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Composition of Coronary Thrombus in Acute Myocardial Infarction

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Objectives We sought to analyze the composition of coronary thrombus in vivo in ST-segment elevation myocardial infarction (STEMI) patients.

Background The dynamic process of intracoronary thrombus formation in STEMI patients is poorly understood.

Methods Intracoronary thrombi (n = 45) were obtained by thromboaspiration in 288 consecutive STEMI patients presenting for primary percutaneous intervention, and analyzed using high-definition pictures taken with a scanning electron microscope. Plasma biomarkers (TnI, CRPus, IL-6, PAI-1, sCD40 ligand, and TNF-α) and plasma fibrin clot viscoelastic properties were measured simultaneously on peripheral blood.

Results Thrombi were mainly composed of fibrin (55.9 ± 18%) with platelets (16.8 ± 18%), erythrocytes (11.5 ± 9%), cholesterol crystals (5.2 ± 8.4%), and leukocytes (1.3 ± 2.0%). The median ischemic time was 175 min (interquartile range: 140 to 297). Ischemic time impacted thrombi composition, resulting in a positive correlation with intracoronary thrombus fibrin content, r = 0.38, p = 0.01, and a negative correlation with platelet content, r = −0.34, p = 0.02. Thus, fibrin content increased with ischemic time, ranging from 48.4 ± 21% (<3 h) up to 66.9 ± 9% (>6 h) (p = 0.02), whereas platelet content decreased from 24.9 ± 23% (<3 h) to 9.1 ± 6% (>6 h) (p = 0.07). Soluble CD40 ligand was positively correlated to platelet content in the thrombus (r = 0.40, p = 0.02) and negatively correlated with fibrin content (r = −0.36; p = 0.04). Multivariate analysis indicated that ischemic time was the only predictor of thrombus composition, with a 2-fold increase of fibrin content per ischemic hour (adjusted odds ratio: 2.00 [95% confidence interval: 1.03 to 3.7]; p = 0.01).

Conclusions In acute STEMI, platelet and fibrin contents of the occlusive thrombus are highly dependent on ischemia time, which may have a direct impact on the efficacy of drugs or devices used for coronary reperfusion. (J Am Coll Cardiol 2011;57:1359–67) © 2011 by the American College of Cardiology Foundation

Acute coronary thrombosis resulting in total occlusion of a coronary artery leads to ST-segment elevation myocardial infarction (STEMI) (1). Exposition of the lipid-rich core after atherosclerosis plaque rupture/erosion into the arterial lumen triggers the formation of unstable platelet aggregates, which may lead to intermittent reduction in coronary flow and cause distal embolization (2). Early fibrin formation strengthens the platelet aggregate, favoring persistent flow obstruction and blood coagulation activation proximally and

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Abbreviations and Acronyms

adjOR = adjusted odds ratio
IQR = interquartile range
MBG = myocardial blush grade
MI = myocardial infarction
PCI = percutaneous coronary intervention
SEM = scanning electron microscopy
STEMI = ST-segment elevation myocardial infarction
TIMI = Thrombolysis In Myocardial Infarction
UFH = unfractionated heparin

distally to the occlusion. The resulting red thrombus is comprised of erythrocytes and inflammatory cells entrapped by the fibrin network. The layered aspect highlights the repeated episodes of thrombus growth (3,4).

It remains unclear how time affects the dynamic process of thrombus formation in the coronary arteries of STEMI patients (5). Data are also scarce on how thrombus architecture correlates with coronary reperfusion after primary percutaneous coronary intervention (PCI) or with peripheral blood biomarkers. The increased use of thromboaspiration in primary PCI for STEMI offers a unique opportunity to study thrombus composition, dynamic formation, and architecture in vivo (6–9).

Ischemic time was hypothesized to be among the strongest independent correlates of thrombus architecture. We therefore evaluated the effect of the following plausible determinants of thrombus architecture (e.g., coronary anatomy and reperfusion, patient clinical presentation, peripheral blood biomarkers, fibrin clot viscoelastic properties, and response to fibrinolysis). Our aim was to characterize the independent correlates of thrombus architecture and to assess whether thrombus architecture per se could affect myocardial reperfusion.

Methods

Study design and thrombus collection. Between January 2007 and April 2008, we prospectively screened all the STEMI patients referred to the catheterization laboratory for primary PCI associated with thromboaspiration performed by a low-profile catheter (Export 6F, Medtronic, Santa Rosa, California). Thromboaspiration was performed whenever possible (when the anatomy of the coronary artery—curve and size—allowed it) in all patients with a Thrombolysis In Myocardial Infarction (TIMI) flow grade 0 and in all patients with a visible thrombus if TIMI flow grade was 1 or more.

Collected thrombi were immediately washed with saline and fixed with 2% glutaraldehyde in 50 mmol/l Na-cacodylate buffer (pH 7.3).

