Journal of Cardiology 69 (2017) 394–400

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Inflammatory cell content of coronary thrombi is dependent on thrombus age in patients with ST-elevation myocardial infarction

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ARTICLE INFO

Article history:
Received 17 June 2016
Received in revised form 16 September 2016
Accepted 12 October 2016
Available online 9 November 2016

Keywords:
Thrombosis
Immunohistochemistry
Myocardial ischemia
Cardiovascular
Inflammation

ABSTRACT

Background: ST-elevation myocardial infarction (STEMI) is typically caused by an occlusive coronary thrombus. The process of intracoronary thrombus formation is poorly understood. It is known that inflammatory cells play a role in the formation and resolution of venous thrombi, however their role in coronary thrombosis is not clear. We therefore analyzed inflammatory cells in thrombi derived from patients with STEMI in relation to histologically classified thrombus age.

Methods: Thrombus aspirates of 113 patients treated with primary percutaneous coronary intervention were prospectively collected and classified (fresh, lytic, or organized) based on hematoxylin and eosin staining. The density of inflammatory cells neutrophils (MPO), monocytes/macrophages (CD68), lymphocytes (CD45), and the platelet area (CD31), were visualized using immunohistochemistry. Patients' history, medication, and laboratory data were registered.

Results: Fresh thrombi (76.1%) were the most abundant as compared to lytic (16.8%) and organized (7.1%) thrombi. Neutrophils were significantly less present in organized (169 cells/mm^2) compared to fresh (327 cells/mm^2) and lytic thrombi (311 cells/mm^2). Monocytes/macrophages were significantly more present in lytic (471 cells/mm^2) than in fresh (312 cells/mm^2) thrombi. We additionally found that thrombi from patients aged <50 years as compared to >50 years old contained significantly more neutrophils and monocytes/macrophages irrespective of thrombus age. Furthermore platelet area was smaller in patients on aspirin again irrespective of thrombus age. No gender differences were found.

Conclusions: The composition of inflammatory cells differs with thrombus age in thrombosuction material of STEMI patients that in part depends on patient age and medication.

Introduction

Acute ST-elevation myocardial infarction (STEMI) is typically caused by occlusive coronary thrombus formation superimposed on a ruptured atherosclerotic plaque [1]. Plaque disruption triggers the formation of initial platelet aggregates that expand in association with an increase in fibrin formation and subsequent trapping of erythrocytes leading to persistent coronary flow obstruction [2]. Neutrophils and monocytes are present in these thrombi at 3 h after onset of complaints. After 6 h T-cell and B-cell infiltration significantly increases [3–5]. Of note, inflammatory cells in thrombi were evaluated in these studies in relation to symptom to balloon time, which provides an inadequate estimate of thrombus age [6,7]. Knowledge of the composition of

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http://dx.doi.org/10.1016/j.jjcc.2016.10.003
0914-5087/

inflammatory cells would be interesting since it is known that inflammatory cells play a role in the formation and resolution of venous thrombi [8,9]. Rittersma et al. [6] described a classification method based on histological characteristics which since then is considered as the gold standard for the estimation of thrombus age of coronary thrombi. They defined thrombi as: fresh (less than 1 day old) when they were composed of layered patterns of fibrin and intact cells (platelets, erythrocytes, and neutrophilic granulocytes); lytic (between 1 and 5 days old) when areas of colliquation necrosis and karyorrhexis of neutrophilic granulocytes were present; organized (more than 5 days old) when areas of ingrowth of smooth muscle cells, with or without depositions of young connective tissue and/or ingrowth of capillary vessels was observed. Several studies have shown that this histological appearance does not relate to clinical infarct duration or symptom to balloon time [6,7,10], indicative for a time interval of days to weeks between plaque disturbance, thrombus formation, and the onset of symptoms [6]. This suggests that sudden coronary occlusion is often preceded by a variable period of plaque instability and/or thrombus formation, underlining the importance of identifying thrombus initiation and early progression before occlusion becomes clinically overt [11,12]. In this light, it is of great importance to further elucidate the timescale of thrombus formation and the factors involved.

It is known that inflammatory cells play an important role in the process of plaque instability [13], but their role in arterial thrombus formation is however less known. For this, we have studied the composition of the aspirated thrombus material in relation to their histologically defined age in a large cohort of STEMI patients undergoing primary percutaneous coronary intervention (PCI) after sudden onset of symptoms. We also set out to evaluate if clinical variables such as sex, age, or medication influence the thrombus composition.

