MINIREVIEWS

Thrombosis in ST-elevation myocardial infarction: Insights from thrombi retrieved by aspiration thrombectomy

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Abstract

In patients with ST-elevation myocardial infarction, recurrent cardiovascular events still remain the main cause of morbidity and mortality, despite significant improvements in antithrombotic therapy. We sought to review data regarding coronary thrombus analysis provided by studies using manual aspiration thrombectomy (AT), and to discuss how insights from this line of investigation could further improve management of acute coronary disease. Several studies investigated the fresh specimens retrieved by AT using techniques such as traditional morphological evaluation, optical microscopy, scanning electron microscopy, magnetic resonance imaging, and immunohistochemistry. These approaches have provided a better understanding of the composition and dynamics of the human coronary thrombosis process, as well as its relationship with some clinical outcomes. Recent data signaling to new antithrombotic therapeutic targets are still emerging.

Key words: Myocardial infarct; Aspiration; Mechanical; Thrombectomy; Thrombus; Immunohistocytochemistry

Core tip: This paper describes the importance of coronary thrombosis as a direct effector of ST-elevation acute myocardial infarction, reviewing important data provided by coronary aspiration thrombectomy regarding thrombus composition and its relationship with clinical variables. The knowledge of such data is an important basis for improving antithrombotic therapy, as it signals for potential new therapeutic targets.

INTRODUCTION

Over the past years, improvements in antithrombotic and reperfusion therapies have been associated with

decreasing mortality in the setting of ST-elevation acute myocardial infarction (STEMI)[1]. However, coronary artery disease (CAD) remains the leading cause of death worldwide[2], so that efforts are still needed in order to better treat this condition. In most cases, STEMI is caused by the disruption of vulnerable atherosclerotic plaques associated with intense inflammatory activity of a dysfunctional endothelium. Such rupture is the trigger for platelet activation and aggregation and thrombin formation, culminating with total occlusion of the coronary artery by thrombus[3].

Because of the pivotal role of thrombus as a final effector of coronary occlusion and ischemic injury in most cases of acute coronary syndromes, many efforts have been made to improve antithrombotic therapy. For example, antithrombotic drugs like prasugrel and ticagrelor, as compared to clopidogrel, have shown to reduce ischemic events and even mortality in STEMI patients[4,5]. Recently, a large clinical trial demonstrated that double antiplatelet therapy with ASA and ticagrelor significantly reduced the risk of cardiovascular death, myocardial infarction (MI), or stroke in patients with previous MI[6].

Despite of these significant improvements in the medical treatment of patients with CAD, recurrent cardiovascular events still remain the main cause of morbidity and mortality, which justifies further studies to better understand the physiopathology of human coronary thrombosis.

ASPIRATION THROMBECTOMY

Percutaneous coronary intervention (PCI) has been shown to be the preferred method of reperfusion in patients with ST-elevation acute MI[7]. The high thrombotic burden and the subsequent compromise of coronary flow after dilatation and stent implantation in many cases stimulated the development of adjunctive devices designed to remove thrombi. The manual aspiration thrombectomy (AT) technique was the most successful of such approaches, and it has gained widespread use after the demonstration of improved angiographic results and clinical outcomes in the TAPAS trial[8]. On the other hand, enthusiasm over this technique has substantially waned after recent reports of lack of benefit in the large TASTE and TOTAL trials[9-11].

The demonstration of lack of clinical benefit of AT in these trials is not fully understood yet. One possible explanation is that manual thrombectomy was not effective enough, which is supported by a recent TOTAL substudy using optical coherence tomography[12]. In this analysis, there was no difference between the two groups of patients randomized (routine upfront manual thrombectomy *vs* PCI alone) with respect to the mean amount of thrombus, although this residual amount was relatively low on average. Another substudy, evaluating angiographic variables, found a 30% reduction in the distal embolization in favor of the thrombectomy group,

being this surrogate endpoint an independent predictor of mortality[13]. Assuming that for every 10 patients who have distal embolization, maybe one or two will die related to that, we would expect a reduction of mortality in the range of 10% or 15%, a difference which no trial was powered to detect.

