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Coronary thrombus composition: Links with inflammation, platelet and endothelial markers

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ABSTRACT

Objectives: We investigated whether markers of platelet, neutrophil and endothelial activation, plasma fibrin clot properties and patient clinical profile may characterize coronary thrombus composition in ST-segment elevation myocardial infarction (STEMI) patients. **Methods**: A total of 40 intracoronary thrombi obtained 4.0–16.5 h since chest pain onset by manual aspiration during primary coronary intervention (PCI) were assessed using scanning electron microscopy. Plasma fibrin clot permeation coefficient (K_s) and clot lysis time (CLT), together with platelet and endothelial activation, fibrinolysis, and inflammation markers, were measured ex vivo in 16 patients on admission (pre-PCI group) and on the next morning in 24 patients (post-PCI group). **Results**: Fibrin, erythrocyte, platelet and white blood cell content in the thrombi were estimated at 49.1%, 24.2%, 11.6% and 3.7% respectively. In the pre-PCI group, in addition to fibrinogen, P-selectin and plasminogen activator inhibitor-1 were positively correlated with thrombus fibrin content. In the post-PCI group, in addition to von Willebrand factor antigen (vWF:Ag), soluble CD40 ligand and myeloperoxidase (MPO) were positively correlated with thrombus fibrin content. After adjustment for fibrinogen and onset-to-thrombectomy time circulating vWF:Ag in both groups, and MPO and P-selectin in the pre-PCI group were the independent predictors of fibrin-rich intracoronary thrombus presence. Other predictors were renal impairment, arterial hypertension and time from symptom onset to thrombus aspiration in all patients. **Conclusions**: In STEMI patients coronary thrombus composition is partly characterized by plasma markers of platelet, neutrophil and endothelial activation, with a varying contribution of these factors over time.

1. Introduction

Mechanical blood clot properties and resistance to lysis are largely determined by fibrin fibre structure and their spatial relations, which depend on several genetic, clinical and environmental factors, including plasma concentrations of fibrinogen and C-reactive protein (CRP) [1,2]. The rate of fibrin degradation is determined by levels of plasminogen, antiplasmin, tissue plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1) and thrombin activatable fibrinolysis inhibitor (TAFI), however architecture of fibrin clots also substantially affects efficiency of fibrinolysis, rendering it impaired in thin, highly cross-linked, dense fibre networks [1,2].

Several studies have demonstrated that stable coronary artery disease (CAD), including a history of myocardial infarction (MI), is associated with unfavourably altered plasma fibrin clots with denser fibrin networks and impaired lysis [3]. It has also been shown that in patients with acute coronary syndrome (ACS), clots formed from plasma obtained within the first 12 h since symptom onset are composed of dense networks that are less susceptible to lysis as compared to those with stable angina [4]. It is however unclear whether plasma fibrin clot properties are associated with intracoronary thrombus composition in acute MI.

Acute intraluminal thrombus formation arising from atherosclerotic plaque rupture, erosion or dissection results in a blood flow cessation and distal embolization in an infarct-related artery (IRA) area and causes most cases of ST-segment elevation myocardial infarction (STEMI) [5]. Aspiration thrombectomy with a 40–55% success rate seems to be a useful technique to improve epicardial flow and to reduce distal embolization [6,7]. Silvain et al. [8] demonstrated using scanning electron microscopy (SEM) of

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intracoronary thrombi from 45 STEMI patients that thrombus composition evolves over time during the first 12 h since pain onset. They found that fibrin content increased from 48.4% in thrombi collected less than 3 h from symptom onset to 66.9% in those collected after 6 h, whereas platelet content decreased from 24.9% to 9.1% [8]. A platelet activation marker, soluble CD40 ligand (sCD40L), was positively correlated with platelet content and negatively with fibrin content [8]. Ischemic time, but not fibrinogen and CRP, was the only predictor of thrombus composition with a 2-fold increase in fibrin content per each ischemic hour [8]. In STEMI patients presenting later that 6 h from symptom onset, insignificantly shorter clotting time, stiffer clot and longer clot lysis time were noted [8]. Similar results on coronary thrombus composition were obtained using histochemical techniques [9,10] or magnetic resonance imaging [11]. It has also been reported that the ruptured plaque is more prone to initiate the fibrin-rich thrombus formation as opposed to the eroded plaque [12]. Moreover, serum myeloperoxidase (MPO) derived from activated neutrophils and monocytes is elevated in ACS and MPO-positive neutrophils represent a larger component of intracoronary thrombi in the ACS patients with eroded plaques [9,13,14].

In this study, we sought to investigate whether the intracoronary thrombus composition, plasma fibrin clots properties and circulating fibrin clot modifiers that are altered during myocardial ischemia reflect pro-coagulable and pro-inflammatory state in STEMI patients.

