debris, 41.4% had fibrous tissue, and 18.0% had calcified particle. The prevalence of lesions in patients with acute coronary syndrome (ACS) was 62.1%. Tissue amount score distribution was as follows: score 1 (tissue invisible), 3.9%; score 2 (tissue clinging to the basket), 52.0%; score 3 (tissue accumulated at the bottom of the basket), 38.5%; and score 4 (tissue accumulated in more than half of the basket), 5.7%. On multivariate analysis, necrotic debris and fibrous tissue were associated with greater tissue amount as well as clinical presentation of ACS.

**Conclusions:** The presence of atherosclerotic plaque component, such as necrotic debris and fibrous tissue, might be a risk for distal embolism during PCI.

**Key Words:** Coronary artery disease; Distal embolization; Distal protection; Percutaneous coronary intervention

Distal embolism during percutaneous coronary intervention (PCI) for coronary artery disease (CAD) is thought to be a cause of microcirculation impairment of the myocardium, and it may influence the clinical outcomes of PCI. Filter-type distal protection devices (DPD) were developed to prevent distal embolism, but the effectiveness of these devices is still controversial. Although several trials failed to prove the benefits of DPD in patients with acute myocardial infarction (AMI), the devices have been shown to be clinically useful in some studies: Baim et al noted the utility of the DPD in PCI for saphenous vein graft, and Mizote et al indicated that the DPD might reduce microcirculation damage and left ventricular dysfunction in patients with AMI. Distal protection has also been shown to improve Blush score at 30 days after primary PCI or long-term outcome when DPD are used during PCI after AMI. Necrotic debris has frequently been identified in the tissue collected in DPD in patients with low-attenuation plaque on coronary computed tomography angiography or large plaque burden on intravascular ultrasound (IVUS), which was associated with distal embolization. Recently Kitagawa et al showed that the volume of the lipid-rich component identified on integrated backscatter-IVUS was significantly associated with a larger amount of debris captured by DPD.

Atherosclerotic plaque components, namely necrotic debris, cause the formation of a coagulation cascade, leading to platelet activation, aggregation and subsequent thrombus formation. Atherosclerotic plaque in the coronary artery has histological heterogeneity and the plaque contains various kinds of tissue components, such as necrotic debris, fibrous tissue, calcified particle and so on.
Plaque Component: Risk Factor for Distal Embolization

Figure 1. Semi-quantitative filter device tissue evaluation. Score 1, almost no visible tissue; score 2, visible tissue clinging to the filter mesh; score 3, apparent collection of tissue at the bottom of the filter (up to half of the basket); score 4, massive tissue protruding from the filter basket (more than half of the basket).

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>26 / 671</th>
<th>349 / 671</th>
<th>258 / 671</th>
<th>38 / 671</th>
</tr>
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<tbody>
<tr>
<td>(%)</td>
<td>(3.9%)</td>
<td>(52.0%)</td>
<td>(38.5%)</td>
<td>(5.7%)</td>
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Figure 2. Histopathological components of tissue captured by distal protection device. (A) Necrotic debris with cholesterol crystals (Masson’s trichrome). (B) Necrotic debris with foam cells (hematoxylin-eosin). (C) Fragments of blue-stained fibrous tissue (Masson’s trichrome). (D) Elastic fibers of fibrous tissue (elastica van Gieson). (E) Tiny calcified particles with crystallization (hematoxylin-eosin). (F) Calcified particles positive for von Kossa. (G) Organized thrombus, with homogeneous eosinophilic material with cell lysis and/or proliferation of endothelial cells in the matrix (hematoxylin-eosin). (H) Platelet thrombus with blue-stained granules (Masson’s trichrome). Scale bars: (A,B,E-H) 50μm; (C,D) 100μm.
Necrotic debris is the main component of atherosclerotic core, and it often contains degenerated cell debris, cholesterol crystals and foam cell. Fibrous tissue is one of the important tissue elements in the vessel wall and it is distributed abundantly in the plaque, including in the fibrous cap. Calcified particles are frequently observed in the advanced atherosclerotic plaques of the coronary artery, particularly in the necrotic core and/or edge of the core; they are generally tiny and may scatter during PCI. These various plaque components may have differing thrombogenic activity, but the question of how these components contribute to the thrombogenic effect during PCI remains unanswered. The aim of this study was therefore to clarify the relationship between the clinical status, pathological characteristics of the tissue captured by DPD, and the amount of tissue accumulated in DPD.