Scanning electron microscope analysis. Sample fixation, dehydration, and preparation were performed according to a previously published method (10). High-definition photographs (3,000× magnification) were obtained using a Philips/FEI XL20 scanning electron microscope 4-nm resolution (FEI, Hillsboro, Oregon) (11). To control for composition heterogeneity in the analysis of the surface of thrombus, we covered several areas (at least 12 according to a grid and the size of the thrombus) for each thrombus (Fig. 1). A semiquantitative analysis and a visual approach were used for the first 10 thrombi. For the semiquantitative analysis, each picture was divided into 400 squares (Image J software, National Institutes of Health, Bethesda, Maryland), and the predominant composition (platelet, fibrin, erythrocytes, leukocytes, cholesterol crystal) of each square was noted (Fig. 1) (12). All the structures visualized were easy to identify, and crystal-like structures were characterized as cholesterol crystals (asterisks in Fig. 2B) on the basis of previous SEM images described in vitro (13,14) and the probability of their presence in the setting of a ruptured plaque exposing a lipid-rich core. The visual approach consisted of a careful observation of each pictures with a subjective evaluation of the percentage of each component. Very low interindividual variability was found in the thrombus composition analysis (<6%) between the 2 approaches; therefore. the time-consuming semiquantitative analysis was not performed for the remaining 35 thrombi (Figs. 2A and 2B). The 2 scientists performing scanning electron microscopy (SEM) and analysis were blinded to the clinical data.

Blood samples and measurement. Blood samples were drawn from the arterial sheath just after insertion and were collected in Vacutainer tubes (Becton Dickinson, Franklin Lakes, New Jersey) containing trisodium citrate 0.129 mol/l (3.2% sodium citrate) for routine blood work and for biomarkers measurements.

Soluble CD40L levels were measured using a human sCD40L ELISA bioassay (Arcus Biological, Modena, Italy). TNF-α and IL-6 levels were measured by Quantikine sandwich enzyme immunoassays (R&D Systems, Abingdon, United Kingdom) according to the manufacturer's instructions. CRP levels were quantified by commercially available ELISA kits (Zymutest, Hypen BioMed, Neuville sur Oise, France). The PAI-1 concentration was measured using an Asserachrom PAI-1 kit (Diagnostica Stago, Asnieres, France). All measures were assessed in duplicate.

Whole blood clot viscoelastic properties and lysis rates. Clots were formed in a thermostable 37°C plastic plate by recalcifying 700 μl of PPP with $CaCl_2$ (10 mmol/l final concentration) and 5 μl of purified recombinant human tissue factor (Innovin, Dade Behring, Deerfield, Illinois). To facilitate lysis, 10 μl of tissue plasminogen activator (rt-PA) (0.5 nmol/l final concentration) (Boehringer Ingelheim, Ingelheim am Rhein, Germany) was also added. Plasma clot viscoelastic properties were measured with a Hemodyne RM-2 Analyser (Hemodyne, Richmond, Virginia). Fibrinolysis was assessed by continuous monitoring of the elastic modulus and was expressed as the lysis rate (s^{-1}). The assay accuracy is reportedly similar to that of determination of the lysis front velocity (or fiber lysis rate). Clot lysis time was defined as the time needed for the maximum elastic modulus to decrease by 50%. The intra-

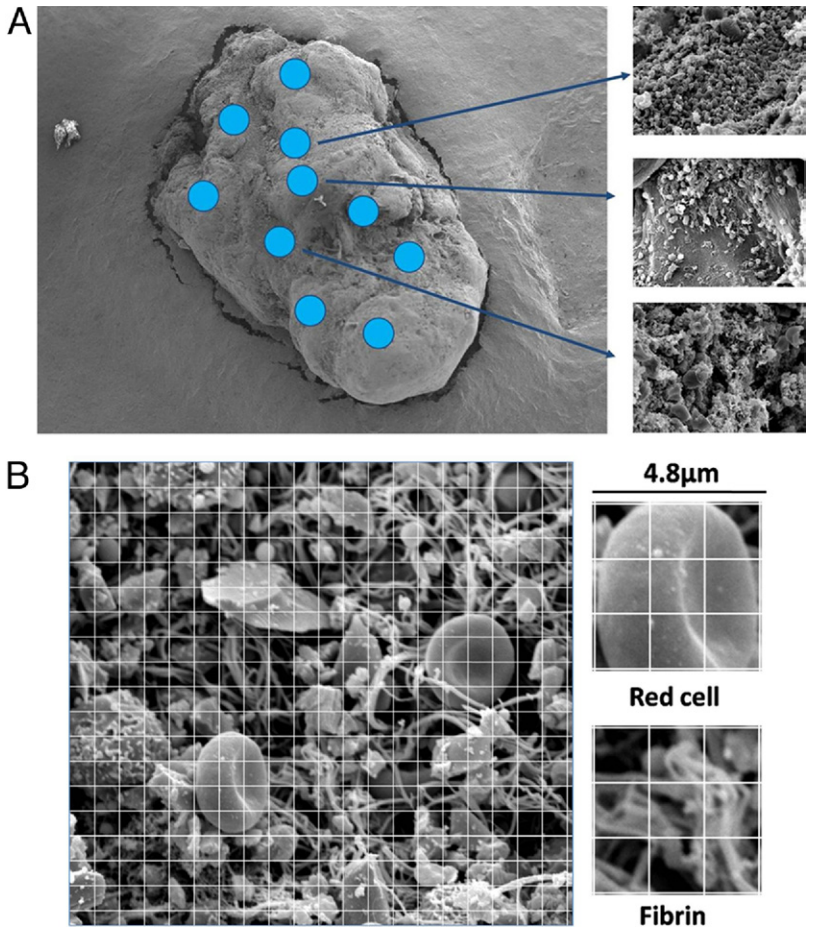

Figure 1 **Analysis of Clot Surface With 10+ Areas**

These areas (indicated by the **blue circles**) were used to average the thrombus heterogeneity **(A)** and perform semiquantitative analysis **(B)**.

individual variability of this technique was published previously (15).

