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Research paper

Immunohistochemical characteristics of coronary thrombi in ST-elevation myocardial infarction

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ABSTRACT

Background and aims: The dynamics and implications of intracoronary thrombus constituency in patients with ST-segment elevation myocardial infarction (STEMI) are not fully understood. We evaluated the expression of CD34, CD61and factor VIII surface markers in thrombi of patients with STEMI and its association with clinical and angiographic characteristics and major adverse cardiovascular events (MACE).

Methods: Patients presenting with STEMI undergoing aspiration thrombectomy during primary percutaneous coronary intervention (pPCI) were included. Morphological, histopathological and immunohistochemical aspects of thrombi were assessed by two pathologists blinded to clinical variables and outcomes.

Results: The mean age of the 245 patients included was 58 ± 12 years old, and 70 % were men. Regarding the thrombi microscopic patterns, 61 % were classified as recent, 20 % as lytic and 19 % as organized. There were higher levels of the CD61 index in patients with a history of heart failure. Smokers presented lower CD61 positive cells and CD61 index, but this association did not remain significant after multivariable analysis. There was an inverse correlation between CD61 positive cells and CD61 index with the time from onset of pain to the first medical contact, but no other significant association amongst clinical characteristics and antigenic expression. There was higher expression of the CD61 antigen in patients with in-hospital MACE, but statistical significance was borderline ($p = 0.06$).

Conclusions: In this cohort of patients with STEMI, immunohistochemistry of coronary thrombus showed a significantly higher platelet content in patients with previous heart failure and a trend in those with in-hospital MACE. Thrombus' platelet content was inversely related to ischemic time.

1. Introduction

Primary percutaneous coronary intervention (pPCI) is the preferred reperfusion therapy in patients with acute ST-elevation myocardial infarction (STEMI) [1]. In this clinical scenario, intracoronary thrombus burden and its composition are associated with a higher risk of distal embolization, stent thrombosis and recurrent cardiovascular events [2–8]. The performance of aspiration thrombectomy (AT) during the pPCI procedure has brought along a unique opportunity for investigating the dynamics of thrombosis *in vivo*, by retrieving fresh specimens of coronary thrombi, and allowing the assessment of its cellular composition, morphology, histology and patterns of protein expression [9,10]. In counterpart, the pathophysiological role of the different thrombus' constituents is still poorly understood.

In order to increase the sensitivity for recognition of thrombus components, immunohistochemistry can be an additional tool to histopathology in the assessment of the thrombi retrieved by AT [11,12]. It is possible to estimate the number of endothelial cells and platelet and coagulation cascade activation in intracoronary thrombi by testing their immunoreactivity to CD34, CD61, and factor VIII, respectively. CD34 expression may be increased in vessels affected by atherosclerosis, as well in thrombi during the propagation and organization/recanalization phases. CD61 expression is associated with platelet glycoprotein IIIa, which is a marker of platelets and megakaryocytes. Finally, activation of factor VIII is an important step in the early events of thrombus formation [11,13–15]. However, available studies have limited samples, provided controversial findings and scarce investigation of the associations between cellular parameters and clinically relevant outcomes

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[13,14,16–28].

Therefore, we aimed to assess the association between protein expression levels of CD34, CD61 and factor VIII in coronary thrombi of patients with STEM with clinical characteristics and outcomes.

2. Methods

2.1. Patients

All consecutive patients with STEMI admitted to our institution between April 2010 and November 2012 were considered for the study. Patients were invited to participate during the index hospitalization for STEMI. All participants signed the informed consent. This study was conducted in accordance with the principles outlined in the 1975 Declaration of Helsinki and it was approved by the local Ethical Research Committee.

The inclusion criteria were age >18 years, STEMI in the first 12 h after pain onset, and indication to pPCI with thromboaspiration during the index STEMI. STEMI was defined as thoracic pain lasting >30 min since pain onset, and ST segment elevation > 0.1 mV in two or more contiguous leads. Exclusion criteria were failure to retrieve thrombi or insufficient thrombus material to be analyzed.

2.2. Percutaneous coronary intervention procedures

Our institution is a high-volume tertiary referral center for interventional cardiology, with an average of 2500 PCIs performed per year. Antiplatelet therapy followed institutional routines for STEMI: 300 mg aspirin and 300–600 mg clopidogrel on hospital admission. Heparin (60–100 U/kg) was administered before coronary guide wire introduction, and all procedures were performed according to standard pPCI techniques [29,30].

