Clinical Significance of Histological Features of Thrombi in Patients with Myocardial Infarction

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Abstract

Background: Percutaneous Coronary Intervention (PCI) is the most common strategy for the treatment of Acute ST segment elevation Myocardial Infarction (STEMI), and thromboaspiration has been increasingly utilized for removal of occlusive thrombi.

Objectives: To analyze the influence of histopathological features of coronary thrombi in clinical outcomes of patients with STEMI, and the association of these variables with clinical, angiographic, and laboratory features and medications used in hospitalization.

Methods: Prospective cohort study. All patients were monitored during hospitalization and thirty days after the event. Aspirated thrombi were preserved in formalin and subsequently stained with hematoxylin-eosin and embedded in paraffin. Thrombi were classified as recent and old. The primary outcome was the occurrence of major cardiovascular events within thirty days.

Results: During the study period, 1,149 patients were evaluated with STEMI, and 331 patients underwent thrombi aspiration, leaving 199 patients available for analysis. It was identified recent thrombi in 116 patients (58%) and old thrombi in 83 patients (42%). Recent thrombi have greater infiltration of red blood cells than old thrombi (p = 0.02), but there were no statistically significant differences between other clinical, angiographic, laboratory, and histopathological features and medications in both group of patients. The rates of clinical outcomes were similar in both groups.

Conclusions: Recent thrombi were identified in 58% of patients with STEMI and it was observed an association with infiltration of red blood cells. There was no association between histopathological features of thrombi and clinical variables and cardiovascular outcomes. (Arq Bras Cardiol. 2013; 101(6):502-510)

Keywords: Coronary thrombosis; Percutaneous coronary intervention; Thrombectomy; Myocardial infarction.

Introduction

Acute ST Segment Elevation Myocardial Infarction (STEMI) is generally caused by sudden occlusion of a coronary artery due to fracture or erosion of atherosclerotic plaque, and it is one of the main causes of deaths worldwide[1-3]. Primary Percutaneous Coronary Intervention (pPCI) is the most common strategy for the treatment of this disease[4,5]. A metanalysis published by Keeley et al[5] in 2003 demonstrated that treatment by PCI results in significant decrease in mortality when compared to thrombolytic treatment. The mortality rates of STEMI have significantly decreased after the use of routine pPCI, representing approximately 7% in contemporary practice[5-7]. These results can further improve with thromboaspiration, and this approach also reduces stent thrombosis and improves myocardial blush[8-10].

Thrombectomy by manual aspiration can be effectively carried out with several devices available in the market[11]. Aspirated thrombi present different histopathological and morphometric features, and it has been suggested that thrombi may be classified as recent, lytic and organized[8,12,13]. Other studies demonstrate that there may be a period of days or weeks between thrombus formation and infarction symptoms[12,14,15]. It was also demonstrated that old thrombi may be present in patients with chest pain only for a couple of hours, and that the age (progression time) of thrombus is related to worst prognosis and partial resolution of ST segment[12,16,17].

Recent studies demonstrated an association between histological features of thrombi and major cardiovascular outcomes[8,13,15,17,18]. However, these studies were performed over ten years ago, and with different thromboaspiration devices, and this limits its applicability in contemporary clinical practice. This study aims at analyzing the influence of histopathological features of coronary thrombi in clinical outcomes of patients with acute ST segment elevation

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myocardial infarction undergoing manual aspiration in contemporary clinical practice, and the association of these variables with clinical, angiographic, and laboratory features and medications administered during hospitalization.

Methods

Study design

Prospective cohort study of patients with STEMI treated at the Cardiology Institute of Rio Grande do Sul, University Cardiology Foundation, Porto Alegre, Brazil, in the period of April, 2010 to January, 2012.

Patients

Inclusion criteria were STEMI and indication of pPCI and thrombectomy by aspiration (at the attending physician's discretion). Exclusion criteria were under age, failure at thromboaspiration and refusal to sign the consent form. The project was approved by the Ethics Research Committee of the Cardiology Institute of Rio Grande do Sul. The patients signed the informed consent form.