Data collection. All patients entered the e-PARIS registry, a prospective web-based registry regrouping clinical and biological data on STEMI patients treated by primary PCI. Our angiographic core laboratory reviewed all angiographic films and noted the size of the infarct-related artery, corrected TIMI frame count, and myocardial blush grade (MBG), as previously described (16). Clinical and biological data were analyzed to identify predictors of thrombus composition. This study was approved by the Institutional Review Board of the Pitié-Salpêtrière Hospital in Paris (CPP), France.

Clinical follow-up. Patients were followed-up for 30 days through out-patient consultation. Physicians noted the occurrence of major cardiovascular and cerebrovascular events, including death, stroke, recurrent myocardial infarction (MI), urgent revascularization, and definite and probable stent thrombosis (Academic Research Consortium definition), as well as major and minor bleeding events. ST-segment resolution data were collected from the patient file.

Statistical analysis. Categorical variables were expressed as percentages, and continuous variables as mean ± SD or medians with the interquartile range (IQR) (25th to 75th percentiles) for time delay. Association between continuous variables was assessed by Pearson's correlation test. The variable ischemic time had an almost normal distribution. Because complementary analyses using Pearson's correlation test after log-transformation and nonparametric Spearman's test found similar results, the variable was considered normally distributed for all other analyses. Potential associations between clinical and biological parameters were tested by Student *t* test and ANOVA test with Dunnett's correction for multiple comparisons. Multivariable logistic and mixed models were used to assess the relationship between ischemic time and both qualitative and quantitative thrombus composition. Covariables (age, diabetes, renal insufficiency, admission troponin fibrinogen and platelet levels, pre-hospital abciximab use, clopidogrel pre-treatment, unfractionated heparin [UFH] versus enoxaparin use, and coronary artery reference diameter) were introduced into the models in addition to ischemic time in a stepwise fashion.

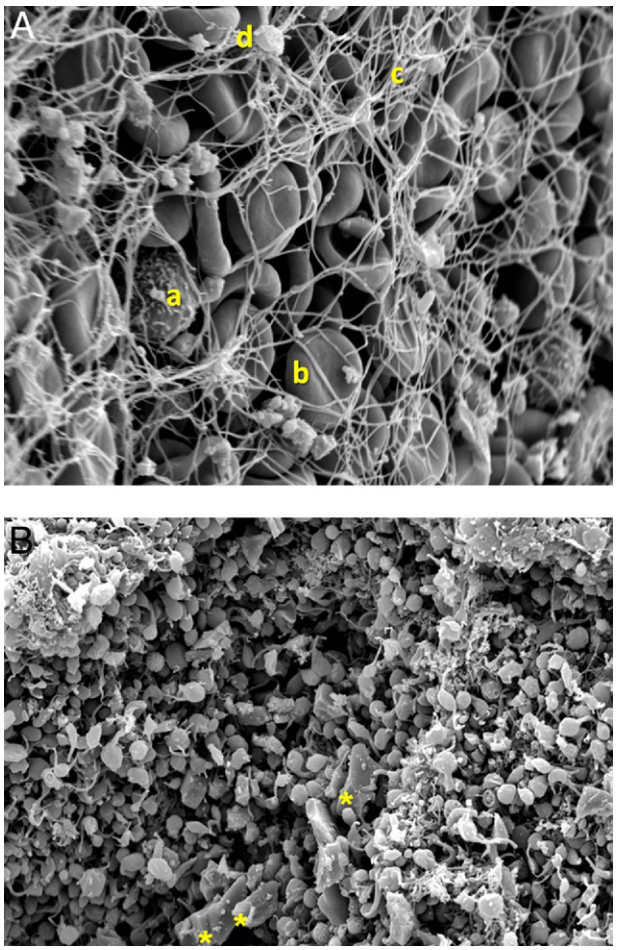

Figure 2 Scanning Electron Micrograph at 3,000× Magnification

(A) This portion is mainly composed of erythrocytes **(b)** trapped in a fibrin mesh **(c)** with few platelet aggregates **(d)** and rare white cells **(a)**. **(B)** This portion is mainly composed of platelet aggregates with a few cholesterol crystals **(yellow asterisks)**.

Diagnostics were systematically conducted for both logistic and mixed models to assess the normal distribution of residuals. Analyses were performed with SAS version 9.2 (SAS Institute, Cary, North Carolina).