Regardless of the clinical appropriateness of AT in current practice, its development has made possible a new line of investigation, with the opportunity of analyzing fresh specimens of *in vivo* coronary thrombi, assessing morphology, histology, immunohistochemistry and others[14,15]. Before the availability of this procedure, studies of coronary thrombi were performed mainly by post-mortem analyzes, angioscopy or *ex-vivo* analysis[16-20]. The information derived from post-mortem studies is reliable, but it is always limited by the selection bias that occur when studying only patients who died. Angioscopy provides *in vivo* information of thrombi morphology and color, but it has been used rarely due to technical difficulties of the method. Experimental studies, like the Badimon chamber[21] and others, are limited by not evaluating the process of human coronary thrombosis *in vivo*.

On the other hand, AT is limited by the relative frequent occurrence of unsuccessful procedures, which have been reported in approximately 25% of the patients[8]. Potential causes for failing to retrieve thrombotic material are partial lyses of thrombi by pharmacological therapy administered before arrival in the catheterization laboratory, non-thrombotic lesions, distal embolization before aspiration and limitations of the current aspiration devices. Challenging anatomies for performing AT include tortuous and/or calcified vessels, bifurcations, very distal lesions and small vessels[22].

Morphology of coronary thrombi

Thrombus varies widely in shape and size. Arterial thrombi usually are about one centimeter long, arising at the site of an endothelial injury (for example, an atherosclerotic ruptured plaque) in the retrograde direction from the point of anchorage. It generally consists of a tangled network of variable amounts of platelets, fibrin, erythrocytes and degenerate leukocytes[23].

In patients with acute coronary syndromes, there are several factors associated with thrombus size, such as the intensity of anticoagulant and antithrombotic therapy[24,25], the age of the thrombus[14,26], and the presence of flow in the infarct-related artery before primary PCI[18]. Thrombus burden is an established predictor of complications during PCI with or without stents[27,28].

Another condition that may influence the characteristics of coronary thrombi is the presence of diabetes mellitus (DM). In this setting, thrombus area seems to be greater[21] and coronary plaques present greater total and distal plaque load than in those subjects without DM[16]. Moreno *et al*[29], evaluating coronary tissue retrieved by atherectomy, found a large content of lipid-rich atheroma, macrophage infiltration and subsequent
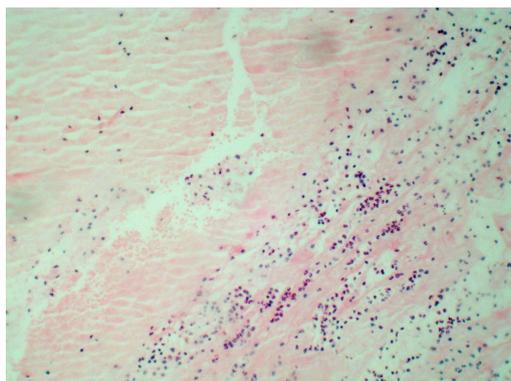
Figure 1 Recent coronary thrombus composed of fibrin, white blood cells and red blood cells, hematoxylin-eosin, 200 ×.
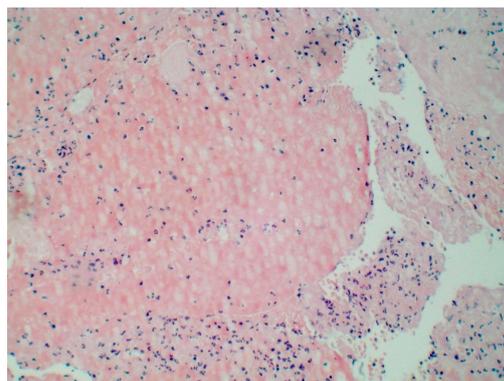

Figure 2 Coronary thrombus with lysis focuses in few neutrophils, hematoxylin-eosin, 200 ×.

thrombosis in patients with DM.