The decision to perform thromboaspiration during the pPCI procedure was made at operator discretion, as were technical aspects such as type and number of stents, use of any other devices, and glycoprotein IIb/IIIa use. Aspiration thrombectomy was performed with one of the following devices: Export (Medtronic Vascular Inc., Santa Rosa, CA), Diver (INVATEC, Brescia, Italy), or Pronto (Vascular Solutions, Minneapolis, MN). In every case, aspiration was attempted before balloon dilatation (as in the TAPAS trial) [31], and several passages at the site of occlusion were performed. Aspirated blood and intracoronary material were collected in the filter.

2.3. Thrombus analysis

Immediately after thrombus removal from the coronary artery, the sample was embedded in 10 % formalin. Next, the samples were fixed in paraffin for 24 h, and then sliced into 3 μm sections and placed on appropriate slides. Tissue sections were stained with hematoxylin-eosin or submitted to the immunohistochemistry protocol in order to evaluate the following parameters: microscopic pattern (recent, lytic, or organized), percentage of fibrin, and expression of CD34, CD61, and factor VIII.

For the immunohistochemistry analysis, 3-μm tissue sections were placed into silanized slides. Next, each slide was submitted to deparaffinization, using xylene and hydration with ethanol. Antigen retrieval was performed in a microwave oven with a 10 mM/pH 6.0 citric acid solution, in two cycles of 9 min each, at 750 W. The endogenous peroxidase blockage was performed with 3 % hydrogen peroxide (10 v·v). The primary antibody was diluted in 1 % albumin and 0.1 % sodium azide in PBS (phosphate-buffered saline), incubated in a moist chamber for 30 min at 37 °C, and then the material was kept under refrigeration at 4 °C for 18 h. The biotinylated secondary antibody was incubated in a moist chamber at 37 °C, for 30 min, as well as the streptavidin-biotin-peroxidase complex (StreptABC). The chromogenic substrate used was 60 mg diaminobenzidine in PBS, and Harris hematoxylin was used as counter-stain.

The following primary antibodies were used: CD34 (monoclonal mouse anti-human QBEn-10, dilution ratio 1:50, DAKO, Santa Clara, CA, USA), CD61 (monoclonal mouse anti-human 2F2, dilution ratio 1:50, Ventana, Oro Valley, Arizona, USA), and von Willebrand factor (factor VIII) (policlonal mouse anti-human A0082, dilution ratio 1:50, Cell Marque, Darmstadt, Germany). In all cases, an immunohistochemistry panel was created. The expression obtained by blood cells and/or components of the specimen was revealed by the presence of brown staining in different areas of the sample (Fig. 1). A strong positive expression was considered when the immunohistochemistry reaction showed strong and diffuse brown staining in several cells of the analyzed tissue. Moderate positive expression was defined when the immunohistochemistry reaction showed a moderately intense brown (light brown) staining in some cells of the analyzed tissue. In each case, the analysis of the entire slide was performed by the pathologist using optical microscopy, considering as a positive result for CD34 any number of positive cells for CD34 antibody. Regarding Factor VIII and CD61 expression, the sum of all positive cells in the slide for their respective antibodies was described. The expression level of each antigen was adapted from the Quickscore method [32] and refers to the product between the number of positive cells and the intensity of the staining.

Recent thrombi were characterized by the presence of fibrin, leukocytes, and erythrocytes (Fig. 2). Thrombi with lysis were characterized by the occurrence of leukocyte apoptosis/degeneration/lysis (Fig. 3). Organized thrombi were characterized by the presence of loose connective tissue [9]. All histological analyses were performed by a pathologist blinded to the clinical characteristics of patients.

2.4. Clinical characteristics and follow-up

Patients were interviewed on hospital admission, and clinical, laboratory, angiographic and procedural characteristics were prospectively assessed and recorded in a dedicated database. During hospitalization, patients were followed by one of the investigators for assessment of cardiovascular events. After hospital discharge, patients were contacted by telephone for outcome assessment.

The diagnosis of previous heart failure was based on history of typical symptoms, use of specific heart failure's pharmacological treatment and/or previous echocardiographic variables indicating elevated left ventricular filling pressures or left ventricular ejection fraction under 50 %.

Major cardiovascular events (MACEs) were assessed and registered. MACE was defined as a combination of death, recurrent myocardial infarction (MI), stroke and urgent revascularization. Recurrent MI was defined by recurrent chest pain with new elevation of serum biomarkers, after the initial decline of the natural curve, with ST-segment elevation or new Q waves. Stroke was defined as a new, sudden-onset focal neurological deficit, of presumably cerebrovascular cause, irreversible (or resulting in death) within 24 h and not caused by another readily identifiable cause. Need for urgent revascularization was defined as a non-scheduled procedure to treat acute myocardial ischemia.