Results

We prospectively screened 349 patients referred for primary PCI. Sixty-one late presenters were excluded because there was a time delay of >12 h from symptom onset to PCI or because symptom onset could not be characterized. Thromboaspiration was used in one-third of the early STEMI presenters (38%), and significant coronary thrombi >1 mm^3 were successfully retrieved in one-third of them (Fig. 3). The baseline characteristics of the patients are shown in Table 1. Patients were treated aggressively, with a high proportion receiving high loading doses of clopidogrel (at least 600 mg) and abciximab in addition to aspirin and low molecular weight or UFH. Median ischemic time (from symptom onset to PCI) was 175 min (IQR: 140 to 297 min). Details of procedures, angiographic findings, biomarker distributions, viscoelastic measurements, and fibrinolysis results are reported in Table 2.

Thrombus composition. Analyses were performed on 44 of the 45 thrombi collected. One thrombus was not analyzed because the SEM preparation had failed. Fibrin fibers were the major thrombus component, representing more than 60% of their composition. Platelets, erythrocytes, cholesterol crystals, and leukocytes together comprised the remaining 40% (Fig. 4). Thrombus components were easily identified in our high-definition SEM images. The 2 examples shown here are good representatives of the broad scope of thrombi components. These include a fibrin-and-erythrocyte composition typical of "old" fibrin-rich thrombi, usually found in patients with an ischemic time of >3 h (Fig. 2A) and a "fresh" platelet-rich thrombus of a patient presenting early, within the first hour following symptom onset (Fig. 2B). Remarkably, we could identify in these high-definition images an unexpected percentage (5%) of cholesterol crystals that were only described previously ex vivo (13).

Effect of time on thrombus composition. Ischemic time was found to have a high impact on thrombus composition, resulting in a positive correlation with intracoronary thrombus fibrin content, $r = 0.38$, $p = 0.01$, and a negative correlation with platelet content $r = -0.34$, $p = 0.02$. Pearson's correlation test after log-transformation and Spearman's nonparametric correlation test showed similar results ($r = 0.39$, $p = 0.01$; $r = -0.34$, $p = 0.025$; and $r = 0.36$, $p = 0.017$; $r = -0.30$, $p = 0.05$, respectively). Additionally, when ischemic time was divided into 3 categories (<3 h, 3 to 6 h, and >6 h), we observed a stepwise increase in fibrin content with respect to prolonged ischemic

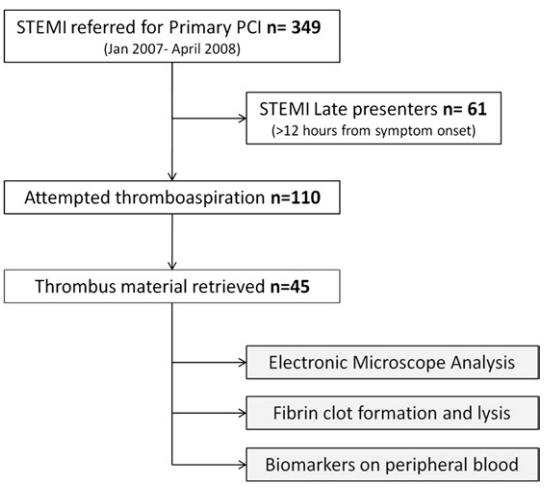

Figure 3 Flow Chart of the Study

Table 1 Baseline Characteristics (n = 45)

Demographics and risk factors	
Age	57.9 ± 13.2
Male	38 (84.4%)
Active smoker	21 (46.6%)
Diabetes	12(26.6%)
Hypertension	16 (35.5%)
Dyslipidemia	30 (66.6%)
BMI	26.4 ± 4.1
Past medical history of	
ACS	7 (15.5%)
Angioplasty	6 (13.3%)
CABG	1 (2.2%)
Clinical presentation	
Anterior MI	21 (46.6%)
Cardiac arrest	4 (11.1%)
TIMI risk score	3.0 ± 2.1
Killip class	1.2 ± 0.6
Cardiogenic shock	2 (4.4%)
Time delay in min	
Symptom onset to medical contact	80 [55–179]
Medical contact to catheterization laboratory	66 [55–128]
Catheterization laboratory to sheath insertion	20 [10–33]
Symptom onset to PCI	175 [140–297]
Biomarkers on admission	
Troponin I, μg/ml	5.1 ± 13.8
Negative troponin	17 (37.7%)
CK, IU/ml	447 ± 828
CRP, mg/l	13.7 ± 38
Fibrinogen, g/l	3.7 ± 1.3
Platelet, mm^3	242 ± 93
Creatinine clearance, ml/min	98 ± 33
Antithrombotic treatment	
ASA	45 (100%)
Clopidogrel	37 (83.5%)
Unfractionated heparin	17 (37.7%)
Enoxaparin	26 (62.3%)
GP IIb/IIIa inhibitor	41 (91.1%)
Pre-hospital	8 (17.7%)
Catheterization laboratory	33 (73.3%)
Thrombolysis	1 (2.2%)

Values are mean ± SD, n (%), or median [interquartile range].