Logistics

All patients were interviewed at hospital admission and followed-up during the study period by one of the investigators. Data collections were performed using paper forms elaborated by the group of investigators involved, and these data were stored in a specific database in Microsoft Access. Data included in the database were verified by another investigator. Clinical, angiographic, laboratory, and histopathological features and medications used during the first 24 hours of hospitalization and cardiovascular outcomes within thirty days were analyzed.

Delta T was defined as the time between onset of chest pain and patient's arrival at the emergency room. Door-to-balloon comprised time between patient's arrival at the emergency room and first balloon inflation inside the infarct-related artery. Total ischemia time was the sum of these two times.

Procedures of primary percutaneous coronary intervention

PPCI procedures were carried out as described in the literature[19,20]. All patients were treated with aspirin 300mg and clopidogrel 300 to 600mg at hospital admission. Heparin (100 U/Kg EV) was administered before PCI. Inhibitors of glycoprotein IIb/IIIa and other technical aspects, such as the number of stents used and pre- and post-dilation, were used at the operators' discretion, as well as the decision of performing thrombectomy by aspiration. In this study, the following manual aspiration devices were used: Export (Medtronic Vascular Inc., Santa Rosa, United States), Pronto (Vascular Solutions, Minneapolis, United States), Diver (Invatec, Brescia, Italy) or Thrombuster (Kaneka Medix Corporation, Osaka, Japan). The aspiration procedure technique was that described in the literature[9], passing the catheter several times through the occlusion site.

Angiographic analyses were performed in two orthogonal projections by experienced operators. The coronary flow was analyzed before and after the procedure according with TIMI criterion[21]. Myocardial perfusion was evaluated by myocardial blush score, previously described[22].

Histopathological analysis

We included in the study 199 thrombi of patients with STEMI subjected to pPCI, 116 recent thrombi and 83 old thrombi. Immediately after thrombus aspiration by manual device, the device filter was placed in 10% formalin, aspirated and fixed for 24 hours. Then, the material was embedded in paraffin, subjected to series of 8μm-thick hemoxylin-eosin-stained histological cuts. In histopathological analysis, we evaluated the quantities of white blood cells, red blood cells and fibrin, described according to their percentage. The number of fragments of each aspirated thrombus was also analyzed. Thrombotic material was classified according with its composition. Thrombi were classified in three groups, accordingly with previously published data: recent thrombus, composed of patterns of layers of platelets, fibrin and white blood cells; lytic thrombi, characterized by areas of necrosis and apoptosis; white blood cells; and organized thrombi, which showed proliferation of smooth muscle cells and/or deposition of connective tissue[12,13]. Thrombi collected in this study were divided in two groups: recent and old (lytic/organized). Pathological analyses were carried out by three pathologists blinded to clinical features.

Clinical follow-up and outcomes

Each patient was monitored during hospitalization by one of the investigators, and telephone call was made within 30 days after STEMI. The study primary outcome was the combination of cardiovascular events: death, AMI and myocardial revascularization. Myocardial infarction was defined as recurrence of chest pain with new ST segment elevation or elevation of biomarkers serum levels. New revascularization was carried out by surgical myocardial revascularization or percutaneous coronary intervention procedure, at the attending physician's discretion. We also evaluated secondary outcomes, such as Cerebrovascular Accident (CVA) and stent thrombosis. Ischemic stroke was considered when there was a rapid loss of neurological function. Thrombosis was defined according to the Academic Research Consortium definitions[23].

Statistical analysis

The data were analyzed using the statistical program SPSS 19.0. Quantitative variables are described as mean $\pm$ standard deviation or median, interquartile range. Categorical variables are described through absolute and relative frequency. The patients were divided in recent thrombus group and old thrombus group, and comparisons were carried out using Chi-square test, Fischer's Exact test, t or Mann-Whitney test, as appropriate. Values considered significant are those with $p < 0.05$.