Materials and methods

Thrombus aspiration and clinical data

Patients who presented at the VU University Medical Center with a STEMI (defined according to the European Society of Cardiology [ESC] guidelines for STEMI [14]) and from whom thrombus was aspirated were included in the study. All PCIs were performed according to current standard guidelines for PCI. After arrival of the ambulance or after arrival at the hospital, patients were treated with 300 mg of aspirin and 5000 IU of heparin intravenously (iv). On admission patients were directly transported to the catheterization laboratory and PCI was performed of the infarct-related artery and a repeat bolus of heparin was given at the cathlab, based on the patient's body weight. After passage of the coronary artery occlusion with a guide wire, manual thrombectomy was performed with the Export AP Aspiration Catheter (Medtronic, Dublin, Ireland). Typically, several suctions at the site of the occlusion were performed after smooth introduction and initial passage of catheter. Aspirated thrombus and intracoronary material were collected in the collection bottle, which was provided with a filter. Additional balloon angioplasty and stent placement were performed at the discretion of the operator, as was the additional use of glycoprotein IIb/IIIa receptor blockers. Upon thrombosuction, specimens were immediately fixed in formalin. The following baseline clinical data of the patients were collected: age, gender, cardiovascular history, medication, cardiovascular risk factors, infarct-related artery, C-reactive protein, and white blood cell count in the blood. The study conforms with the principles outlined in the Declaration of Helsinki. The study protocol was approved by the medical ethical research committee at the VU University Medical Center.

Histology

The aspirated coronary thrombus material was embedded in paraffin after overnight fixation (4% formalin). Tissues were deparaffinized, rehydrated, and stained with a standard hematoxylin/eosin (HE)-staining. Thrombi were histopathologically classified based on the observation of two independent observers (H.W.M.N. and A.C.W), as described before [6]: fresh (less than 1 day old): completely composed of layered patterns of platelets, fibrin, erythrocytes, and intact granulocytes; lytic (between 1 and 5 days old): areas of colliquation necrosis and karyorrhexis of granulocytes; organized (more than 5 days old): areas of ingrowth of smooth muscle cells, with or without depositions of young connective tissue and ingrowth of capillary vessels. Thrombus material with a heterogeneous composition was graded according to the age of the oldest part.

Immunohistochemistry

For immunohistochemical analysis tissues were deparaffinized, rehydrated, and incubated in methanol/H_2O_2 (0.3%) for 30 min to block endogenous peroxidases. Antigen retrieval was performed by boiling the slides (MPO, CD68, CD31, but not for CD45) for 10 min in a citrate pH 6.0 buffer. The sections were incubated with either rabbit anti-human myeloperoxidase (1:500, A0398 Dako, Glostrup, Denmark), mouse anti-human CD68 (1:400, M0814 Dako), mouse anti-human CD31 (1:40, M0823 Dako), or mouse anti-human CD45 (1:50, M0701 Dako) antibody for 1 h at room temperature (RT). Next, the sections were incubated with anti-mouse/rabbit Envision (Dako) for 30 min at RT. The stainings were visualized with 3,30-diaminobenzidine (0.1 mg/ml, 0.02% H_2O_2) for 10 min. The slides were subsequently counterstained with hematoxylin, dehydrated, and covered. With each staining a phosphate-buffered saline control was included. All these controls yielded negative results (not shown). All the slides were scanned with the MIRAX scan (3DHISTECH Ltd, Budapest, Hungary). With the MIRAX Viewer software snapshots of all tissue were made and stored for further processing with ImageJ. Areas in thrombi (n = 11; 9.7%) that contained plaque material were left out of further analysis. The surface area of CD31-positive thrombocytes was quantitatively measured and divided by the total surface area of the scored tissue. For the slides stained with MPO, CD68, and CD45 the numbers of positive cells were scored and divided by the total surface area of the scored tissue.

Statistics

Continuous variables are expressed as mean ± standard deviation (SD) for normally distributed or median (interquartile range) for non-normally distributed ones. Differences between the groups were tested by Student's t-test for normally distributed or by the Mann–Whitney U (two groups) and Kruskal–Wallis (>2 groups) test for non-normally distributed continuous variables. The Pearson or Spearman rank correlation coefficients were calculated to test the association between two variables with a normal or non-normal distribution. A value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed with SPSS software (Windows version 20, IBM corp., Armonk, NY, USA).

Results

Thrombus age and the number of inflammatory cells

Intracoronary-derived thrombus material was collected from 113 patients (see Table 1 for patients' characteristics). Histological evaluation of the aspirated material in tissue sections showed that