**Methods**

**Subjects**
This was a single-center retrospective observational study. We examined 671 consecutive patients who underwent PCI using a filter-type DPD (Filtrap™, Nipro, Osaka, Japan) at Osaka Police Hospital from January 2010 to August 2015. Patients who underwent PCI to the bypass graft were excluded. This study complied with the Declaration of Helsinki, regarding investigation in humans. There was no industry involvement in the design, conduct, financial support, or analysis of the study.

**Coronary Intervention and Angiography**
All patients were pretreated with a maintenance dose of 75 mg clopidogrel 21 week before angioplasty with 100 mg aspirin, or a loading dose of 300 mg clopidogrel immediately before angioplasty. All patients received i.v. heparin before the procedure to keep activated clotting time >250 s. Distal protection during the procedure was provided with a filter-type device, Filtrap™, which consists of a 190-cm-long, 0.014-inch guidewire with a spindle-shaped spiral wire basket with a porous polyurethane filter membrane. The filter was placed at approximately 20 mm distal to the lesion, and expanded automatically to suit the vessel dimensions. When the procedure was completed, the basket was removed using a 3.9-Fr retrieval sheath. Intracoronary thrombus was defined on angiography as reduced contrast density, haziness, irregular lesion contour, smooth convex meniscus, or total occlusion. The filter no-reflow phenomenon was defined as impaired blood flow after successful completion of PCI during deployment of the DPD, followed by a sudden increase >1 in Thrombolysis in Myocardial Infarction (TIMI) flow grade immediately after device removal, in the absence of pharmacological treatment between the 2 contrast injections.18
Pathology
The total amount of collected tissue in the DPD was assessed semi-quantitatively (score, 1–4), as follows: score 1, almost no visible tissue; score 2, visible tissue clinging to the filter mesh; score 3, apparent collection of tissue at the bottom of the filter (up to half of the basket); score 4, massive tissue protruding from the filter basket (more than half of the basket; Figure 1). The tissues in the filter were gently removed from the device, fixed with 10% buffered formalin, embedded in paraffin and prepared for histological sectioning at 4μm thickness. Sections were stained with hematoxylin-eosin (HE), Masson’s trichrome, Elastica van Gieson and von Kossa. Identification of calcified particles on HE was confirmed on von Kossa staining (Figure 2). Immunohistochemistry for fibrin (mouse monoclonal antibody to fibrin IIb chain antibody; Accurate Chemical and Scientific Corporation, Westbury, NY, USA), platelets (sheep anti-human platelet glycoprotein IIb/IIIa; Affinity Biologicals, Ontario, Canada) and endothelial cells (anti-CD34, Leica Biosystems, Newcastle upon Tyne, UK) was performed to identify the tissue components of thrombus (Figure 3). The amount of necrotic debris, fibrous tissue, calcified particle, platelet thrombus, and organized thrombus was also assessed semi-quantitatively. The scale was defined as follows: grade 0, absent; grade 1, occupied less than one-third of the total tissue area on histological section; grade 2, occupied more than one-third but less than two-thirds of the total tissue area; grade 3, occupied more than two-thirds of the total tissue area. Necrotic debris was frequently accompanied by cholesterin crystals and foam cells, and organized thrombus was defined as homogenous eosinophilic material with cell lysis and/or having the features of endothelialization.19 Endothelial cell ingrowth was confirmed on CD34 immunohistochemistry.

Both the tissue identification and quantification were performed by 3 examiners (A.F., H.H., S.S.), independently, and, in the case of disagreement, they discussed until they reached a consensus.

Statistical Analysis
Categorical variables are presented as frequency and were compared using chi-squared statistics or Fisher’s exact test, as appropriate. Continuous variables are presented as median and first and third quartiles and were compared using the Wilcoxon rank sum test. Logistic regression modeling including pathological findings and clinical risk factors was used to predict greater amount of tissue in DPD. Two-sided $P<0.05$ indicated statistical significance. Statistical analysis was performed using JMP version 8.0 (SAS Institute, Cary, NC, USA).