Results

In the study period, we evaluated 1,149 patients with STEMI, and 331 patients underwent thrombi aspiration. The success rate was of 69%, and 199 samples were available for analysis. The study flowchart is shown in Figure 1.

Recent thrombi were identified in 116 patients (58%), and due to the small number of lytic and organized thrombi (N = 43 and N = 40, respectively), we formed a group of old thrombi with 83 patients (42%). Comparisons between clinical and angiographic features of both groups of patients are shown in Table 1. Patients with recent thrombi presented a more frequent history of coronary artery disease (p = 0.09) and 2/3 TIMI flow after the procedure (p = 0.09). Patients with old thrombi present higher abdominal circumference (p = 0.09). Delta T did not present a statistically relevant association with histological features of thrombi.

Laboratory features of these two groups of patients are shown in Table 2. There were no statistically relevant differences regarding fibrogen, C-Reactive Protein (CRP), Creatinine phosphokinase (CK), Creatinine phosphokinase-MB (CKMB), Ultra-sensitive Troponin (USTN) and other analyses.

In Table 3 are shown the medications used during the first 24 hours of hospitalization, and there was no statistically relevant difference between these two groups of patients.

Histopathological analyses are shown in Table 4. Patients with recent thrombus presented significantly higher infiltration of red blood cells (p = 0.02), and the group of old thrombus had a tendency for higher fibrin infiltration (p = 0.07). As for other histopathological and morphometric characteristics, such as the number of fragments removed, dimension and volume of thrombus and infiltration of white blood cells, there were no statistically relevant differences between these two groups of patients.

In the 30-day clinical follow-up there were no statistically relevant differences regarding death (recent thrombus = 7.8% vs. old thrombus = 4.8%; p = 0.59), AMI (recent thrombus = 2.6% vs. old thrombus = 2.4%; p = 1), ischemic stroke (recent thrombus = 0.9% vs. old thrombus = 2.4%; p = 0.77), stent thrombosis (recent thrombus = 1.7% vs. old thrombus = 2.4%; p = 1) and combined outcomes (recent thrombus = 9.5% vs. old thrombus = 6%; p = 0.53). Results of the clinical follow-up are described in Figure 2.

Recent and old thrombi are illustrated in Figures 3 and 4, respectively.

Discussion

In this study we demonstrated that there was no correlation between the histopathological classification of thrombus as recent or old and the progression time of infarction, other clinical, angiographic, and laboratory features and clinical progression of patients. The infiltration of white blood cells was significantly higher in thrombi classified as recent and there was a tendency for higher infiltration of fibrin in old thrombi, which is consistent with the criteria used to define these thrombi.

The absence of an association between histological pathology of thrombus and progression time of infarction had already been previously described by other authors[12,15]. Kramer et al[15], in 2009, reported that the time between plaque rupture and thrombi formation is still unpredictable, and Rittersma et al[12] have also demonstrated a discrepancy between thrombotic process and onset of infarction symptoms. However, another study recently published by Silvain et al[24] showed that ischemic time is a determining factor in thrombus composition, its formation during infarction coronary occlusion is a rapid evolution process, changing its composition rapidly during ischemic process[24]. But our results corroborate the findings of Kramer et al[15] and Rittersma et al[12] because they showed that coronary thrombi in our analysis have no relation with ischemic time.

On the other hand, our results are different from other previous studies that demonstrated that recent thrombi