2. Material and methods

2.1. Study design and thrombus collection

Between January 2012 and December 2012 consecutive 319 all-comer STEMI patients eligible for primary PCI according to the current guidelines [6] were prospectively screened for the ability of manual thrombus aspiration. To avoid the interference of inflammation- and coagulation/fibrinolysis-induced factors a total of 247 cases were excluded for the following reasons: estimated glomerular filtration rate less than 30 ml/min (11 cases), acute infection (7 cases), treatment with systemic corticosteroids (8 cases) or oral anticoagulants (22 cases), malignancy (9 cases), and in-stent thrombosis (7 cases), no thrombus visible on angiography (72 cases), and lack of consent to participate (94 cases). Arbitrarily, we decided to exclude patients in whom the time delay was >24 h (17 cases) given a major potential impact of endogenous fibrinolysis after such a long time. Arterial hypertension was defined as blood pressure at rest ≥140/90 mmHg or receiving antihypertensive medication. Dyslipidaemia was defined as serum total cholesterol ≥5.2 mmol/l or receiving hypolipidaemic agents. Diabetes was defined as receiving either oral antidiabetic agents or insulin alone or in combination. Renal impairment (chronic kidney disease stage 3) was defined as estimated glomerular filtration rate ≤ 60 ml/min. A positive family history was defined as evidence of CAD in a first degree relative in men before the age of 55 years and women before the age of 65 years. This study was approved by the Ethics Committee. All patients gave written informed consent.

All patients received pretreatment with 600 mg of clopidogrel and 300 mg of acetylsalicylic acid orally and 5000 IU of unfractionated heparin intravenously at first medical contact. All patients underwent primary PCI via the femoral approach with heparin infusion at activated clotting time of 250–300 s. Depending on coronary anatomy thrombectomy was performed by 6 or 7F catheter (Export, Medtronic, USA) in 72 patients with Thrombolysis in Myocardial Infarction (TIMI) flow grade 0 or with TIMI grade greater than 0 and thrombus visible on angiography. Thrombi were washed with 0.9% saline and then fixed in 2.5% glutaraldehyde (Sigma–Aldrich, St. Louis, MO, USA) in a phosphate-buffered saline (PBS, pH 7.4) for 24 h.

2.2. Scanning electron microscopy

Fixed clots were washed with PBS, and then dehydrated in graded water-ethanol solutions, dried by the critical point procedure, and sputter coated with gold. High-definition images (3500× magnification) were obtained using a JEOL JSM 5410 scanning electron microscope (JEOL, Tokyo, Japan). Due to thrombus heterogeneity, samples were scanned in ≥10 different areas depending on the thrombus size. Each image was divided into 400 squares (Photo Filter 7 software). A major component, i.e. fibrin, platelet, erythrocyte, leukocyte, cholesterol crystals, or fibrin mixed with cells/platelets (on average, 50:50), was recorded in each square and then a median value calculated for each thrombus was used for further statistical analyses. Fibrin-rich thrombus was defined as a thrombus containing >70% of fibrin (fibrin as a major component in >70% of the squares).

2.3. Blood samples and measurement

On admission we measured blood cell counts, glucose, creatinine, fibrinogen, CRP, cardiac troponin T (high-sensitive, hsTnT) and creatine kinase MB (CK-MB) activity using routine laboratory techniques. In patients who presented between 7 a.m. and 3 p.m., blood from the antecubital vein was collected immediately for additional measurements including plasma fibrin clot properties (the pre-PCI group). In those who were admitted during off-hours, peripheral blood collection was performed the next morning at 6 a.m. (the post-PCI group, median PCI-to-blood collection time: 720 min). Blood samples were mixed with 3.2% sodium citrate (9:1), centrifuged once for 20 min and stored at −80 °C.

Commercially available enzyme-linked immunosorbent assays (ELISAs) were used to determine serum MPO (Calbiochem Millipore, Billerica, MA, USA), and plasma plasminogen activator inhibitor type-1 (PAI-1) and tissue plasminogen activator (t-PA; both, Hyphen BioMed, Neuville-Sur-Oise, France), thrombin activatable fibrinolysis inhibitor (TAFI; Chromogenix, Lexington, MA, USA), and two platelet markers, i.e. soluble CD40 ligand (sCD40L), and P-selectin (both, R&D Systems, Minneapolis, MN, USA). vWF antigen (vWF:Ag) was measured by latex immunoassay on a STAR coagulation instrument (Diagnostica Stago, Asnieres, France). The inter-assay and intraassay coefficients of variation for all the ELISAs were <8%.

2.4. Plasma fibrin clot permeability

Clot permeation was assessed using a pressure-driven system, with calculation of a permeation coefficient (K_s) using the tissue factor (TF)- and thrombin-based (Thr) assays. K_s was calculated as follows: $K_s = Q \cdot L \cdot \eta / t \cdot A \cdot \Delta p$, where Q is the flow rate in time t, L is the length of a fibrin gel, η is the viscosity of liquid (in poise), A is the cross-sectional area (in cm^2), and Δp is a differential pressure (in dyne/cm^2). Lower K_s values indicate reduced clot permeability [15,16].

To calculate K_s-Thr, 60 μl of citrated plasma were mixed with 60 μl of activation mixture at final concentrations of 1 U/ml human thrombin (Calbiochem, San Diego, CA, USA) and 20 mmol/l $CaCl_2$. Plasma gels were generated in mechanically scratched tubes (prepared from 1 ml serological pipette, Sarstedt, Nümbrecht, Germany) and incubated for 2 h at room temperature in a wet chamber. Later the tubes were connected via silicone tube to a reservoir containing Tris-buffered saline (TBS, 50 mmol/l Tris–HCl and