Results
After excluding the patients who underwent PCI to the bypass graft, 671 patients with 671 lesions were analyzable. Patient and lesion characteristics are listed in Table 1. A total of 417 of 671 patients had acute coronary syndrome (ACS; 62.1%), and 254 had stable CAD (37.9%). Approximately half (335/671) of the lesion were in the left anterior descending artery. Procedural success was obtained in all lesions. Necrotic debris was observed in 40.7% (273/671) of the lesions, fibrous tissue in 41.4% (278/671), calcified particle in 18.0% (121/671), platelet thrombus in 93.1% (625/671), and organized thrombus in 58.3% (391/671). All specimens with fibrous tissue and calcified particle were identified as grade 1 (less than one-third of the total tissue area) when present (i.e., no specimens had fibrous tissue or calcified particle more than one-third of the total tissue area). The prevalence of necrotic debris and fibrous tissue in patients with ACS and in those with stable CAD was similar (ACS, 41.7% vs. stable CAD, 39.0%, $P=0.48$; ACS, 39.8% vs. stable CAD, 44.1%, $P=0.27$, respectively). In contrast, calcified particle was observed significantly more frequently in patients with ACS than in those with stable CAD (20.9% vs. 13.4%, $P=0.01$). Although organized thrombus was equally identified in lesions in ACS and stable CAD (59.7% vs. 55.9%, $P=0.33$), platelet thrombus was significantly frequently observed in lesions in ACS compared with stable CAD (95.2% vs. 89.8%, $P<0.01$). Furthermore, the area occupied by platelet thrombus on the sections was significantly larger in lesions in ACS compared with stable CAD (ACS: grade 1, 18.2%; grade 2, 27.1%; grade 3, 49.9%; stable CAD: 25.6%, 29.1%, 35.0%, respectively, $P<0.01$). Immunohistochemistry for platelets was specifically positive, corresponding to the light green granules identified as platelets on Masson’s trichrome. In contrast, immunohistochemistry for fibrin was specifically positive, corresponding to the red fibrin net in Masson’s trichrome.

Semi-quantitative tissue amount score distribution was as follows: score 1, 3.9% (26/671); score 2, 52.0% (349/671);...
diabetes mellitus, current smoking, chronic kidney disease, prior myocardial infarction, prior percutaneous coronary intervention, prior coronary artery bypass grafting, and use of thienopyridine, anti-coagulants, or statin on admission. ACS, acute coronary syndrome; DPD, distal protection device.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Regression coefficient</th>
<th>95% CI</th>
<th>P-value</th>
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<tr>
<td>Necrotic debris</td>
<td>0.07</td>
<td>0.01–0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Fibrous tissue</td>
<td>0.08</td>
<td>0.03–0.12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Platelet thrombus</td>
<td>0.13</td>
<td>0.03–0.23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Organized thrombus</td>
<td>0.05</td>
<td>0.01–0.10</td>
<td>0.051</td>
</tr>
<tr>
<td>Clinical presentation of ACS</td>
<td>0.08</td>
<td>0.02–0.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Aspirin use on admission</td>
<td>−0.09</td>
<td>−0.16 to −0.02</td>
<td>0.01</td>
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Variables included in the model, but which were not significant: calcified particle, age, gender, hypertension, dyslipidemia, diabetes mellitus, current smoking, chronic kidney disease, prior myocardial infarction, prior percutaneous coronary intervention, prior coronary artery bypass grafting, and use of thienopyridine, anti-coagulants, or statin on admission. ACS, acute coronary syndrome; DPD, distal protection device.

Discussion

To the best of our knowledge, there have been few histopathological studies to evaluate tissue obtained by DPD. In the present study, a larger area occupied by pathological necrotic debris was significantly associated with a larger amount of emboli captured by DPD. Moreover, on multi-
variante analysis, the presence of necrotic debris was an independent predictor of greater amount of captured tissue in the distal DPD. Based on the results of previous studies and the present one, lipid-rich plaque diagnosed on intracoronary imaging may accelerate thrombus formation, and, subsequently, it may generate a larger amount of tissue that causes distal embolization.