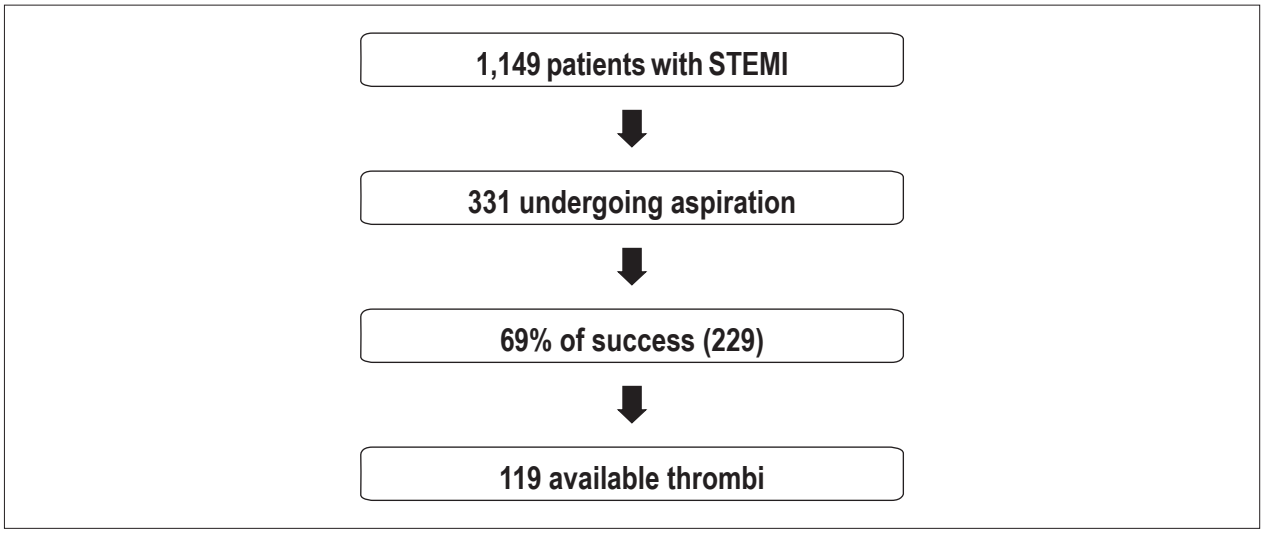

Figure 1 - *Study flowchart. STEMI: Acute ST segment elevation Myocardial Infarction.*

Table 1 - Clinical and angiographic features according to thrombus histopathological classification

Variables	Recent (N = 116)	Old (N = 83)	p
Age, years	58.37 ± 12.35	58.67 ± 10.89	0.86
Male, %	72	68	0.64
Caucasian, %	87.4	92.6	0.35
Weight	77.09 ± 14.75	78.64 ± 13.67	0.46
Height	167.9 ± 8.35	169.11 ± 9.07	0.35
AC, %	94.01 ± 13.71	97.58 ±14.32	0.09
Hypertension, %	58.6	54.3	0.66
Diabetes, %	18	21	0.74
Dyslipidemia, %	24.3	34.6	0.16
Smoking, %	48.6	49.4	0.92
History CAD, %	36	23.5	0.09
Medical history			
PCI, %	14.4	11.1	0.65
MRS, %	0	3.7	0.15
AMI, %	12.6	13.6	1
CCI, %	1.8	4.9	0.42
CRI, %	1.8	3.7	0.72
CVA, %	6.3	8.6	0.74
Previous infarction, %	49.5	42	0.37
Delta T, hours	4 (1.97;6.67)	3.97 (2.54;6.47)	0.51
Door-to-balloon, hours	1.25 (0.92;1.62)	1.25 (0.92;1.5)	0.96
Ischemic time, hours	5.24 (0;8)	5.35 (3.77;8.12)	0.51
Use ASA, %	25.2	19.8	0.47
Compromised vessels:			
One, %	50.9	59	
Two, %	35.3	32.5	0.38
Three, %	13.8	8.4	
ADA, %	49	43	0.51
XCA, %	11.2	9.6	0.9
RCA, %	39.7	44.6	0.58
Bypass, %	0	2.4	0.34
Reference diameter, mm	3.36 ± 0.51	3.35 ± 0.53	0.96
Extension, mm	19.28 ± 10.97	18.3 ± 7.11	0.5
2/3 TIMI flow:			
Pre, %	7.8	10.8	0.62
Post, %	99.1	93.9	0.09
Blush 2/3			
Pre, %	5.2	1.2	0.26
Post, %	71.6	70.4	0.98
TIMI score	3 (2;5)	3 (2;5)	0.53

AC: abdominal circumference; CAD: Coronary Artery Disease; PCI: percutaneous coronary intervention; MRS: myocardial revascularization surgery; AMI: acute myocardial infarction; CCI: congestive cardiac insufficiency; CRI: chronic renal insufficiency; CVA: cerebrovascular accident; ASA: acetylsalicylic acid; ADA: anterior descending artery; XCA: circumflex coronary artery; RCA: right coronary artery.

