Inflammation and oxidative stress play key roles in atherosclerotic plaque instability, and plaque rupture/erosion and subsequent thrombus formation constitute the principal mechanisms of total vessel occlusion and acute ST-elevation myocardial infarction (STEMI). Plaque disruption triggers the formation of initial platelet aggregates that grow in association with an increase in fibrin formation, leading to persistent coronary flow obstruction and blood coagulation. The fibrin network may trap large numbers of erythrocytes and inflammatory cells to form an erythrocyte-rich thrombus. In fact, previous clinical studies have shown that not only platelet-rich white thrombi, but also erythrocyte-rich red thrombi can be visualized using angioscopy in patients with acute coronary syndrome. Recently, the development of thrombus aspiration and distal protection devices has significantly improved the clinical outcomes of percutaneous intervention in STEMI patients and has enabled the evaluation of antemortem coronary artery thrombi. This is important because previous autopsy studies were unable to differentiate coronary thrombi responsible for myocardial ischemia from postmortem clots. Using frozen samples of aspirated thrombi and specific monoclonal antibodies, we investigated the cellular components of thrombi (platelets, erythrocytes, fibrin and inflammatory cells, such as myeloperoxidase-positive cells) and pathologically evaluated the relationships between erythrocyte-rich thrombi and inflammation, oxidative stress and clinical outcomes in STEMI patients. Therefore, this review article focuses on the efficacy of thrombus aspiration therapy and the components of aspirated intracoronary thrombi in STEMI patients and presents the results of recent studies regarding the relationship between the composition of aspirated intracoronary thrombi and clinical outcomes.


Key words: Coronary thrombus, Erythrocyte, Neutrophil, ST-segment elevation, Myocardial infarction
Coronary Thrombus in STEMI

Role of Tissue Factor and Microparticles in Thrombus Formation

In atherosclerotic plaques, macrophages and smooth muscle cells produce large amounts of tissue factor, a trigger of the coagulation system, and abundant tissue factor is expressed in advanced atherosclerotic lesions. Therefore, plaque disruption leading to tissue factor exposure to flowing blood triggers coagulation activation and initiates platelet-fibrin thrombus formation. In fact, the plasma tissue factor levels and the expression/activity of tissue factor by circulating monocytes have been shown to be elevated in patients with ACS or metabolic syndrome. These findings suggest that abundant tissue factor within plaque is the major factor responsible for the formation of large-size fibrin-rich thrombi and that local/systemic activation of monocytes increases the thrombogenic potential of the blood flow, thus leading to the onset of ACS.

Microparticles (MPs) are 0.1-1-μm membrane vesicles that are released into the extracellular space following cell activation or apoptosis. Several cardiovascular risk factors, such as oxidative stress and elevated levels of lipoproteins and cytokines, increase the release of MPs from vascular/circulation cells, and a large number of studies have proposed that MPs may contribute to atherosclerotic plaque development and thrombus formation/progression. In fact, circulating MPs (primarily endothelial and leukocyte MPs), which contribute to the initiation of atherosclerosis due to endothelial dysfunction by decreasing nitric oxide synthesis, have been reported to be significantly higher in ACS patients and are considered to be a primary source of the blood-borne tissue factor involved in thrombus propagation at the site of vascular injury. In addition, human atherosclerotic plaques contain high levels of MPs, and a large number of human plaque MPs express CD40 ligand and harbor tissue factor, increasing their procoagulant activity. Human plaque MPs are reported to be significantly prothrombogenic because they generate twice as much thrombin as circulating MPs in the same patient. At the time of plaque disruption, locally released plaque MPs (particularly tissue factor-positive MPs), circulating tissue factor-bearing MPs and platelet-derived MPs contribute to the concentration of tissue factor activity at the thrombus edge, leading to fibrin-rich thrombus formation.
Thrombus Aspiration/Distal Protection Therapy and Its Efficacy