2.5. Statistical analysis

Data were analyzed using SPSS for Windows (IBM, version 26.0). Quantitative variables were expressed as mean ± standard deviation or as median and interquartile range (25–75 %). *t*-Test and ANOVA were used to analyze differences between antigenic expression according to clinical and angiographic characteristics and clinical outcomes. Correlations were evaluated by Pearson's or Spearman's method. Multivariate regression analysis was used to assess potentially confounding factors on antigenic expression. Statistical significance was $p < 0.05$.

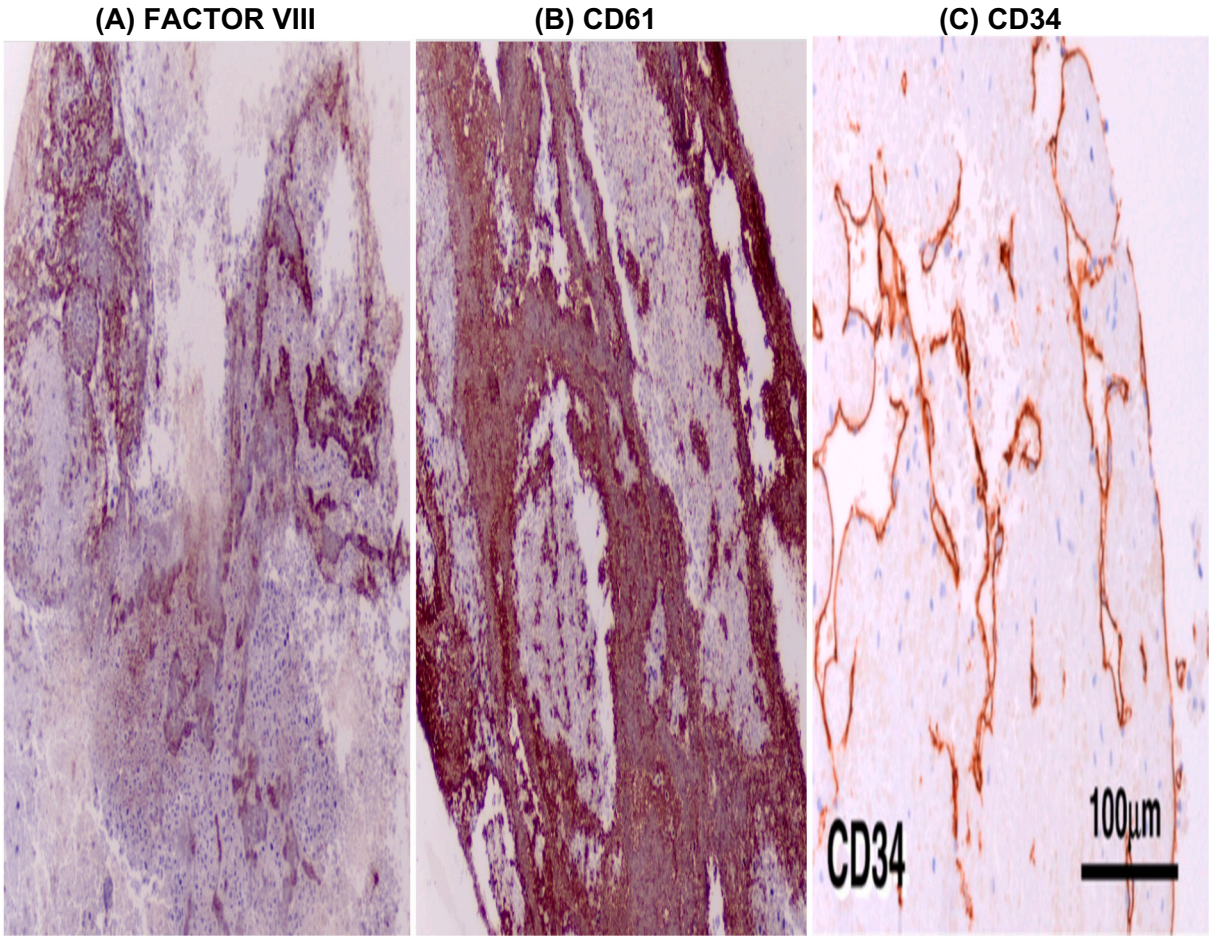

Fig. 1. Immunohistochemistry panels using CD34 (C), CD61 (B) and factor VIII (A) primary antibodies in coronary thrombi.

3. Results

3.1. Patients and procedures

In the study period, 1548 STEMI patients were admitted at our center, and AT was performed in 495 patients (32 %). Of these, 245 patients with aspirated coronary thrombi material available for immunohistochemical analysis were included in this study (Fig. 4).

Clinical and angiographic characteristics of study patients are shown in Table 1. The mean age was 58 ± 12 years, most patients were male, half of the patients had hypertension and one quarter presented diabetes mellitus. One third had dyslipidemia and half of the patients were smokers. The median time from the onset of chest pain to first medical contact was 3.2 h [1.4–6], while the median ischemic time between the onset of symptoms and pPCI was 4.4 h [3.1–7.2].