Table 2 - Laboratory features according to thrombus histopathological classification

Variables	Recent (N = 116)	Old (N = 83)	p
Blood glucose, mg/dL	169.88 ± 64.47	156.26 ± 54.14	0.15
HbA1c, %	5.44 ± 0.22	5.75 ± 0.7	0.12
Cholesterol, mg/dL	200.8 ± 50.54	209.5 ± 48.59	0.27
HDL, mg/dL	39.56 ± 12.11	41.02 ± 10.06	0.42
Triglycerides, mg/dL	105 (64.5;195)	108 (69;173.5)	0.8
CRP, mg/dL	0.45 (0.24;0.89)	0.42 (0.2;0.88)	0.39
Fibrinogen, mg/dL	218.7 ± 56.6	226.11 ± 83.23	0.51
Hematocrit, %	40.51 ± 4.55	41.22 ± 3.36	0.25
Red blood cells, g/dL	13.61 ± 1.52	13.97 ± 1.37	0.11
White blood cells, mm^3	13643.73 ± 4604.43	14402.69 ± 447.78	0.26
Platelets, mm^3	258542.55 ± 76548.06	254828.57 ± 64027.16	0.74
Creatinine, mg/dL	1.02 ± 0.35	0.98 ± 0.31	0.37
CK, U/L	160 (70;464)	129 (59;473.25)	0.45
CK-MB, ng/mL	12 (5;38)	12 (6;35)	0.93
Troponin US, ng/dL	384 (50;2767)	251 (44.5;1218)	0.49

HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; CRP: C reactive Protein; CK: creatinine phosphokinase; CK-MB: creatinine phosphokinase MB; TNT-US: ultras-sensitive.

Table 3 - Medications administered in the first 24 hours of hospitalization according to thrombus histopathological classification

Variables	Recent (N = 116)	Old (N = 83)	p
Aspirin, %	95.5	100	0.14
Clopidogrel			
300 mg, %	16.4	12	0.52
600 mg, %	82	88	0.33
IIb/IIIa Inhibitor	534	43.4	0.21
Heparin, %	100	97.6	0.34
Statin, %	85.6	83.8	0.88
Beta blocker, %	73	67.5	0.51
ECA inhibitor, %	72.1	68.8	0.74
Nitrate, %	24.3	20	0.5

ACE: angiotensin-converting enzymes.

Table 4 - Histopathological and morphometric features according to thrombus classification

Variables	Recent (N = 116)	Old (N = 83)	p
Number of fragments, n	2 (1;5)	3 (2;5)	0.28
Dimension (width), mm	4 (3;6)	4 (3;7)	0.93
Volume, μm^3	14 (6.5;24)	12 (7;28)	0.77
Red blood cells, %	40 (10;50)	25 (10;40)	0.02
White blood cells, %	10 (5;20)	10 (10;20)	0.36
Fibrin, %	50.9±22.86	56.7±20.45	0.07