According to the macroscopic appearance, thrombi can be classified as white, red or mixed. White thrombi are mainly composed of platelets and fibrin[30]. Mizuno and cols showed that white thrombi occur when blood flow was not completely interrupted in the vessel[18]. In patients with STEMI, we have previously demonstrated that white thrombus has a smaller size when compared to red thrombus, and is associated with high fibrin infiltration, shorter ischemic times and lower mortality[31]. Red thrombi are wet, gelatinous and resemble a blood clot being formed by fibrin, erythrocytes and platelets[30], causing complete occlusion of the vessel[18].

Thrombi can also be classified according to its age: (1) recent (newly formed), composed primarily of fibrin, white blood cells and red blood cells (Figure 1); (2) lytic (intermediate), characterized by the presence of apoptosis of leukocytes (Figure 2); and (3) organized thrombi, classified mainly by presenting collagen and connective soft tissue[14,17].

Rittersma *et al*[14] assessed coronary thrombi age in 199 STEMI patients submitted to AT within 6 h after onset of chest pain. The authors found that in at least 50% of patients, coronary thrombi were days or weeks old, indicating a variable period of plaque instability and thrombus formation initiated before onset of symptoms. These findings were later confirmed by another report by Kramer *et al*[26]. In an important study with more than 1300 STEMI patients, fresh thrombus was identified in approximately 30% of the patients. The mortality rates at the 4-year follow-up were significantly higher in patients with older thrombi (16%) when compared to those with fresh thrombus (7%)[32].

Silvain *et al*[15] used magnetic resonance imaging to evaluate the composition of coronary thrombus and its association with ischemic time. It was found that fibrin content increased with ischemic time, ranging from 48% (< 3 h) up to 67% (> 6 h), whereas platelet content decreased from 21% (< 3 h) to 9% (> 6 h). Multivariate analysis indicated that ischemic time was the only predictor of thrombus composition, with a 2-fold increase of fibrin content per ischemic hour[15].

Immunohistochemical analysis

Immunohistochemistry detects surface proteins in the cells of tissues using the principle of antibodies binding specifically to antigens. It is used in specimens removed surgically or in autopsies. In the assessment of thrombi retrieved by AT, this can also be an additional tool to histopathology, in order to increase the sensitivity for recognition of thrombus components[33,34].

Ikuta *et al*[35] compared thrombotic material from individuals with stable or unstable angina with immunohistochemistry analysis. The patients with unstable coronary syndromes presented higher platelet aggregation and activation, and also increased immunoreactivity of GP Ⅱb/Ⅲa and P-selectin[35].

Iwata *et al*[36] analysed the cellular constituents of 108 thrombi aspirated from coronary lesions in 62 patients who underwent emergent intervention for the treatment of acute (< 24 h) or recent (24-72 h) STEMIs. The content of platelets, as determined by immunostaining for CD42a, presented a negative correlation with the time since the onset of chest pain. The ratio of CD34-positive cells in intracoronary thrombi had a significant positive correlation with restenosis at follow-up coronary angiography. This finding indicates that the early accumulation of primitive cells in platelet aggregates may play a role in neointimal growth after successful coronary intervention in patients with acute coronary syndromes.

Sambola *et al*[37] compared the content of thrombotic and fibrinolytic factors in thrombi of patients submitted to rescue PCI to those with successful thrombolysis. Thrombi resistant to lysis showed higher content of platelets, fibrin, P-selectin and Von Willebrand Factor, demonstrating a disturbance in thrombus structure of these patients.