During the index hospitalization, 19 patients died (7.7 %), 8 patients had re-infarction (3.3 %) no patients sustained stroke and 7 patients required urgent revascularization (2.8 %). In-hospital MACE occurred in 24 patients (9.8 %).

3.2. Histopathological and immunohistochemical analyses

Antigenic expression according to clinical characteristics is shown in Table 2. We identified higher levels of the CD61 index (216 ± 113 *vs.* 145 ± 101; p = 0.03) in patients with a history of heart failure. During the index hospitalization, LV function was assessed in almost half of the study sample (109 patients). In this subgroup, the mean LV ejection fraction was lower in patients with CD34 positive thrombi than in those with CD34 negative (50,2 ± 13,6 × 56,3 ± 12,9; p = 0.018). CD61 positive cells were lower in smokers (smokers: 41 ± 26 cells; non-smokers: 48 ± 27 cells; p = 0.03), as well as CD61 index (smokers: 133 ± 94; non-smokers: 164 ± 107; p = 0.01). However, this association did not remain significant after multivariable analysis (p = 0.618). There was no other significant association amongst clinical characteristics and antigenic expression.

Antigenic expression according to angiographic and procedural aspects is shown in Table 3. There was an inverse correlation between CD61 positive cells and CD61 index with the time from onset of pain to the first medical contact (r = −0.12, p = 0.05; r = −0.13, p = 0.04) and with ischemic time (r = −0.13, p = 0,05; r = −0.14, p = 0.03). There were no other significant association amongst other characteristics and antigenic expression.

Regarding the thrombi microscopic patterns, 61 % were classified as recent, 20 % as lytic and 19 % as organized. With respect to fibrin content, we found a 54 % mean percentage in the thrombotic material. There were no differences regarding antigenic expression according to specific histological patterns (Table 4).

Table 5 shows antigenic expression according to the occurrence of in-hospital cardiovascular events. There was higher expression of the CD61 antigen in patients with in-hospital MACE, but statistical significance was borderline. The other clinical events assessed did not present statistically significant associations with the antigens studied.

4. Discussion

In the present study, we evaluated the expression of CD34, CD61and factor VIII surface markers in thrombi of patients with STEMI and its association with clinical characteristics and MACE. Our main findings

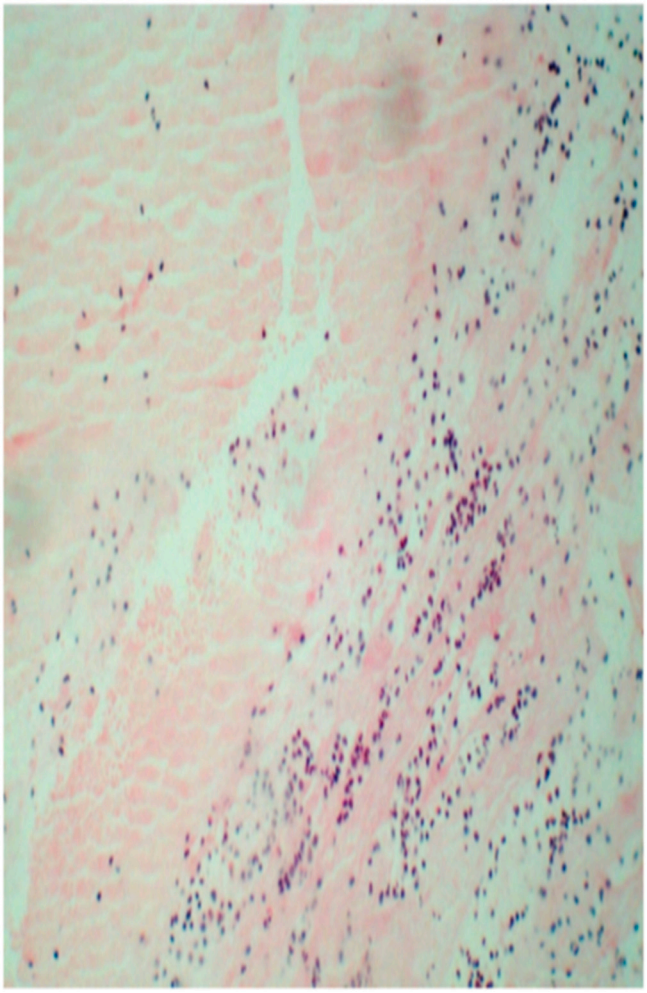

Fig. 2. Recent thrombi were characterized by the presence of fibrin, leukocytes, and erythrocytes.