ACS = acute coronary syndrome(s); ASA = acetylsalicylic acid; BMI = body mass index; CABG = coronary artery bypass grafting; CK = creatine kinase; CRP = C-reactive protein; GP = glycoprotein; MI = myocardial infarction; PCI = percutaneous intervention; TIMI = Thrombolysis In Myocardial Infarction.

time ranging 48.4 ± 21% (<3 h) to 66.9 ± 9% (>6 h) (p = 0.02). Platelet content decreased from 24.9 ± 23% (<3 h) to 9.1 ± 6% (>6 h) (p = 0.07) (Fig. 5). The evolution of each thrombus component relative to ischemic time is represented in Figure 6.

Effect of antithrombotic treatment on thrombus composition. Aspirin was administrated before thromboaspiration in 100% of patients, and abciximab was used either in a pre-hospital setting or in the catheterization laboratory in 91% of patients, limiting the analysis of their impact on thrombus composition. Clopidogrel pre-treatment was administered in 29 patients (66%) and did not induce any significant changes on platelet composition (24.1 ± 6% vs. 15.5 ± 3%; p = 0.17) when compared with patients without pre-hospital inhibition of the $P2Y_{12}$ receptor.

Biomarker distribution, viscoelastic properties, and fibrinolysis. The peripheral measure of the platelet activation marker sCD40 ligand was positively correlated with platelet content in the thrombus (r = 0.40, p = 0.02) and negatively correlated with fibrin content (r = −0.36; p = 0.04). The simultaneous measure of the more classic biomarker of myocardial infarction, namely troponin I, was a strong biomarker of ischemic time (r = 0.56, p < 0.01), but was not correlated with thrombus composition. No correlation was seen between ischemic time and inflammation biomarkers (IL-6, TNF-α, CRPus) or PAI-1, and neither platelet count nor fibrinogen level was correlated with thrombus composition (data not shown). Patients presenting after 6 h had a nonsignificant trend towards faster clotting time, stiffer

Table 2 Angiographic Results, Revascularization Procedure, and Biological Data (n = 45)

Radial approach	42 (93.3%)
Diameter of the infarct-related artery	3.15 ± 0.5
Acute stent thrombosis	5 (11.1%)
Patients with more than 1 significant lesion	13 (28.8%)
Infarct-related artery	
LAD	21 (46.6%)
RCA	17 (37.7%)
Cx	6 (13.3%)
CABG	1 (2.2%)
Successful PCI	41 (91.1%)
Ejection fraction post-MI (%)	48.9 ± 12
Markers of reperfusion	
TIMI flow grade 3 before PCI	3 (6.6%)
TIMI flow grade 3 after PCI	40 (88.8%)
cTFC before PCI	133 ± 45
cTFC after PCI	25 ± 22
TIMI MBG3 before PCI	4 (8.8%)
TIMI MBG3 after PCI	18 (40%)
ST-segment resolution >70%	25 (55.3)
Biomarkers	
CRPus, μg/ml	8.5 ± 3.9
IL-6, pg/ml	14.2 ± 34.8
PAI-1, ng/ml	70.2 ± 51.2
sCD40-ligand, ng/ml	1.2 ± 0.6
TNF-alpha, pg/ml	8.6 ± 1.1
Viscoelastic properties and response to fibrinolysis	
PCF, kdynes/s	1.4 ± 1.4
Elastic modulus max, kdynes/s	8.7 ± 8.8
Clotting time, s	691 ± 651
Clot lysis time, s	347 ± 204
Lysis rate × 10^4, s^{-1}	2.9 ± 4.1

Values are n (%) or mean ± SD.

cTFC = corrected Thrombolysis In Myocardial Infarction frame count; CRPus = ultra-sensitive C-reactive protein; Cx = circumflex coronary artery; IL = interleukin; LAD = left anterior descending coronary artery; MBG = myocardial blush grade; PAI = plasminogen activator inhibitor; PCF = platelet contractile force; RCA = right coronary artery; sCD40 = soluble CD40; TNF = tumor necrosis factor; other abbreviations as in Table 1.

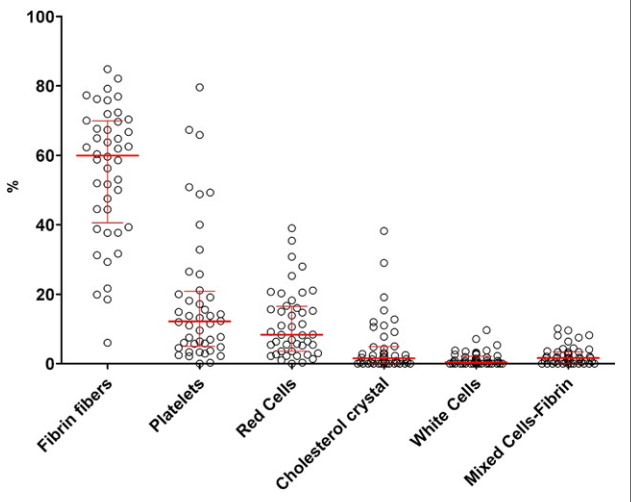

Figure 4 Thrombi Composition in 44 STEMI Patients (Early Presenters)

Red lines indicate median and interquartile range (25% to 75%). PCI = percutaneous intervention; STEMI = ST-segment elevation myocardial infarction.

clot (as indicated from the elastic modulus), and longer clot lysis time (Fig. 7).