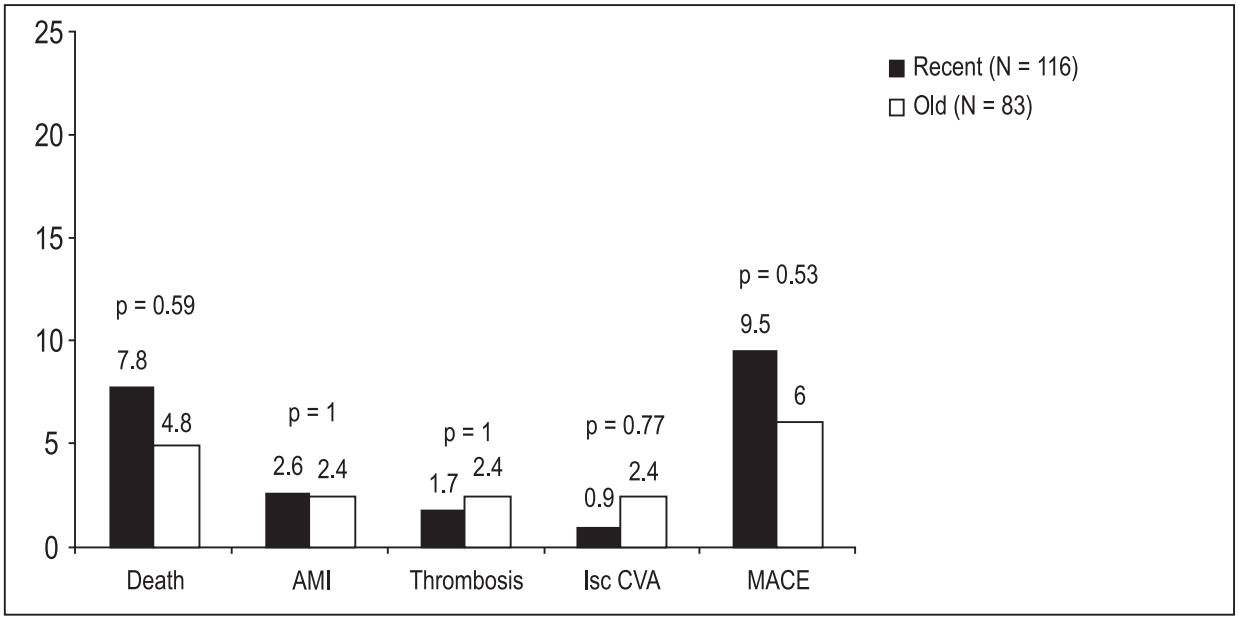

Figure 2 - *Cardiovascular outcomes in the 30-day follow-up (%) according to histopathological classification. AMI: acute myocardial infarction; Isc CVA: ischemic cerebrovascular accident; MACE: major adverse cardiac events: death, AMI, MRS, and PCI.*