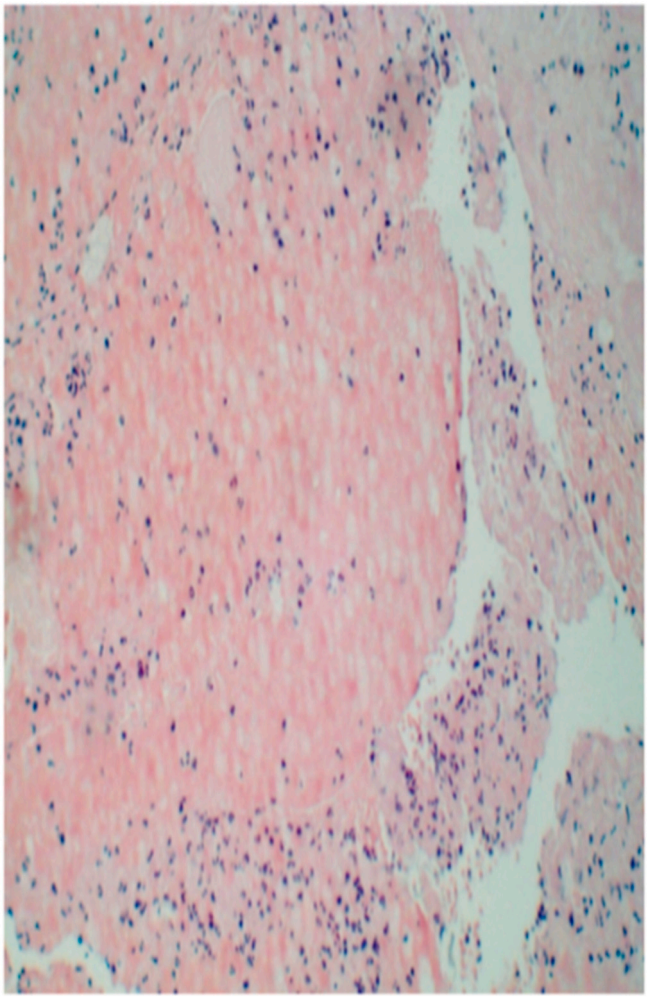

Fig. 3. Thrombi with lysis were characterized by the occurrence of leukocyte apoptosis/degeneration/lysis.

were a higher expression of CD61 in thrombus of patients with previous heart failure presenting with STEMI, an inverse correlation between CD61 expression and ischemic time and a trend toward higher CD61 levels in patients with MACE. CD61 was less present in thrombus of smoker patients, but this did not remain significant after multivariable analysis. There were no other significant association or correlations amongst clinical, angiographic and procedural aspects and molecular markers, as well there was no association between those markers and histological thrombus type. Our study is amongst the largest reports addressing histological and immunohistochemistry aspects of thrombus in patients with STEMI in contemporary practice.

An important finding of our study was the increased expression of CD61 index in patients with previous systolic or diastolic heart failure, which represents a higher platelet content associated with this situation. Also, the mean LV ejection fraction was lower in patients with CD34 positive thrombi than in those with CD34 negative. It has been previously demonstrated that tissue responsiveness to direct nitric oxide (NO) donors in blood vessels and platelets are diminished when heart failure is present [33,34]. Borgognone et al. compared platelet responses to nitrite and the NO donor sodium nitroprusside (SNP) in age-matched healthy volunteer controls (n = 12), HF preserved ejection fraction–atrial fibrillation (AF) patients (n = 29), and chronic AF patients (n = 8). Anti-aggregatory effects of nitrite in the presence of NO scavengers/soluble guanylate cyclase (sGC) inhibitor were determined and vasodilator-stimulated phosphoprotein (VASP) phosphorylation was assessed using western blotting. The authors showed for the first time that HFpEF-AF (but not chronic AF without HF) was associated with marked impairment of platelet NO responses due to sGC dysfunction [35].

The present study was not designed to address potential mechanisms of increased platelet function in patients with myocardial infarction and previous heart failure. However, as far as we know, our study is amongst the first to demonstrate increased platelet content, instead of platelet reactivity, in aspirated coronary thrombi of patients with a previous heart failure diagnosis, in a “real-world” STEMI setting. These findings may contribute to explain the significant impact of advanced Killip class on myocardial reperfusion observed in STEMI patients undergoing mechanical reperfusion [36,37],as well an additional explanation for the increased STEMI mortality in patients with CHF [38]. Besides that, a potentially important clinical implication of this study would be to encourage decision-making clinical trials testing more intensive antithrombotic strategies in patients with acute myocardial infarction and history of heart failure.