Independent correlates of thrombus composition and of reperfusion. Ischemic time was the only correlate of thrombus composition in the multivariable stepwise logistic analysis. None of the other covariables met the 0.05 significance level for entering the model. Every additional ischemic hour led to a 2-fold increase in the rates of fibrin-rich thrombus—<30% platelet and >70% fibrin—(adjusted odds ratio [adjOR]: 2 [95% confidence interval (CI): 1.03 to 3.7]) and a 50% reduction in platelet content (adjOR: 0.5 [95% CI: 0.27 to 0.94]) ($p = 0.001$ for both). Similar results were obtained when the fibrin content (%) was analyzed quantitatively in a multivariable mixed model with the same covariables introduced in the model in addition to ischemic time. "Fresh" platelet-rich thrombus was defined as >30% platelet and <70% fibrin, and "old" fibrin-rich thrombi as <30% platelet and >70% fibrin. No differences in reperfusion were seen between "fresh" platelet-rich thrombi and "old" fibrin-rich thrombi assessed by ST-segment resolution and TIMI MBG. Independent correlates of successful reperfusion (ST-segment resolution of >70%) included a low plasma fibrinogen level (adjOR: 0.33 [95% CI: 0.12 to 0.91] [per g/l], $p = 0.003$) and the use of enoxaparin versus UFH (adjOR: 10.3 [95% CI: 1.7 to 62], $p = 0.02$). High and low counts for plasma fibrinogen level were 8 and 1.8 g/l, respectively.

Discussion

Thrombus aspiration is a newly recommended technique that facilitates thrombus removal from the culprit coronary artery in myocardial infarction. The method reportedly improves mortality in STEMI patients presenting within 12 h of symptoms onset when used in conjunction with primary PCI (7,8). This life-saving technique allowed us to study prospectively the composition of occlusive thrombi in STEMI patients admitted for mechanical coronary reperfusion.

We report the results of a new analysis method for intracoronary thrombi obtained in vivo that allows the determination of their precise composition using SEM, associated here with an analysis of cardiac biomarkers, fibrin clot viscoelastic properties, and fibrinolysis obtained simultaneously from patients' peripheral blood. The analysis of high-definition SEM images that we produced with respect to ischemic time (from symptom onset to thrombus retrieval) revealed that the dynamic formation of the thrombus during an acute coronary occlusion is a fast-evolving process. The platelet and fibrin fiber components of the thrombus, which are both therapeutic targets, changed rapidly with respect to ischemic time. These observations of thrombus structure and formation in vivo demonstrate the relationship between thrombus composition and ischemic time, which was found to be the only major predictors of thrombus composition. Our results also demonstrate that thrombus composition is an indicator of coronary occlusion time and, therefore, of the beginning of myocardial damage.

Our findings add to the body of knowledge on thrombus formation in acute MI. First, they confirm, at the pathophysiological level, the generally accepted pathogenesis of clot formation in vivo in STEMI patients. "Fresh" thrombi have the highest proportion of platelets, whereas the proportion of fibrin fibers increases over time, as the level of thrombin increases, leading to "older" fibrin-rich thrombi. These results confirm previous in vivo animal studies showing the very early stage of thrombus formation, just after endothelial injury, when the initial thrombus is primarily composed of activated platelets, rapidly stabilized by fibrin

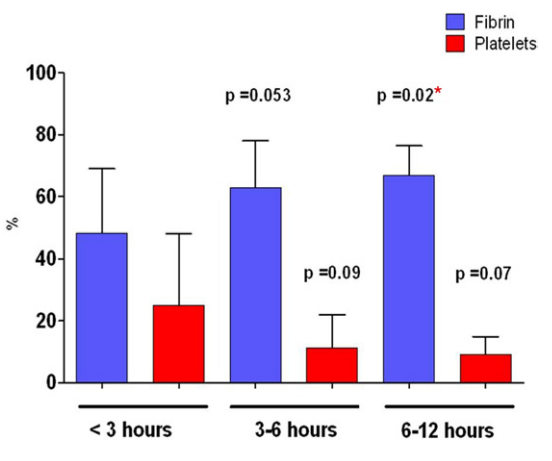

Figure 5 Impact of Time on Thrombus Composition

The p values are given for comparison with the group <3 h as a reference with multiple Student *t* test. *$p < 0.05$. Multiple comparisons were done with the Dunnett's test, resulting in a $p = 0.044$ for fibrin and nonsignificant for platelet content.