The presence of an intracoronary thrombus at the culprit lesion site may increase the risk of distal embolization (DE), resulting in microvascular damage and left ventricular dysfunction during primary percutaneous coronary intervention (p-PCI)\(^{43-47}\). In this regard, the use of a mechanical device for thrombus removal or trapping to improve clinical outcomes after p-PCI is attractive, and the efficacy of these devices has been tested in many clinical trials. Although some data, especially those regarding the use of distal protection devices, have failed to show clinical benefits due in part to patient and device selection\(^{48-53}\), many recent large-scale randomized trials have demonstrated significant improvements in myocardial perfusion\(^{45, 54-67}\) and the left ventricular function\(^{64, 65}\) in addition to reduced mortality\(^{68}\) (Table 1). De Luca et al. demonstrated using a meta-analysis of the results of 2,417 STEMI patients evaluated in nine randomized trials that adjunctive manual thrombectomy was associated with a significantly higher frequency of postprocedural thrombolysis in patients with a myocardial infarction (TIMI) 3 flow (87.1% vs. 81.2%, \(p<0.0001\)) and a postprocedural myocardial blush grade (MBG) of 3 (52.1% vs. 31.7%, \(p<0.0001\)) in addition to reduced DE (7.9% vs. 19.5%, \(p<0.0001\)) and significant benefits in terms of 30-day mortality (1.7% vs. 3.1%, \(p<0.04\))\(^{69}\). In addition, Burzotta et al. showed using a meta-analysis of the results of 3,180 STEMI patients evaluated in 18 prospective randomized trials comparing adjunctive manual thrombectomy with standard PCI that adjunctive manual thrombectomy was associated with lower rates of angiographically evident DE [odds ratio (OR) 0.54, 95% confidence interval (CI) 0.37-0.81, \(p=0.003\)] and reduced rates of ST-segment resolution (STR) (OR 0.60, 95% CI 0.48-0.96, \(p=0.028\)) during a mean follow-up of 9.9 ± 3.8 months\(^{72}\).

Based on these findings, if not anatomically contraindicated, adjunctive manual thrombectomy devices should be widely and routinely used in STEMI patients undergoing p-PCI.

Pathological Analysis of Aspirated Intracoronary Thrombi

The results of histopathological evaluations of aspirated intracoronary thrombi obtained from STEMI patients have recently been presented\(^{73-77}\). Rittersma et al. investigated 199 aspirated intracoronary thrombi obtained from STEMI patients within six hours of symptom onset and pathologically classified them into fresh thrombi (<1 day old) composed of layered patterns of fibrin and intact platelets, erythrocytes and granulocytes and older thrombi with lytic (1 to 5 days) or organized (>5 days) changes\(^{73}\). In that study, 50% of the thrombi were days or weeks old. Moreover, Nagata et al. evaluated 77 thrombi aspirated from STEMI patients within 24 hours of symptom onset and demonstrated that the dominant cell type of thrombi in the early phase of STEMI was platelets and that large thrombi in the right coronary artery contained more erythrocytes\(^{74}\). However, these studies were based on thrombi fixed in formalin, and the thrombus components (platelets, erythrocytes, fibrin and inflammatory cells) were not evaluated using immunohistochemical methods with specific antibodies. Our study was based on frozen thrombus samples because monoclonal antibodies against glycoprotein (GP) IIb/IIIa and P-selectin only work well on frozen sections\(^{78}\). Using frozen samples of aspirated thrombi and these monoclonal antibodies, we evaluated the components of thrombi obtained from 178 STEMI patients\(^{78}\). In the erythrocyte-rich thrombi, the majority of the areas were stained positively for glycoprotein-A (Fig. 1A, B), while the GP IIb/IIIa- and P-selectin-positive areas were small. In these thrombi, the erythrocyte-positive areas were the inverse of the GP IIb/IIIa- and P-selectin-positive areas, and abundant myeloperoxidase (MPO)-positive neutrophils (Fig. 1C) and CD68-positive macrophages were found within the thrombi. In the thrombi, fibrin was also detected, and staining positivity for fibrin was present in the sites of platelets with GP IIb/IIIa and P-selectin.
### Table 1. Summary of recent randomized studies showing the effects of thrombus aspiration and distal protection on clinical outcomes

#### Thrombus aspiration vs. conventional PCI

<table>
<thead>
<tr>
<th>Authors (Study name)</th>
<th>Diagnosis (Onset time)</th>
<th>Type of devices</th>
<th>N</th>
<th>Methods for evaluating clinical outcome (primary endpoint) and p-value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Svilas et al. 45)</td>
<td>STEMI (&lt;12 hours)</td>
<td>6Fr Export Catheter (Medtronic)</td>
<td>1071</td>
<td>MBG 0 or 1 (17.1% vs. 26.3%, p &lt; 0.001)</td>
<td>better</td>
</tr>
<tr>
<td>Kaltoft et al. 46)</td>
<td>STEMI (&lt;12 hours)</td>
<td>Rescue Catheter (Boston Scientific/Scimed)</td>
<td>215</td>
<td>Myocardial salvage assessed by Tc 99m sestamibi imaging (13% vs. 18%, p = 0.12)</td>
<td>n.s</td>
</tr>
<tr>
<td>Ali et al. 49)</td>
<td>STEMI (&lt;12 hours)</td>
<td>AngioJet RT Catheter (Possis Medical)</td>
<td>480</td>
<td>Infarct size assessed by Tc 99m sestamibi imaging (9.8 ± 10.9% vs. 12.5 ± 12.1%, p = 0.03)</td>
<td>worse</td>
</tr>
<tr>
<td>Guetta et al. 41)</td>
<td>STEMI (&lt;12 hours)</td>
<td>7 or 8Fr X-Sizer Catheter (EndiCOR Medical)</td>
<td>66</td>
<td>Postprocedural TIMI 3 flow (90% vs. 84%)</td>
<td>n.s</td>
</tr>
<tr>
<td>Liistro et al. 42)</td>
<td>STEMI (&lt;12 hours)</td>
<td>AngioJet RT Catheter</td>
<td>100</td>
<td>Ratio of early STR (90% vs. 72%, p = 0.022)</td>
<td>better</td>
</tr>
<tr>
<td>Ikari et al. 50)</td>
<td>STEMI (&lt;24 hours)</td>
<td>7Fr TransVascular Aspiration Catheter (TVAC) (Nipro)</td>
<td>355</td>
<td>TIMI myocardial perfusion grade &lt; 3 (12.4% vs. 19.4%, p = 0.07)</td>
<td>n.s</td>
</tr>
<tr>
<td>Napondano et al. 57)</td>
<td>STEMI (&lt;12 hours)</td>
<td>X-Sizer Catheter (ev3)</td>
<td>92</td>
<td>Postprocedural MBG 3 (71.7% vs. 36.9%, p = 0.006)</td>
<td>better</td>
</tr>
<tr>
<td>Burzotta et al. 58)</td>
<td>STEMI (&lt;12 hours)</td>
<td>6Fr Diver CE Catheter (Invasive)</td>
<td>99</td>
<td>MBG ≥ 2 and STR ≥ 70% (46.0% vs. 24.5%, p = 0.025)</td>
<td>better</td>
</tr>
<tr>
<td>Lefèvre et al. 59)</td>
<td>STEMI (&lt;12 hours)</td>
<td>X-Sizer Catheter</td>
<td>201</td>
<td>Magnitude of STR (7.5 mm vs. 4.9 mm, p = 0.033)</td>
<td>better</td>
</tr>
<tr>
<td>Silva-Orrego et al. 60)</td>
<td>STEMI (&lt;12 hours)</td>
<td>6Fr Pronto Catheter (Vasc.solutions)</td>
<td>148</td>
<td>Complete (&gt;70%) STR (68% vs. 50%, p &lt; 0.05)</td>
<td>better</td>
</tr>
<tr>
<td>Chevalier et al. 61)</td>
<td>STEMI (&lt;12 hours)</td>
<td>Export Catheter</td>
<td>249</td>
<td>MBG 3 (2.84 ± 0.32 vs. 2.38 ± 0.59, p &lt; 0.001)</td>
<td>better</td>
</tr>
<tr>
<td>Sardella et al. 62)</td>
<td>STEMI (&lt;9 hours)</td>
<td>Export Catheter</td>
<td>175</td>
<td>MBG ≥ 2 (88% vs. 59%, p &lt; 0.0001)</td>
<td>better</td>
</tr>
<tr>
<td>Duck et al. 63)</td>
<td>STEMI (&lt;6 hours)</td>
<td>6Fr Diver CE Catheter</td>
<td>196</td>
<td>Magnitude of STR (63% vs. 39%, p = 0.001)</td>
<td>better</td>
</tr>
<tr>
<td>De Luca et al. 64)</td>
<td>STEMI (&lt;12 hours)</td>
<td>7Fr Diver CE Catheter</td>
<td>76</td>
<td>Complete (&gt;70%) STR (60 min: 53.7% vs. 35.1%, p = 0.027)</td>
<td>better</td>
</tr>
<tr>
<td>Liistro et al. 65)</td>
<td>STEMI (&lt;12 hours)</td>
<td>6Fr Export Catheter</td>
<td>111</td>
<td>Immediate after PCI: 41% vs. 26%, p = 0.037</td>
<td>better</td>
</tr>
<tr>
<td>Vlaar et al. 66)</td>
<td>STEMI (&lt;12 hours)</td>
<td>6Fr Export Catheter (TAPAS trial)</td>
<td>1071</td>
<td>Cardiac death or non-fatal reinfarction at 1 year (5.6% vs. 9.9%, p = 0.009)</td>
<td>better</td>
</tr>
</tbody>
</table>

#### Distal protection vs. conventional PCI

<table>
<thead>
<tr>
<th>Authors (Study name)</th>
<th>Diagnosis (Onset time)</th>
<th>Type of devices</th>
<th>N</th>
<th>Methods for evaluating clinical outcome (primary endpoint) and p-value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stone et al. 60)</td>
<td>STEMI (&lt;6 hours)</td>
<td>GuardWire Plus (Medtronic)</td>
<td>252</td>
<td>STR ≥70% (63.3% vs. 61.9%, p = 0.78)</td>
<td>n.s</td>
</tr>
<tr>
<td>Gick et al. 61)</td>
<td>STEMI (&lt;48 hours)</td>
<td>FilterWire EX (Boston Scientific)</td>
<td>200</td>
<td>Inferior perfusion assessed by Tc 99m sestamibi imaging (12.0% vs. 9.5%, p = 0.15)</td>
<td>n.s</td>
</tr>
<tr>
<td>Cura et al. 62)</td>
<td>STEMI (&lt;12 hours)</td>
<td>Spider RX (ev3)</td>
<td>140</td>
<td>Maximal adenosine-induced Doppler flow velocity (38 ± 17 vs. 36 ± 20 cm/s, p = 0.46)</td>
<td>n.s</td>
</tr>
<tr>
<td>Guetta et al. 33)</td>
<td>STEMI (&lt;24 hours)</td>
<td>FilterWire EZ (Boston Scientific)</td>
<td>100</td>
<td>Final TIMI 3 flow (88.2% vs. 93.9%, p = n.s.)</td>
<td>n.s</td>
</tr>
<tr>
<td>Muramatsu et al. 66)</td>
<td>STEMI (&lt;12 hours)</td>
<td>GuardWire Plus</td>
<td>341</td>
<td>Final procedural MBG 3 (68% vs 66%, p = n.s.)</td>
<td>n.s</td>
</tr>
<tr>
<td>Ito et al. 67)</td>
<td>STEMI (&lt;24 hours)</td>
<td>Filtrat (Nipro)</td>
<td>36</td>
<td>Cardiac death or non-fatal reinfarction at 1 year (5.6% vs. 9.9%, p = 0.009)</td>
<td>better</td>
</tr>
</tbody>
</table>

STEMI = ST-elevation myocardial infarction; TIMI = thrombolysis in myocardial infarction; MBG = myocardial blush grade; STR = ST-segment resolution; LVEF = left ventricular ejection fraction; DE = distal embolization; CTFC = corrected TIMI frame count; n.s. = not significant.
positivity. In the platelet-rich thrombi, the erythrocyte-positive areas were small, and most thrombi were stained positively for GP IIb/IIIa and P-selectin. In these platelet-rich thrombi, there were only a few MPO-positive neutrophils and macrophages. Furthermore, fibrin was also detected, and staining positivity for fibrin was present in the platelet sites that stained positive for GP IIb/IIIa and P-selectin.

**Coronary Thrombus Components and the Ischemic Time**

Recently, Silvain et al. analyzed 45 intracoronary thrombi obtained by aspiration from 288 consecutive STEMI patients using electron microscopy and clearly showed a relationship between the coronary thrombus components and the ischemic time. That study demonstrated that the fibrin content increased in association with the ischemic time, ranging from $48.4 \pm 21\%$ (3 hours) up to $66.9 \pm 9\%$ (>6 hours) ($p=0.02$), whereas the platelet content decreased from $24.9 \pm 23\%$ (<3 hours) to $9.1 \pm 6\%$ (>6 hours) ($p=0.07$). Moreover, a multivariate analysis indicated that the ischemic time was the only independent predictor of thrombus composition. Similarly, Iwata et al. reported that the platelet content of intracoronary thrombi, as determined with immunostaining for CD42a, was negatively correlated with the time from the onset of chest pain ($r=-0.683$, $p<0.01$). However, the “actual ischemic time” in clinical practice is defined as the time from the coronary event to the onset of reperfusion, which is not necessarily the same as the time from symptom onset. In fact, Rittersma et al. evaluated 199 aspirated thrombi obtained from 211 consecutive STEMI patients within six hours after the onset of chest pain and reported that the coronary thrombi were several days or weeks old in at least 50% of the patients with acute STEMI. These results reflect the fact that aspirated thrombi may be older than expected based on the duration of the ischemic time and indicate that partial vessel occlusion leading to ACS frequently occurs days or weeks before

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**Fig. 1.** Representative Micrographs of an Aspirated Thrombus Obtained from a Patient with an Erythrocyte-rich Component

(A) Hematoxylin-eosin staining. The boxed area is enlarged in B-C. (B) Immunostaining for erythrocytes (glycophorin-A). (C) Immunostaining for myeloperoxidase (MPO-7). Bar: (A) 500 μm, (B, C) 100 μm.
symptom onset. In addition, our recent study including STEMI patients presenting within 12 hours of symptom onset revealed three types of aspirated coronary thrombi: 1) platelet-rich, low-erythrocyte thrombi, 2) platelet and erythrocyte-mixed thrombi and 3) erythrocyte-rich, low-platelet thrombi. These three types were observed despite a similar time from symptom onset to reperfusion among the three groups. Moreover, many of the patients with erythrocyte-rich thrombi were smokers and had high plasma levels of oxidative stress markers (MPO and oxidized low-density lipoprotein). Another report demonstrated that circulating soluble CD40 ligand (sCD40L: an index of platelet activation) rather than demonstrated that circulating soluble CD40 ligand (sCD40L: an index of platelet activation) rather than demonstrated that circulating soluble CD40 ligand (sCD40L: an index of platelet activation) rather than demonstrated that circulating soluble CD40 ligand (sCD40L: an index of platelet activation) rather than demonstrated that circulating soluble CD40 ligand (sCD40L: an index of platelet activation) rather than demonstrated that circulating soluble CD40 ligand (sCD40L: an index of platelet activation) rather than demonstrated that circulating soluble CD40 ligand (sCD40L: an index of platelet activation) rather than demonstrated that circulating soluble CD40 ligand (sCD40L: an index of platelet activation) rather than demonstrated that circulating soluble CD40 ligand (sCD40L: an index of platelet activation). These findings suggest that thrombus components may be dependent on not only the ischemic time, but also multiple factors influencing inflammation, oxidative stress and blood viscosity.

**Coronary Thrombus and Plaque Morphology**

There are three reports related to coronary thrombus and plaque morphology. Sato et al. investigated the proportion of fibrin and platelets in thrombi of ruptured and eroded coronary atherosclerotic plaques obtained from patients who had died of acute MI within three days of onset and reported that fibrin was more abundant than platelets in the thrombi of ruptured plaques (74±19% vs. 35±20%, p<0.01), whereas platelets tended to be more abundant than fibrin in the thrombi of eroded plaques (51±6% vs. 70±21%, p<0.07). The second report was on the relationship between thrombus age (healing) and plaque morphology in sudden coronary death victims. In that study, Kramer et al. immunohisto-pathologically evaluated 111 sudden death victims who had coronary lesions (ruptures, n=65; erosions, n=50) with thrombi and determined the relationship between plaque morphology and thrombus healing classified as early (<1 day) or late characterized in phases of lytic (1 to 3 days), infiltrating (4 to 7 days) or healing (>7 days). The results showed that late-stage thrombi were present in 79 (69%) of the 115 culprit plaques and that the majority of early thrombi (<1 day) and lytic thrombi (1 to 3 days) were present in plaque ruptures as compared with erosions (46% vs. 12%, p<0.0001), whereas the majority of thrombi in erosions were infiltrating (4 to 7 days) or healing (>7 days) (46% vs. 9%, p<0.001). Moreover, women more frequently had erosions with a greater incidence of late-stage thrombi (44 of 50, 88%) than ruptures (35 of 65, 54%, p<0.0001). The third study was a clinicopathological study by Ferrante et al. That study consisted of two substudies including 25 ACS patients and 22 sudden coronary death victims. The first subsudy investigated the relationship between culprit lesion morphology assessed with optical coherence tomography (OCT) and the serum MPO levels in 25 ACS patients (12 STEMI patients and 13 non-STEMI patients) and found that the serum MPO levels were significantly higher in the patients with eroded plaques (n=7) than in those with ruptured plaques (n=18) [erosion vs. rupture: 2,500 ng/ml (range: 1,415-2,929) vs. 707 ng/ml (range 312-943), p=0.001]. In the second subsudy, the authors immunohistochemically analyzed the relationship between coronary plaque morphology and the density of MPO-positive cells within thrombi overlying plaques in 22 sudden coronary death victims. The results showed that the number of MPO-positive cells (neutrophils and some macrophages) within thrombi was significantly higher in the lesions with erosion (n=11) than in the lesions with rupture (n=11) [erosion vs. rupture: 1,584 cells/mm² (range: 1,088-2,135) vs. 579 cells/mm² (range: 442-760), p=0.0012].

All of these previous studies performed postmortem analyses of coronary thrombi rather than ante-mortem assessments of culprit plaque morphology using imaging modalities, such as intravascular ultrasound (IVUS) or OCT, and pathological analyses of aspirated intracoronary thrombi in living patients were not performed. Our study used antemortem aspirated intracoronary thrombi and showed that erythrocyte-rich thrombi contain more MPO-positive cells; however, we did not fully assess plaque morphology in patients with acute STEMI.

**Coronary Thrombus Components and Clinical Outcomes**

Several recent studies have demonstrated associations between coronary thrombus characteristics and DE/myocardial perfusion, restenosis, the left ventricular (LV) function/remodeling and long-term mortality (Table 2).

**Distal Embolization and Myocardial Perfusion**

Plaque volume and composition, particularly the necrotic core volume, has been shown to be associated with DE in patients with STEMI. The presence
of intracoronary thrombi at the lesion site has also been shown to increase the risk of DE. Napodano et al. reported that DE during p-PCI occurs more often in the presence of high thrombus burden lesions (DE vs. No-DE, TIMI thrombus score ≥ 3; 95.3% vs. 71.7%; p < 0.0001). Moreover, Fokkema et al. showed that thrombi aspirated from patients with DE were larger (p = 0.0002) and contained an erythrocyte component more often than thrombi aspirated from patients without DE (50.0% vs. 15.7%, p < 0.001). Moreover, we recently demonstrated that the erythrocyte component of intracoronary thrombi, as assessed with immunohistochemical staining of glycophorin-A (a protein specific to erythrocyte membranes), is an independent predictor of DE based on angiography in patients with STEMI. In addition, our study also showed that signs of impaired myocardial reperfusion, as indicated by an incomplete STR (<70%) and lower MBG (≤ 1), are frequently observed in patients with erythrocyte-rich thrombi (STR, p = 0.056; MBG, p < 0.01). Furthermore, Arakawa et al. demonstrated that a high neutrophil density in an aspirated thrombus (>100 neutrophils/0.025 mm²) is associated with impaired coronary microcirculation, as assessed according to an MBG ≤ 1 and STR < 50%. These findings suggest that not only the thrombus size (burden), but also the thrombus components (erythrocytes and MPO-positive neutrophils) are associated with myocardial reperfusion in STEMI patients.

### Restenosis

Iwata et al. analyzed 108 aspirated thrombi obtained from acute (<24 hours) or recent (24-72 hours) of intracoronary thrombi at the lesion site. Napodano et al. and Fokkema et al. showed that DE occurs more often in association with high thrombus burden lesions and is associated with a larger thrombus size and an erythrocyte component. Furthermore, Arakawa et al. demonstrated that a high neutrophil density in an aspirated thrombus is associated with impaired coronary microcirculation. Therefore, the thrombus size (burden) and thrombus components (erythrocytes and MPO-positive neutrophils) are associated with myocardial reperfusion in STEMI patients.
hours) STEMI patients and reported that the presence of CD34-positive primitive cells in intracoronary thrombi positively correlated with restenosis on follow-up CAG (r = 0.76, p = 0.01).80

LV Function and Remodeling

Ours and other studies have reported a relationship between the thrombus components and the LV function/remodeling. Arakawa et al. demonstrated that the LV ejection fraction (LVFE) at six months after MI was significantly lower in a group of patients with a high neutrophil density in the aspirated thrombus (>100 neutrophils/0.025 mm² thrombus) than in a group with a low neutrophil density (42.4 ± 1.4% vs. 47.1 ± 1.5%, p < 0.05).77 In contrast, we showed that the LVFE on admission and at six months after STEMI are not significantly different among patients with platelet-rich, mixed and erythrocyte-rich thrombi; however, the progression of LV remodeling is frequently observed in patients with erythrocyte-rich thrombi (p < 0.01).78

Long-Term Mortality

Recently, Kramer et al. showed that the presence of older thrombi is an independent predictor of long-term mortality in patients with STEMI treated with thrombus aspiration. In that study, they classified the thrombi aspirated from 1,315 STEMI patients into fresh thrombi (<1 day old) or older thrombi with lytic (1 to 5 days) or organized (>5 days) changes immunohistochemically and showed that all-cause mortality was two-fold higher in the patients with older thrombi than in the patients with fresh thrombi at four years of follow-up (hazard ratio 1.82, 95% CI 1.17-2.85, p = 0.008).

Possible Mechanisms of Thrombus Growth

In the initial phase, the activation of platelets and the coagulation system plays an important role in thrombus formation and growth. Under conditions of a rapid flow in atherosclerotic arteries, von Willebrand factor has been shown to play an important role in platelet aggregation/adhesion and fibrin-rich thrombus formation. Moreover, abundant tissue factor expressed in plaque and the vascular wall may also play a crucial role in the activation of the coagulation cascade and the formation of platelet-fibrin thrombi at the time of plaque disruption.8-15