One of our main findings was an inverse correlation between CD61 expression and ischemic time, indicating a lesser proportionally platelet content contribution in the total amount of the thrombus as a function of time. Silvain et al. [10], used magnetic resonance imaging to evaluate the composition of coronary thrombus and its association with ischemic time, and 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). Iwata et al. [13] analyzed the cellular constituents of 108 thrombi aspirated from coronary lesions in

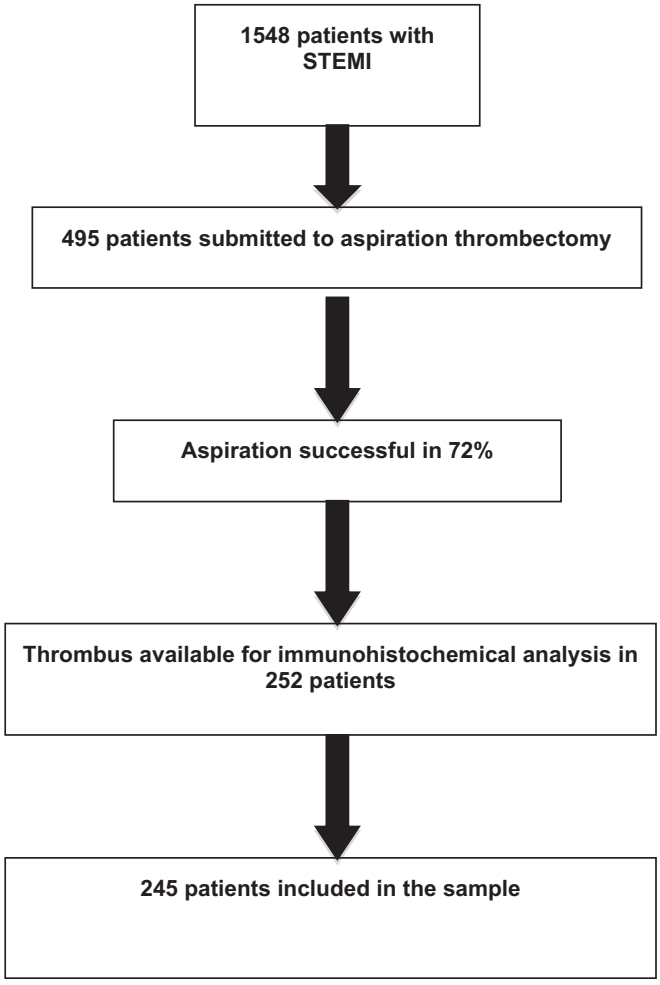

Fig. 4. Study flowchart.

Table 1
Clinical, angiographic and procedural aspects of the study patients.

Male, n (%)	172 (70)
Age (years)	58 ± 12
Body mass index (kg/m^2)	27.33 ± 4.10
Hypertension, n (%)	140 (57)
Diabetes, n (%)	57 (23)
Dyslipidemia, n (%)	86 (35)
Smoking, n (%)	120 (49)
Time from pain to first medical contact (h)	3,24 [1,40–6]
Total ischemic time (h)	4,36 [3,10-7,20]
Target vessel, n (%)	
LAD	108 (44)
LCx	26 (11)
RCA	104 (42)
Bypass grafts	7 (2.8)
Type of pPCI, n (%)	
Predilation + thrombectomy + stenting	95 (39)
Thrombectomy + stenting	139 (57)

Legend - LAD: left anterior descending artery; LCx: left circumflex artery; RCA: right coronary artery.

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 time from chest pain onset. Paradoxically, Fuijkschot and colleagues have shown moderate positive correlation between thrombus CD31 area and ischemic time (r = 0.244; p = 0.020) [21]. Our study strengthens the concept of an inverse relationship between platelet content and ischemic time, since is amongst the largest reports addressing histological and immunohistochemistry aspects of thrombus in patients with STEMI in contemporary practice. Our data help to suggest further pathophysiological explanations on the impact of ischemia time on myocardial reperfusion and mortality [39,40] and on the observed ischemia time-dependent benefits from Gp IIb-IIIa inhibitors as compared to Bivalirudin in patients undergoing primary angioplasty [41].

Finally, the expression of CD34 (endothelial function), CD61 (platelet function) and factor VIII (coagulation cascade) surface markers in thrombi of patients with STEMI was not significantly associated with major clinical events, such as mortality, re-infarction or stroke. However, we demonstrate, with borderline significance, increased platelet activity (expressed by higher mean CD61 index) in those patients who developed combined major cardiovascular events (185 ± 104 *vs.* 144 ± 101; p = 0.06). A possible explanation for the lack of statistical significance is the low number of patients presenting MACE in our sample. Blasco et al., evaluating thrombi of 142 STEMI patients submitted to primary PCI, found 5-year MACE-free survival rates of and 82.2 % in patients whose samples contained plaque (evidenced by CD68 immunohistochemical stain) *vs.* 66.0 % in those with plaque-free aspirates (p = 0.03) [42]. The limited follow-up in our study may also have contributed to the lack of statistical significance.

Despite these important insights, several aspects of the *in-vivo* intracoronary thrombus dynamics are not fully understood yet. One controversial topic is the potential role of diabetes mellitus in the physiopathology of acute coronary thrombosis. Yamashita et al. [28] found a paucity of CD34-positive cells in patients with DM and MI, suggesting a lower capacity to down-regulate thrombus formation. In contrast, Sambola et al. found that CD34 was slightly increased in thrombi of diabetic patients, as well as fibrin, platelets, P-selectin, PAI-1 and vWF [43]. We have previously reported lack of difference between morphologic and histopathologic constituency of coronary thrombi in STEMI patients with or without DM [44]. In the present study, we did not find associations between the expression of CD34, CD61 and factor VIII antigens and DM or other baseline characteristics of the sample.

Aspiration thrombectomy has provided new and interesting insights into myocardial infarction physiopathology through the study of human coronary thrombi retrieved from patients with STEMI. We have previously demonstrated that white thrombus has a smaller size when compared to red thrombus, and it is associated with high fibrin infiltration, shorter ischemic times and lower mortality [45]. Rittersma et al. [9] found that coronary thrombi were days or weeks old in at least 50 % of STEMI patients submitted to aspiration thrombectomy within 6 h of chest pain onset, indicating highly heterogeneous period of plaque instability and thrombus formation processes. Kramer et al. [46], evaluating >1300 STEMI patients, identified fresh thrombus in approximately 30 % of the sample. Moreover, the mortality rates at the 4-year follow-up were significantly higher in patients with older thrombi (16 %) when compared to those with fresh thrombi (7 %).

4.1. Limitations

The criterion of including only patients with available thrombi introduces a potential selection bias because the decision to perform AT was at the discretion of the pPCI operator. On the other hand, this aspect makes this cohort representative of patients submitted to AT in the daily practice. The specimens retrieved by aspiration thrombectomy during pPCI may not represent the whole thrombotic burden inside the coronary artery. However, we believe that the investigation of thrombus characteristics *in vivo* and in real-world clinical practice is an important aspect of our study. Another limitation of our study is the lack of measures of platelet reactivity, once CD61 expression indicates only the platelet content in the total amount of the thrombus. The lack of association between several immunohistochemistry characteristics and other clinical characteristics and outcomes could be related to

Table 2
Thrombus antigenic expression according to clinical characteristics.

		CD34 positivity (%)	p	Factor VIII, cells	p	Factor VIII, index	p	CD61, cells	p	CD61, index	p
Gender	Male	55 %	0.54	53 ± 25	0.53	192 ± 103	0.48	46 ± 27	0.33	153 ± 103	0.31
	Female	59 %		51 ± 25		182 ± 98		42 ± 26		139 ± 99	
Age	> or =60 years	44 %	0.99	53 ± 24	0.74	192 ± 99	0.62	45 ± 26	0.99	152 ± 103	0.55
	<60 years	55 %		52 ± 27		186 ± 106		45 ± 27		144 ± 101	
Race	Caucasian	56 %	0.82	51 ± 25	0.33	185 ± 102	0.35	44 ± 26	0.60	145 ± 101	0.44
	Black	50 %		57 ± 22		207 ± 91		47 ± 30		164 ± 120	
Hypertension	Yes	56 %	0.94	56 ± 26	0.13	180 ± 105	0.09	45 ± 27	0.84	151 ± 104	0.73
	No	56 %		55 ± 24		202 ± 96		44 ± 26		146 ± 100	
Current smoking	Yes	52 %	0.34	50 ± 25	0.13	180 ± 97	0.13	41 ± 26	0.03	133 ± 94	0.01
	No	58 %		55 ± 26		199 ± 106		48 ± 27		164 ± 107	
Dyslipidemia	Yes	53 %	0.60	51 ± 24	0.63	186 ± 99	0.64	42 ± 26	0.26	138 ± 95	0.23
	No	57 %		53 ± 26		192 ± 104		46 ± 27		155 ± 106	
Diabetes	Yes	62 %	0.26	50 ± 25	0.40	181 ± 106	0.50	47 ± 27	0.35	162 ± 108	0.26
	No	54 %		53 ± 25		192 ± 101		44 ± 26		105 ± 100	
Family history	Yes	52 %	0.46	50 ± 24	0.34	175 ± 93	0.16	45 ± 28	0.92	157 ± 107	0.44
	No	57 %		53 ± 26		196 ± 105		45 ± 26		146 ± 100	
Previous MI	Yes	66 %	0.16	48 ± 22	0.20	170 ± 91	0.18	43 ± 26	0.67	142 ± 98	0.64
	No	54 %		53 ± 26		193 ± 104		45 ± 27		150 ± 103	
Previous PCI	Yes	66 %	0.21	49 ± 23	0.35	168 ± 91	0.17	44 ± 25	0.96	148 ± 98	0.83
	No	54 %		53 ± 26		193 ± 103		45 ± 27		149 ± 103	
Previous HF	Yes	80 %	0.11	50 ± 25	0.82	175 ± 97	0.66	60 ± 26	0.06	216 ± 113	0.03
	No	55 %		52 ± 25		189 ± 102		44 ± 26		145 ± 101	

MI: myocardial infarction; PCI: percutaneous coronary intervention; HF: heart failure.