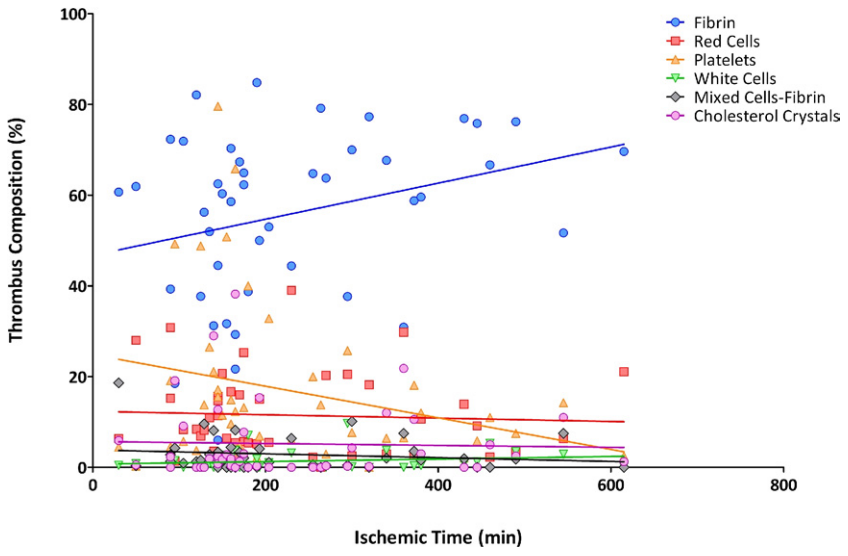

Figure 6 **Evolution of the Percentage Thrombus Composition for Each Component**

The components in percentages (y-axis) are shown relative to ischemic times in minutes (x-axis). **Lines** represent linear regression for correlation with ischemic time.

fibers, with a decreasing proportion of platelets over time (17–19). At a clinical level, our results are along the same lines as those reported by Kramer et al. (20), who showed that the thrombus composition itself seems to be a prognostic marker and is linked to short- and long-term mortality in a large study in STEMI. In this previous study, thrombus composition was evaluated by classical light microscopy showing 2 types of thrombi, "fresh thrombi" (<1 day) composed of layered patterns of fibrin and intact platelets, erythrocytes, and granulocytes, and "older thrombi" (>1 day) comprised of lytic and/or organized areas. Our own analysis differs from this previous classification by the age of clots (earlier period), the method used (scanning electronic microscope), the structure analyzed (we studied the thrombus surface, which reflects more the latter layers of thrombus formation), and the results reported (focused on the proportion of each component). The high-resolution imaging facilitated the detailed characterization of all structures present in the thrombi, despite the difficulties of this task, because of their remarkable heterogeneity. This allowed us, for example, to identify cholesterol crystals that were most likely released by the ruptured plaque. It should also be noted that fibrin is a major component of the thrombus even at the relatively early stages studied here. Additionally, simultaneous measurements of biomarkers allow us to confirm the role of sCD40 ligand on platelet accumulation in the intracoronary thrombus at the early phase of formation.

Our findings also help interpret results obtained from previous clinical trials. Indeed, fibrinolysis treatment has been demonstrated to have a narrow therapeutic time window and seems to be most efficient during the first 3 h of STEMI (21,22). According to our study, this represents a period when the clot has a small and accessible amount of fibrin fibers and when fibrinolysis has not yet been overcome by the intense fibrin formation. Furthermore, the fast accumulation of fibrin fibers in the thrombus followed by its stabilization by FXIII cross-linking is likely to increase clot stiffness, resulting in resistance to fibrinolysis as shown in vitro (15,23). Regarding antiplatelet agents, even though glycoprotein (GP) IIb/IIIa inhibitors, such as abciximab, have been shown to reduce mortality in primary PCI (vs. placebo), the potential benefit is mostly observed with an early administration (24–29). In contrast, negative studies with GP IIb/IIa inhibitors seem to relate to late presentation of patients (30,31). These clinical results suggest a time limit under which these molecules are efficient, and correspond according to our study to the time when the occlusive thrombus contains a high proportion of platelets, shortly before it evolves into a compact, fibrin-rich structure.

In our study, the effect of antithrombotic pretreatment by aspirin and abciximab was initially included in the analysis, but due to the fact that almost all patients received these 2 fast-acting agents before thromboaspiration, we could not demonstrate any potential effect of such drugs on thrombus composition. We believe that the impact of pre-hospital $P2Y_{12}$ inhibition on platelet composition should be studied in a dedicated study.

Study limitations. First, although erythrocytes were the second most common cell identified in our thrombi, we were surprised not to find an increased percentage of erythrocytes with time. Erythrocytes in clots were the origin of the word "red" thrombi for old thrombi as opposed to recent platelet-rich "white" thrombi. Erythrocytes may contribute more to thrombus composition at later stages and