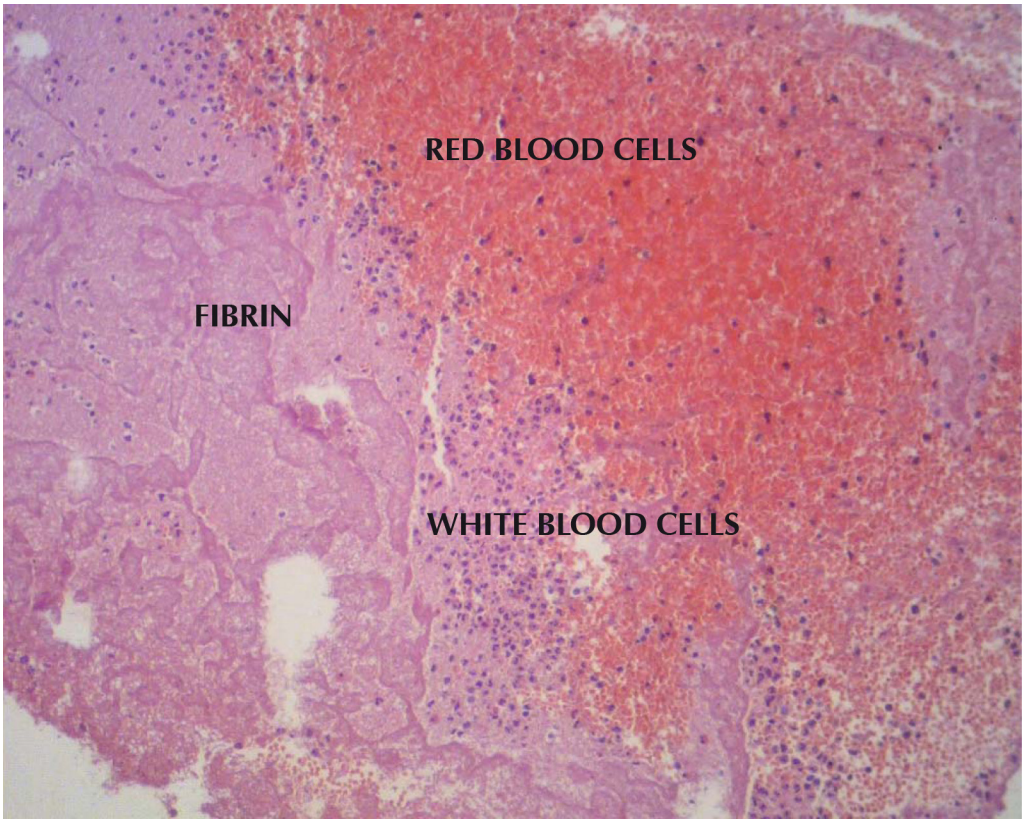

Figure 3 - *Photograph with optical microscope of recent thrombi, characterized by the composition of red blood cells, fibrin and white blood cells.*

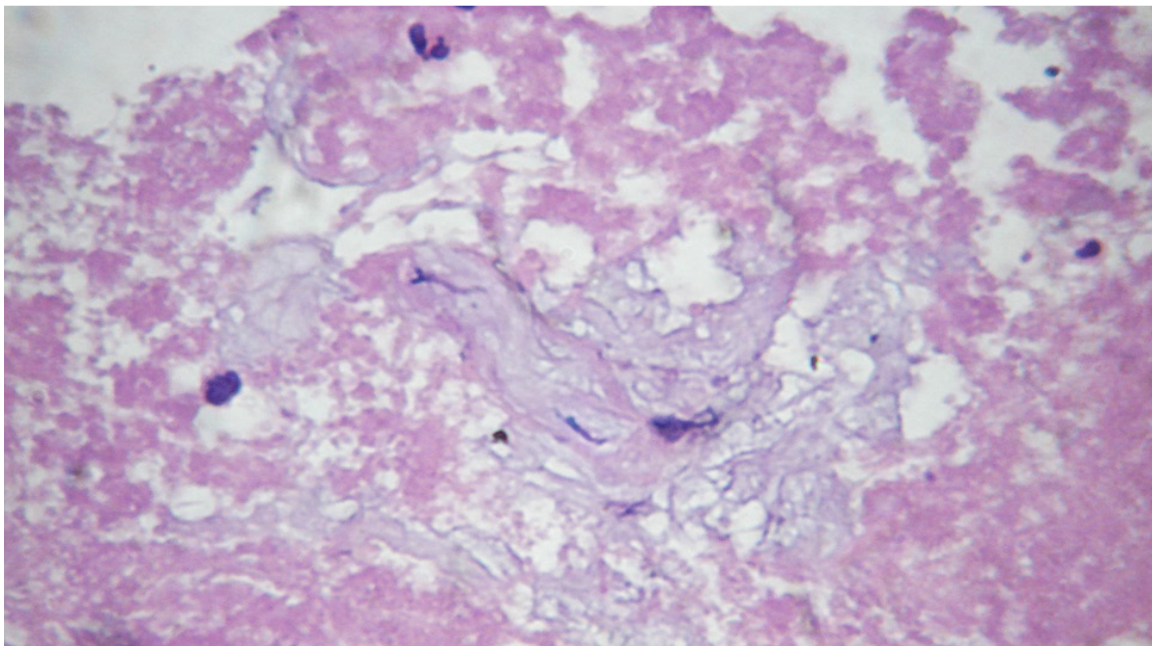

Figure 4 - *Photograph with optical microscope of an old thrombus, characterized by the presence of loose connective tissue.*

