Thrombosis Research 135 (2015) 760–766

Contents lists available at ScienceDirect

Thrombosis Research

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

Regular Article

Ultrastructure and composition of thrombi in coronary and peripheral artery disease: Correlations with clinical and laboratory findings

András Kovács [a,1], Péter Sótonyi [b,1], Anikó Ilona Nagy [b], Kiril Tenekedjiev [c,d], Nikolett Wohner [a], Erzsébet Komorowicz [a], Eszter Kovács [a], Natalia Nikolova [c,d], László Szabó [e], Ilona Kovalszky [f], Raymund Machovich [a], Zsolt Szelid [b], David Becker [b], Béla Merkely [b], Krasimir Kolev [a,*]

[a] Department of Medical Biochemistry, Semmelweis University, Budapest, Hungary
[b] Heart and Vascular Center, Semmelweis University, Budapest, