The vulnerable plaque: From plaque instability towards thrombus instability

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Coronary atherothrombosis – what have we learned from the histopathological investigations of thrombus aspirates of patients with acute myocardial infarction?

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Chapter 5
Plaque disruption followed by acute thrombotic occlusion in the coronary artery is the primary cause of acute myocardial infarction (AMI).\textsuperscript{1} Presently, thrombus aspiration (thrombectomy) during primary PCI is introduced as class IIA recommendation for treatment of ST-segment elevation myocardial infarction (STEMI) within 12 hours.\textsuperscript{2} The aspirated atherothrombotic materials provide a unique opportunity to study in detail the various pathophysiological aspects of atherothrombosis at the site of culprit lesions in large cohorts of STEMI patients. Up to recently, most pathological studies on coronary thrombus were performed on autopsy cases of patients who died of AMI or who witnessed a sudden cardiac death. However, the ex vivo thrombus aspirates, which are now derived from large cohorts of well documented STEMI patients, allow to correlate histopathological features of the aspirates with the clinical and the angiographic characteristics of patients, including the short-term and long-term follow up after a coronary event. New insights retrieved from these investigations are reviewed in this article.

Successful retrieval of thrombus materials and sizes of aspirated samples
The rate of successful retrieval of thrombus materials varies from 41 to 95\% of the thrombus aspiration procedures.\textsuperscript{3-8} This large variation can be explained by the different approaches to study the retrieved samples, the patient population under investigation (unstable angina, NSTEMI or STEMI), or the intervals between onset of symptoms and thrombus aspiration. For the same reason, the numbers (0 to 8; mean 2.5) and also the sizes (1 to 25 mm; mean 5.1 mm) of the samples may vary considerably.\textsuperscript{5} Coronary thrombus can also be obtained by means of filtered protection devices, which contain more fragmented and smaller (mean 0,52 mm) particles.\textsuperscript{9} Based on these dimensions, more than 60\% of the aspired particles have at least the potential to cause obstruction of distal small vessels even before they reach the microcirculation.\textsuperscript{9} Until now, the largest study on thrombectomy was performed by Kramer et al. who retrieved histologically confirmed materials from 1009 out of 1362 STEMI patients (74\%).\textsuperscript{7} About 45\% of the samples were retrieved from left the anterior descending artery (LAD), 40\% from the right coronary artery (RCA) and 10\% from the left circumflex branch (LCx); some samples were obtained from a coronary bypass graft.\textsuperscript{7}

Tissue heterogeneity and component make up
The first study on thrombectomy materials was performed in 1998 by Murakami et al,\textsuperscript{5} who treated AMI with thrombus aspiration during primary PCI. The aspirated materials (n=39) were semisolid red, white or brown colored tissues, histologically
composed of two main compartments: 1) thrombus: fibrin, platelets, erythrocytes and "healing tissue" containing leucocytes, endothelial cells and smooth muscle cells; 2) plaque components: necrotic material, macrophages (foam cells), fibrous tissue and sometimes calcifications. This composition varied from patient to patient, illustrating marked tissue heterogeneity of the atherothrombotic mass at the coronary culprit site. Nagata et al\textsuperscript{3} further classified the aspirated thrombi into 3 histologically distinct categories, based on ratios between their relative contents of platelets and erythrocytes: erythrocyte-rich or mixed thrombi were usually larger than platelet-rich thrombi and more often present in RCA. On the other hand, LAD derived thrombi were mainly platelet-rich thrombi\textsuperscript{3}. Such erythrocyte-rich types of thrombus arose frequently in patients with low initial epicardial flow with a longer ischemic time, and were more often associated with distal embolization\textsuperscript{10}. Hence, distal embolization tended to occur more frequently in RCA than in LAD. Basically the same results could be extracted from the patient group that was also involved in the randomized TAPAS study.\textsuperscript{10, 11}

An alternative way to study the structural composition of retrieved thrombus was recently applied by Silvain et al. by using scanning electron microscopy,\textsuperscript{8} which allows to study a thrombus surface under high magnification. They observed fibrin as the main component (more than 60% of the thrombus surface), followed by platelets (17%), erythrocytes (12%), atherosclerotic plaque tissue (5%) and leucocytes (1%). Fibrin content appeared to be positively associated and platelet content negatively associated with ischemic time in this study.\textsuperscript{8} Such findings could have potential therapeutic implications, since large amount of fibrin on thrombus could alter the accessibility of anti-platelet or fibrinolytic medications.

\textit{The significance of leukocytes in thrombus}
After the onset of a thrombotic event, leucocytes will be recruited into the thrombus mass: leucocyte counts in thrombus are on the average nearly 18-fold higher than in peripheral blood.\textsuperscript{12} The majority of the leucocytes are neutrophils, and their recruitment starts immediately after plaque disruption, probably due to local activation of the classical complement system.\textsuperscript{13} Their numbers peak at approximately 13 hours after onset of anginal symptoms and gradually decrease thereafter.\textsuperscript{14} Several studies demonstrated that the densities of neutrophils associate positively with unfavorable clinical parameters such as low myocardial blush grade (MBG\textleq1) and inadequate ST-segment resolution, indicating impaired coronary microcirculation and resultant myocardial necrosis.\textsuperscript{14} In addition to neutrophils, also eosinophils have been observed in up to 64% of
thrombectomy samples, preferentially at boundaries between white (platelet-rich) and red (erythrocyte-rich) thrombus parts. The density of eosinophilic infiltration increases with the size of red thrombi, but not with that of white thrombi, implying that neutrophils probably stimulate the growth of red thrombus by stimulating the formation of fibrin nets.

**Progenitor cells in coronary thrombus**
The potential clinical impact of primitive stem cells (side-population cells) in AMI patients was evaluated only recently by Iwata et al. These cells, characterized in immune stains by their expression of both CD34 and bcrp-1/ABCG2 antigens, could be identified only in low numbers (2.7%) in thrombus aspirates, but their presence correlated positively with angiographic restenosis at follow-up. However, the cell surface markers (CD34 and bcrp-1/ABCG2) used in this study are not entirely specific for primitive cells since they can also be expressed on endothelial cells. It could be that these findings relate to a process of angiogenesis, which is an integral part of the process of thrombus organization overtime. Endothelial progenitor cells are also operative in the recanalization process of deep venous thrombus. Further immunohistochemical analysis of these cells population may shed light on the backgrounds of these interesting observations.

**Atherosclerotic plaque components in the thrombus mass**
Atherosclerotic plaque fragments released from ruptured lesions can be observed in 40% of the aspirated thrombus samples. Nearly all fragments are derived from soft lipid cores, which are extruded or easily suctioned out of the damaged plaque. Plaque constituents most often encountered in these materials are foam cells, lymphocytes and lipids including cholesterol crystals, which further confirm the concept of the vulnerable (high risk) plaque as a precursor lesion of the complicated thrombosed plaque. In 171 thrombus aspirates that contained such soft plaque fragments, we identified a relationship between the presence of disperse microcalcifications and intraplaque inflammatory activity. Microcalcifications were found in 39% of the samples, had a median size of 9 μm, (range 4-170 μm) and were positively associated with C-reactive protein and osteopontin, in the presence of large fields of macrophages. Since calcifications can be easily detected in plaques, and osteopontin and CRP are both strong inflammatory biomarkers for cardiovascular disease, we suggested that these distinct calcification patterns could serve as surrogate marker for plaques with high inflammatory activity.
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Presence of old coronary thrombus in STEMI patients

Thrombus organization is a process that converts a friable thrombus into stable fibrovascular-, and later even fibrosclerotic (scar) tissue, that will be incorporated in the plaque mass. Similar to wound healing, the “healing” of a thrombus is a tightly regulated multistep event overtime. Its histological parameters are well studied, especially in forensic pathology where the estimation of thrombus age can have medico legal consequences. For the purpose of studies on thrombosed coronary plaques, a 3-scale classification was applied by Rittersma et al.\textsuperscript{6}: Fresh thrombus (up to 1 day old) composed of intact blood cells; Lytic thrombus: (1–5 days) contains necrosis and fragmented nuclei (karyorrhexis); Organized thrombus (older than 5 days) shows (myo) fibroblasts or even collagen deposition and microvessels. The authors demonstrated 49\% completely fresh thrombus 35\% lytic thrombus and 16\% organized thrombus in a series of thrombus aspirates obtained from STEMI patients. In other words, old thrombus was present in nearly 50\% of the patients group, despite the acute onset of their symptoms and immediate therapeutic intervention.\textsuperscript{6} These observations suggest that coronary occlusion likely is a final result of successive episodes of plaque instability and thrombus formation that are initiated already days or even weeks before onset of STEMI. A follow up study on 1362 aspirated samples of STEMI patients showed the same outcome, old thrombus (lytic or organized) 40\% of the patients.\textsuperscript{7} The impact of these older thrombi in aspirates on the clinical outcome of STEMI patients has now been evaluated in several studies. Presence of older thrombus (either lytic or organized) in aspirates appears to be a strong predictor (1.82; CI: 1.17 to 2.85; \(P=0.008\)) of increased mortality in STEMI patients, independent of conventional risk factors such as age, diabetes mellitus or cardiac shock.\textsuperscript{4} Pathophysiological mechanisms that underlie these findings are still largely unknown and obviously require further investigation.

Thrombus age and distal embolization

A frequent and potentially important complication during primary PCI procedure is dislodgment of friable thrombus mass, which can lead to distal embolization.\textsuperscript{21, 22} Further studies on thrombus aspirates showed that distal embolization occurred more frequently in STEMI patients who had lytic (necrotic) components in their thrombus mass (21\%) than those who had either completely fresh or with organized thrombi (12\% resp 15\%). Patients with older thrombus appeared to have worse ST-segment recovery after PCI than patients with fresh thrombus, which probably relates to their increased risk of distal embolization.\textsuperscript{23} These findings show that there is a time window of several days after plaque disruption for increased risk on this complication.
Figure 1.
A – Thrombectomy of fresh thrombus, showing layered structure of completely intact blood cells (platelets, erythrocytes and leucocytes); Hematoxylin and Eosin stain
B – Detail of a thrombus with eosinophils (arrow) which are probably involved in thrombus growth; Hematoxylin and Eosin stain
C – Fragment of old thrombus, showing ingrowth of smooth muscle cell (brown); anti-α actin immunostain
D – Fragment of old thrombus, showing ingrowth of microvessels; anti-CD31/anti-D34 immunodouble stain
E – Plaque fragment in thrombectomy, showing microcalcifications (red, Alizarin red S stain) in close apposition to macrophages (blue, anti-HAM56 immunostain)
F – Plaque fragment in thrombectomy, containing large amount of tissue bound C-reactive protein (brown) in close apposition to macrophages (red); anti-CD68/anti-CRP immunodouble stain
## Table 1. Overview of histopathological studies on aspirated coronary thrombus material (1998-2011)

<table>
<thead>
<tr>
<th>First Authors</th>
<th>Year</th>
<th>Clinical characteristics</th>
<th>Nr. of patients</th>
<th>Nr. of retrieved thrombi</th>
<th>Devices</th>
<th>Major findings on thrombus aspirates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murakami23</td>
<td>1998</td>
<td>AMI, onset time not mentioned</td>
<td>91</td>
<td>30</td>
<td>Probing11**</td>
<td>Description of heterogeneous composition of coronary thrombectomy materials</td>
</tr>
<tr>
<td>Kotera24</td>
<td>2002</td>
<td>AMI of &lt;72h and UAP</td>
<td>46</td>
<td>55</td>
<td>Rescue22*</td>
<td>Plaque materials released during angioplasty can be associated with no-reflow phenomenon</td>
</tr>
<tr>
<td>Nagata25</td>
<td>2004</td>
<td>AMI &lt;24h</td>
<td>231</td>
<td>77</td>
<td>Rescue22*</td>
<td>Erythrocyte-rich thrombi are larger than platelet-rich ones and more frequently present in RCA.</td>
</tr>
<tr>
<td>Angelini26</td>
<td>2004</td>
<td>SAP and UAP</td>
<td>39</td>
<td>37</td>
<td>Angioguard23*</td>
<td>Numerous tissue fragments released during PCI, which are large enough to cause distal embolization. Fragments of UAP are larger than those of SAP</td>
</tr>
<tr>
<td>Rittersma27</td>
<td>2005</td>
<td>STEMI &lt;24h</td>
<td>211</td>
<td>199</td>
<td>Rescue22*</td>
<td>Old thrombi (&gt;1 day) in 50% of thrombectomy materials of acute STEMI patients</td>
</tr>
<tr>
<td>Admbrecht28</td>
<td>2007</td>
<td>STEMI, onset time not mentioned</td>
<td>52</td>
<td>35</td>
<td>X-Sizer29*</td>
<td>Large numbers of leukocytes in aspirates. Substantial vasoconstriction probably relates to presence of endothelin in the aspirated thrombus</td>
</tr>
<tr>
<td>Vlaar29</td>
<td>2008</td>
<td>STEMI &lt;12h</td>
<td>160</td>
<td>115</td>
<td>Diver30*</td>
<td>Large-intimalumen catheter (Diver) does not result in retrieval of larger thrombotic particles, nor better clinical outcomes. Erythrocyte-rich thrombus is associated with lower patients</td>
</tr>
<tr>
<td>Kramer31</td>
<td>2009</td>
<td>STEMI, onset time not mentioned</td>
<td>1362</td>
<td>1009</td>
<td>Rescue22*</td>
<td>Lytic thrombi are associated with distal embolization</td>
</tr>
<tr>
<td>Sakai32</td>
<td>2009</td>
<td>ACS, onset time not mentioned</td>
<td>209</td>
<td>165</td>
<td>Rescue22*</td>
<td>Density of eosinophils in the thrombus is associated with thrombus size and percentage of red thrombus area</td>
</tr>
<tr>
<td>Distelmajer33</td>
<td>2009</td>
<td>AMI, variable onset times not mentioned</td>
<td>47</td>
<td>Not mentioned</td>
<td>Thrombuster34*</td>
<td>High neutrophil density in aspirated thrombus is associated with impaired coronary microcirculation and resultant myocardial function</td>
</tr>
<tr>
<td>Fokkema35</td>
<td>2009</td>
<td>STEMI x 12h</td>
<td>883</td>
<td>300</td>
<td>Export36*</td>
<td>The presence of erythrocyte component and the sizes of aspirated materials are positively associated with angiographically visible distal embolization after PCI</td>
</tr>
<tr>
<td>Kramer31</td>
<td>2009</td>
<td>STEMI &lt;12h</td>
<td>1362</td>
<td>1009</td>
<td>Rescue22*</td>
<td>Old thrombi (&gt;1 day) in 40% aspirated materials from STEMI.</td>
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</tr>
<tr>
<td>Verouden37</td>
<td>2010</td>
<td>STEMI &lt;12h</td>
<td>1176</td>
<td>601</td>
<td>Rescue22*</td>
<td>Better ST-segment recovery at the end of PCI in patients with aspirated fresh thrombus than in patients with aspirated old thrombus</td>
</tr>
<tr>
<td>Iwata38</td>
<td>2010</td>
<td>STEMI &lt;72h</td>
<td>62</td>
<td>108</td>
<td>Thrombuster34*</td>
<td>Identification of primitive cells in the coronary thrombus, which are associated with restenosis</td>
</tr>
<tr>
<td>Silvain39</td>
<td>2011</td>
<td>STEMI &lt;12h</td>
<td>288</td>
<td>45</td>
<td>Export36*</td>
<td>Fibrin content correlates positively and platelet content correlates negatively with ischemic time</td>
</tr>
<tr>
<td>Barba40</td>
<td>2011</td>
<td>STEMI, onset time not mentioned</td>
<td>100</td>
<td>59</td>
<td>Pronto31*</td>
<td>MRI is unable to assess the age of a thrombus</td>
</tr>
<tr>
<td>Li41</td>
<td>2011</td>
<td>STEMI &lt;24h</td>
<td>562</td>
<td>171</td>
<td>Rescue22*</td>
<td>Microcalcifications in aspirates identify plaques with high inflammatory burden</td>
</tr>
</tbody>
</table>

ACS: acute coronary syndromes; AMI: acute myocardial infarction; MRI: magnetic resonance imaging; PCI: percutaneous coronary intervention; RCA: right coronary artery; SAP: stable angina pectoris; STEMI: ST-segment elevation myocardial infarction; UAP: unstable angina pectoris
It can be speculated that during this time the thrombus is very friable due high apoptotic and proteolytic activity that initiates the lytic changes in the thrombus, and increases the risk of dislodgment.

Role of embolic plaque debris in no-reflow phenomenon
Kotani et al. evaluated the role of embolic plaque debris on occurrence of no-reflow phenomenon during mechanical reperfusion. Thrombus aspirates were retrieved before and after mechanical angioplasty. The aspirates obtained before the angioplasty showed no difference between patients with and without no-reflow phenomenon, but in aspirates collected after the angioplasty, there were significantly larger amounts of plaque debris in patients with no-reflow phenomenon. Most no-reflow phenomena were ameliorated when the intraluminal atherosclerotic debris was removed, which shows also plaque debris contributes significantly to a post-angioplasty no-reflow phenomenon.

Can thrombus composition be detected in vivo?
As mentioned above, thrombus compositions itself may serve as a prognostic marker for not only peri-procedural complications but also long-term mortality of STEMI patients treated with primary PCI. This implies that determination of the component make up of thrombus for example with the use of clinical imaging tools could potentially contribute to stratification of risk profiles of patients and guide clinical decisions on treatment.

Barba et al tried to assess the histological composition of thrombus materials aspirated from STEMI patients with use of magnetic resonance imaging (MRI), but could not differentiate between fresh, lytic and organized thrombi. However, other high resolution techniques are presently available for the virtual histological imaging of intracoronary tissues. Optical computed tomography (OCT) is such a high resolution tech-
nique with a resolution of 10 μm and is widely now used in the catheterization unit. With use of this new modality, the components make up of intracoronary thrombus can probably be evaluated in vivo in the near future, which is now under intense investigation.

Conclusion
Ex vivo histological examination of thrombus materials with thrombectomy in STEMI patients provide a unique opportunity to obtain information on the pathology of coronary atherothrombosis. The aspirates derived from STEMI patients have revealed that there appears to be a time window of several days to perhaps weeks after plaque disruption in many patients, in which there is a clear risk of thrombus embolization and/or sudden thrombotic occlusion. Moreover, the presence of older thrombus in aspirates in the same patient population can be seen as an independent predictor of long-term mortality. Further investigations on thrombus aspirates are needed, and are in progress, in order to elucidate the pathophysiological mechanisms that underlie these interesting findings, which have the potential to serve as a biomarker or may otherwise have an impact on clinical decision making and clinical outcome in near future.
References


Chapter 5