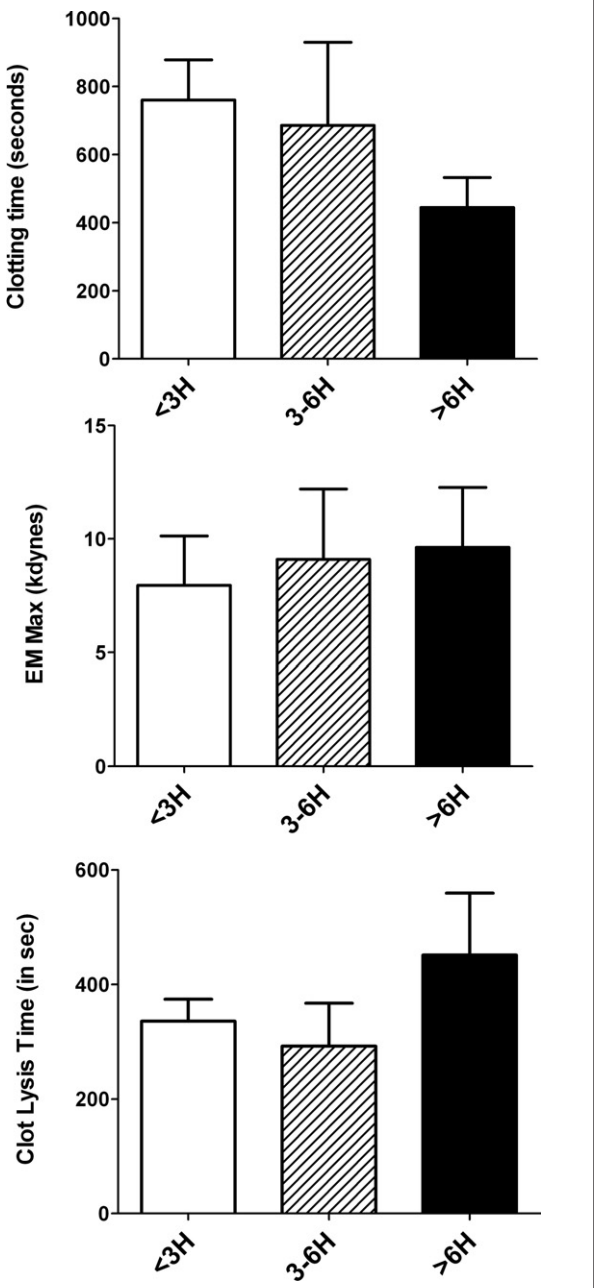

Figure 7 **Results of Fibrin Clot Viscoelastic Properties and Response to Fibrinolysis According to Ischemic Time**

No significant differences were found between groups with the Dunnett's test. EM Max = maximum elastic modulus.

not in the time window of acute reperfusion of STEMI. The role of erythrocytes in thrombi remains to be studied more extensively, since they also appear to mediate the formation of thicker fibrin fibers and influence clot viscoelasticity by increasing the viscous component of the sample relative to the elastic component (32). Second, due to the in vivo nature of our study, the analysis was performed on the main aspirated piece of the thrombus and did not include the total volume of retrieved fragments or the distal embolic debris. Because the retrieval of the thrombus is done by aspiration through the catheter lumen, the high-definition analysis, made by scanning the surface of the piece of thrombus, can correspond, in fact, to any part of the thrombus (head or tail, surface or core, luminal or abluminal), turning this potential limitation into a major strength of this approach. To our knowledge, there is no known technique (beside surgical removal or postmortem autopsy) that can analyze the whole thrombus, including the part of the thrombus that might have embolized, or allows analyzing the 3-dimensional structure of a thrombus. Additionally, the number of thrombi retrieved, and the random localization of the pictures that were analyzed, should guarantee an unbiased measurement, and control for composition heterogeneity in the pieces of thrombi that were obtained. Third, potential distortion of the samples might have occurred during thromboaspiration. However, we believe that damage was limited, knowing that thrombi are stiffer than in vitro clots and that fibrin fibers and clots bear outstanding elastic properties (33,34). The preserved appearance of cells and fibrin fibers in the microscopy images confirmed that distortion was minimal. Fourth, the choice of SEM can be criticized since material cannot be stained as in histopathologic analysis, but was motivated by its ability to provide high-resolution images of thrombi in humans who had had MI (5). Fifth, the aspirated thrombus may be older than expected from the duration of the ischemic time, and younger thrombus could be superimposed onto an older thrombus, thereby potentially confusing our observations (20). Finally, even if thrombi were retrieved randomly in a subset of patients representative of our STEMI population, the absence of successful thromboaspiration in all STEMI presenters could have biased our results. A methodological limitation also needs to be underlined, as the multivariable analysis should be considered as only exploratory, because the model is not parsimonious.

Our work helps characterize thoroughly the components of coronary thrombi in STEMI and thereby contributes to the current understanding of coronary thrombus formation, which is relevant to the efficacy of various reperfusion treatments. The composition of the aspirated thrombus could also be a potential surrogate end point in clinical trials assessing the benefit of a new antithrombotic regimen (new drugs or new combination of drugs). Ongoing research should elucidate the mechanistic relationships between thrombus composition, myocardial perfusion, and microvascular obstruction.

Conclusions

Thrombus aspiration, an integral component of mechanical reperfusion of acute MI, allowed us to describe in detail the composition of the occlusive thrombus and its changes over

time. Our study shows that the platelet and fibrin contents of the occlusive thrombi of acute STEMI evolve rapidly. Even within a population of patients treated with aggressive antithrombotic regimens, ischemic time is a key determinant of thrombus composition.

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