Table 3
Thrombus antigenic expression according to angiographic and procedural aspects.

		CD34+ (%)	p	Factor VIII, cells	p	Factor VIII+, index	p	CD61+, cells	p	CD61, index	p
Trivascular disease	Yes	69 %	0.11	52 ± 26	0.89	178 ± 98	0.52	46 ± 26	0.71	151 ± 104	0.82
	No	53 %		52 ± 25		191 ± 102		44 ± 26		147 ± 101	
Clinical success	Yes	54 %	0.38	52 ± 25	0.61	190 ± 99	0.47	44 ± 25	0.42	146 ± 97	0.21
	No	62 %		50 ± 27		178 ± 110		48 ± 31		168 ± 124	
Angiographic success	Yes	56 %	0.91	45 ± 28	0.11	158 ± 111	0.09	46 ± 34	0.78	166 ± 138	0.37
	No	57 %		53 ± 25		193 ± 99		45 ± 25		147 ± 96	
Lesion extension, mm				0.01*	0.88	0.01*	0.90	0.01*	0.93	0.01*	0.90
Vessel diameter, mm				−0.06*	0.40	−0.03*	0.65	−0.10*	0.15	−0.10*	0.13
Time to first medical contact, h				−0.09*	0.16	−0.11*	0.09	−0.12*	0.05	−0.13*	0.04

* = use of correlations.

Table 4
- Antigenic expression according to thrombus histological pattern.

	Recent	Lytic	Organized	p
CD34+ (%)	52 %	57 %	70 %	0.76
Factor VIII+, cells	51 ± 26	52 ± 25	56 ± 26	0.49
Factor VIII+, index	186 ± 105	187 ± 96	204 ± 105	0.59
CD61+, cells	44 ± 27	48 ± 25	43 ± 26	0.68
CD61+, index	147 ± 106	159 ± 94	143 ± 106	0.74

insufficient statistical power due to a small sample. On the other hand, our sample size is larger than most previous reports addressing coronary thrombi retrieved by aspiration thrombectomy in patients with STEMI. At the study baseline, we were not able to make the characterization into systolic or diastolic previous heart failure. Regarding assessment of LV function during the index hospitalization, we do not have data on all patients because it was not included as part of our study protocol and according to our institution routines.

Table 5
Thrombus antigenic expression according to the occurrence of in-hospital cardiovascular events.

		CD34+ (%)	Factor VIII, cells	Factor VIII, index	CD61, index	CD61, cells
Urgent revascularization	Yes	2.2	50 ± 33	180 ± 134	173 ± 142	48 ± 33
	No	1.9	52 ± 25	189 ± 101	148 ± 101	44 ± 26
	p	0.85	0.8	0.8	0.7	0.8
Re-infarction	Yes	5.1	50 ± 19	176 ± 71	134 ± 97	41 ± 23
	No	6.6	53 ± 26	190 ± 103	149 ± 102	45 ± 27
	p	0.60	0.7	0.6	0.6	0.6
Death	Yes	8.8	57 ± 29	204 ± 113	167 ± 123	49 ± 30
	No	6.5	52 ± 25	188 ± 101	147 ± 100	44 ± 26
	p	0.51	0.8	0.5	0.4	0.4
MACE	Yes	11.2	56 ± 29	197 ± 114	185 ± 114	53 ± 26
	No	8.3	52 ± 25	187 ± 100	144 ± 101	44 ± 26
	p	0.46	0.5	0.7	0.06	0.09

MACE: major adverse cardiovascular events.

5. Conclusion

In patients with ST- elevation acute myocardial infarction submitted to primary percutaneous coronary intervention and aspiration thrombectomy, CD61 expression was higher in patients with previous heart failure and inversely correlated with the ischemic time. There was a trend toward higher CD61 expression in patients presenting in-hospital MACE. There were no other significant associations between thrombus immunohistochemistry and clinical characteristics or thrombus histology.

CRediT authorship contribution statement

All authors have contributed to the analysis design and oversight, manuscript conception and drafting, statistical analysis, and/or editorial review. All authors have also reviewed and approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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