On pathology in the present study, DPD-captured tissue was identified as consisting of various kinds of plaque components, such as fragmented fibrous tissue, and calcified particles other than the necrotic debris. As previously discussed, scattering of necrotic debris may play an important role in the distal embolism. The present pathological study, involving a relatively large number of cases, is consistent with the previous evidence. Furthermore, in the present study the presence of fragmented fibrous tissue was found to be independently associated with greater tissue amount in the DPD, suggesting that it might stimulate the coagulation cascade, which may lead to the microcirculation impairment of myocardium. This novel finding indicates that fragmented fibrous tissue derived from the plaque, probably from the fragile fibrous cap, may have high thrombogenic activity, along with the necrotic debris. The thrombogenicity of the microfibrillar structures associated with elastin in the arterial wall, has been demonstrated both in vivo and in vitro. Furthermore, elastin microfibril interface located protein 2 (EMILIN2), which is important for the structure of elastin, has been shown to play an important role in thrombogenesis. EMILIN2 is abundantly expressed in thrombus and regulates platelet activation and thrombus formation. In addition, collagen in fibrous tissue may contribute to platelet adhesion, activation and aggregation. Platelets have 2 major receptors for collagen: the integrin α2β1, with a major role in adhesion and platelet anchoring; and the immunoglobulin superfamily member, glycoprotein VI, principally responsible for signaling and platelet activation. Thereby, PCI to the lesions with thin-cap fibroatheroma might carry a risk of peri-procedural myocardial injury, regardless of the amount of necrotic debris.

Organized thrombus was frequently identified in both lesions in ACS and in stable CAD in this study. In general, organized thrombus is frequently observed in ACS lesions, as well as in stable lesions with subclinical silent rupture, which causes plaque progression. In the present study, the amount of tissue in the DPD basket tended to be associated with a greater amount of organized thrombus, suggesting that the lesions that contain previously ruptured plaque may be a risk factor for distal embolism or subsequent adverse cardiac events.

In addition, it is of great interest that the calcified particle in DPD was frequently identified in lesions in ACS than in stable CAD. In a recent in vitro study of cultured vascular smooth muscle cells, addition of phosphate in culture medium led to the formation of calciprotein particles. This calcium phosphate-containing nano-aggregate produced calcification and enhanced the release of tumor necrosis factor-α (TNF-α). We speculate that the presence of tiny calcified particles in the plaque may also stimulate the secretion of inflammatory cytokines, such as TNF-α, thereby initiating plaque vulnerability and rupture. The DPD-captured calcified particles were tiny: their diameter was <20 μm. Using current imaging modalities, it is difficult to distinguish the calcified particle, which is synonymous with microcalcification, from other components.

Although the calcified particles are tiny relative to the plaque components, the biological role of these particles in plaque progression and plaque vulnerability should be further investigated in future studies.

Last, the effect of chronic aspirin therapy has been reported to be associated with reducing angiographic thrombus or infarct size in ACS. Consistent with these previous studies, in the present study it was found that ongoing aspirin use may be effective in decreasing the amount of scattering tissue and/or thrombus in the distal vessels. Aspirin requires 7–10 days for complete suppression of platelet thromboxane biosynthesis; therefore, contentious aspirin use may be beneficial to reduce the risk of myocardial injury during PCI.

Study Limitations
First, this was a single-center retrospective study, and included only patients treated with DPD, which could be a selection bias. Second, it is possible that the tissue in the filter device might not have been derived from the target lesion, but might have developed within the device. A higher prevalence of platelet thrombus in lesions in ACS, however, suggests that the tissue in the DPD was mainly derived from the target lesions.

Conclusions
The presence of atherosclerotic plaque component, such as necrotic debris and elastic fiber, might be a risk factor for distal embolism during PCI. This phenomenon tends to occur more frequently in patients with ACS.

Acknowledgments
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Disclosures
The authors declare no conflicts of interest.

References
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