In the progressive phase of thrombus growth, both fibrin accumulation and platelet-fibrin stabilization and blood thrombogenicity/viscosity and erythrocyte properties are important; therefore, tissue factor-positive MPs, local and systemic activation of inflammatory cells and various cardiovascular risk factors and immune-inflammatory/oxidative stress-related factors influencing blood/erythrocyte properties may play important roles. At the time of plaque disruption, locally released tissue factor-positive and leukocyte-derived MPs play an important role in fibrin-rich thrombus formation. Moreover, activated inflammatory cells, such as neutrophils, macrophages and eosinophils, contribute to the growth of an erythrocyte-rich thrombus. Our study also demonstrated that erythrocyte-rich thrombi often contain more inflammatory cells, such as MPO-positive neutrophils, thus leading to increased plasma MPO levels, which reflects a high thrombus burden. These results suggest that local accumulation of activated inflammatory cells and a systemic inflammatory status contribute to thrombus growth, which is understandable when considering the mechanisms of thrombus formation and growth, as previously reported.8-15 Moreover, MPO itself and MPO-derived reactive oxygen species have been shown to increase the expression of tissue factor, leading to a thrombotic state, and influence erythrocyte deformation and aggregation, leading to membrane generation, rouleau formation and higher blood viscosity. In addition, other cardiovascular risk factors (cigarette smoking, hyperglycemia) and immune-inflammatory/oxidative stress-related factors (C-reactive protein, CD40 ligand, fibrinogen and D-dimer, lipoprotein (a)) have been reported to influence the platelet-fibrin clot structure and blood viscosity/erythrocyte aggregation. These findings are of considerable interest, since they suggest that some factors influencing blood thrombogenicity/viscosity and erythrocyte properties (deformability and/or aggregability) may play important roles in the evolution of erythrocyte-rich large thrombi. Schemas of the possible mechanisms of thrombus growth and the evolution of erythrocyte-rich thrombi are presented in Fig.2.

Conclusions

Thrombus aspiration in ACS patients has provided important histopathological information regarding thrombus composition, which may contribute to furthering our understanding of the pathophysiology of ACS. In studies based on the examination of thrombus specimens, sampling bias must be considered; nevertheless, evaluating the thrombus burden or components using both coronary angiography and intravascular imaging modalities, such as IVUS, angioscopy or OCT, may contribute to risk stratifica-
Interactions between the severity of atherosclerotic plaques, the lumen diameter/area and blood properties may play a role in determining the thrombus components and growth. (A) In patients with a large plaque area and severe lumen narrowing, initial platelet aggregation following plaque disruption may lead to subtotal vessel occlusion resulting in acute coronary syndrome. (B) In contrast, in patients with intermediate plaque areas and less severe narrowing, erythrocytes and inflammatory cells play a crucial role in blood properties, thrombus formation and growth, leading to total vessel occlusion. (C) At the time of plaque disruption, von Willebrand factor, tissue factor and microparticles play important roles in the activation of platelets and the coagulation cascade and the formation of platelet-fibrin thrombi. Moreover, systemic activation of neutrophils, C-reactive protein, local accumulation of activated inflammatory cells (myeloperoxidase-positive cells), the oxidative stress status and other cardiovascular risk factors (smoking, hyperglycemia, CD40 ligand, fibrinogen/D-dimer, etc.) have been shown to influence the fibrin clot structure, blood viscosity/thrombogenicity and erythrocyte properties (deformability and/or aggregability), leading to the evolution of erythrocyte-rich thrombi. CRP: C-reactive protein, DM: diabetes mellitus, MPO: myeloperoxidase, ROS: reactive oxygen species, vWF: von Willebrand factor, TF: tissue factor.

**Fig. 2.** Schematic Representation of the Possible Mechanisms of Thrombus Growth and the Evolution of Erythrocyte-rich Thrombi

| interaction regarding thrombus features in order to determine whether the thrombus should be aspirated or a distal protection device should be used during p-PCI. Furthermore, future studies using a combination of pathological assessment and cardiovascular imaging should be conducted to confirm the association between thrombus characteristics and underlying plaque morphology in STEMI patients.

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**Conflicts of Interest**

None declared.
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