are associated with lower mortality. In spite of not being a statistically relevant difference, our study demonstrated a higher mortality rate in patients with recent thrombi than with old thrombi in the 30-day follow-up, as opposed to a study of Kramer et al[16], which demonstrated an association between the age of thrombi and mortality. The presence of old thrombi in that study was an independent predictor of long-term mortality, and there was a higher risk of death during the first 14 days in patients with old thrombi compared to patients with recent thrombi. After 14 days, both groups had similar mortality rate, demonstrating the difference in death occurs primarily within a few weeks after pPCI[16]. Unlike the study abovementioned, our series reflects a contemporary medical practice, in which thrombi were obtained exclusively by means of manual aspiration devices, which could contribute to the difference in results.

To date, there are few studies on histopathological analyses of thrombi aspirated during pPCI in patients with STEMI, some with small samples of patients and/or others with long duration[16,24,25]. Our study analyzed 199 thrombi aspirated by thrombectomy in pPCI in almost two years of collection, exceeding many published studies. This is an important aspect to interpret these data. Difficulties related to the study of thrombi aspirated in pPCI procedures are due to the relatively small number of these procedures in most centers, to partial penetration of thromboaspiration procedure, to success rates of approximately 70% and to logistic problems resulting from collection and processing of material.

Confirming our results with the literature, 2/3 TIMI flow post-pPCI presented a tendency for association with the recent thrombi group, and there were studies demonstrating that recent thrombus can be formed within one day[12,13,15], therefore, it can contribute to improve myocardial reperfusion and myocardial blush grade after thromboaspiration and rapid recanalization of the injured artery.

As previously mentioned, in the recent thrombi of this study we found more red blood cells than in the old thrombi group, with a statistically relevant difference. Red blood cells are one of the most common components found in thrombi, and may contribute more in the composition of thrombi in later stages and not during acute STEMI phase[24,26]. Our findings related to infiltration of red blood cells in the recent group and tendency for infiltration of fibrin in the old group corroborate the literature findings, claiming that recent thrombi have higher composition of red blood cells, and over time, fibrin fibers increase and transform in old thrombi, rich in fibrin[24,27,28]. In addition to red blood cells and other cell constituents, plasma compartment supports the fibrin formation and, consequently, thrombus formation[29,30]. Red blood cells need to be further studied, since they seem to mediate the formation of fibrin fibers and influence in clot viscoelasticity[24,26], reinforcing the importance of studies with histopathological analysis of coronary thrombi aspirated during STEMI.

Our study merits validation, for it confirms many findings in the literature. However, for a clearer understanding of the influence of coronary thrombi histopathological features in STEMI in the contemporary clinical practice, it is necessary to carry out further studies.

Limitations

The small number of patients is one of the limitations of this study. However, it is important to consider that our series compares favorably to several recently published studies, with less than 100 patients. Studies dedicated to collection and analysis of coronary thrombi by aspiration thrombectomy have been limited by a relatively small number of patients undergoing primary coronary intervention even in reference centers, through incomplete penetrance of this procedure in clinical practice, its success rates around 70% and difficulties in processing and analysis of material. Due to the restrict number of patients included in this analysis, it was not possible to carry out a multivariate analysis for the evaluation of possible confounding variables.

Conclusion

In this study, most patients with STEMI had recent thrombi, which confirms the findings in literature. Recent thrombi showed a significantly higher infiltration of red blood cells, and there was no relation between thrombus histopathological classification and clinical or angiographic features. Clinical outcomes in patients with old or recent thrombi were similar.

Author contributions

Conception and design of the research and Critical revision of the manuscript for intellectual content: Sebben JC, Cambruzzi E, Gottschall CAM, de Quadros AS; Acquisition of data: Sebben JC, Cambruzzi E, Avena LM, Gazeta CA; Analysis and interpretation of the data: Sebben JC, Cambruzzi E, Avena LM, Gazeta CA, de Quadros AS; Statistical analysis and Writing of the manuscript: Sebben JC, de Quadros AS.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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