Yamashita *et al*[38] examined thrombi removed within 24 h of acute MI with immunohistochemistry techniques, focusing on possible mechanisms of thrombosis in patients with DM. There was a paucity of CD34-positive cells in the specimens analyzed, suggesting that the ability of these cells to down-regulate thrombus formation and facilitate thrombus organization was

Table 1 Studies evaluating aspirated thrombus characteristics of ST-elevation acute myocardial infarction patients

Ref.	Main comparison/subject	n	Results
Quadros *et al*[31]	White *vs* red thrombus	113	Mortality (0% *vs* 10.1%; P = 0.05), size (0.4 ± 0.2 *vs* 0.6 ± 0.4 mm, P < 0.001), fibrin (68% ± 19% *vs* 44% ± 18%, P < 0.001), ischemic time (4.5 ± 2.3 h *vs* 6.1 ± 3.1 h, P = 0.01)
Rittersma *et al*[14]	Age of intracoronary thrombi	199	Organized: 9%, lytic changes: 35%, fresh: 49%, both fresh and organized: 7%
Kramer *et al*[26]	Older *vs* fresh thrombus	1315	All-cause mortality at 4 yr (16.2% *vs* 7.4%, hazard ratio: 1.82, 95%CI: 1.17-2.85, P = 0.008)
Silvain *et al*[15]	Composition of coronary thrombus and its association with ischemic time	45	Fibrin content: 48.4% ± 21% (< 3 h) up to 66.9% ± 9% (> 6 h) (P = 0.02)
Iwata *et al*[36]	Restenosis *vs* without Restenosis	108	CD34-positive primitive cells (5.10% ± 0.66% *vs* 1.88% ± 0.24%, P < 0.01)
Sambola *et al*[37]	Thrombus resistant to fibrinolysis *vs* sensible to lysis	20	Rescue PCI: Significantly higher levels of fibrin (P = 0.016), P-selectin (P = 0.03) and VWF (P = 0.03) than patients who were underwent to primary PCI
Yamashita *et al*[38]	Thrombosis in diabetics *vs* non diabetics	50	Paucity of CD34-positive cells and higher expression of HMGB-1 in diabetics

PCI: Percutaneous coronary intervention; VWF: Von willebrand factor; HMGB-1: High-mobility group box-1.

compromised in diabetic patients. On the other hand, the higher expression of HMGB-1 found in those with DM, in association with the thrombin-induced microvascular thrombosis accelerated by HMGB-1, may contribute to the adverse events frequently seen in these patients[38].

FUTURE PERSPECTIVES

In the previous sections of this paper, we have described several studies that aimed to investigate the physiopathology of human coronary thrombosis by studying specimens of thrombi retrieved by AT (Table 1). The majority of those studies used techniques such as traditional morphological evaluation, optical microscopy, scanning electron microscopy, magnetic resonance imaging, and immunohistochemistry. More recently, novel approaches have been described.

Ramaiola *et al*[39] applied principles of proteomics and advanced cellular microscopy to evaluate retrieved coronary thrombi. The authors showed that profilin-1 (Pfn-1) levels in the systemic circulation are directly correlated to the duration of coronary artery thrombotic occlusion. Thrombus age is an independent predictor of long-term mortality[32], and these results may suggest that measuring Pfn-1 levels could be used to assess ongoing thrombosis and occlusion time in clinical practice[39].

The immune response mediated by lymphocytes is involved in the pathogenesis of the acute coronary syndromes[3], but there is few evidence of the role of T cells in thrombus composition. Regulatory T cells (Treg) are an inherent anti-inflammatory component of adaptive immunity which exerts atheroprotective effects[40-44]. Treg were frequently identified among T cell subsets present in coronary thrombi of patients presenting with ACS[45], which raises the hypothesis of a local compensatory mechanism to attenuate inflammation[46]. The concept of expanding antigen-specific Treg to diminish vascular inflammation and atherothrombosis by immunotherapy is appealing and may represent a new line of investigation[45].

CONCLUSION

Thrombosis plays a central role in acute coronary syndromes. A better understanding of the human coronary thrombosis process *in vivo* and its relationship with clinical outcomes could be obtained by analyzes of specimens obtained by AT. Recent data signaling to new therapeutic targets has been recently provided, and insights from this line of investigation will help to further improve management of acute coronary disease.

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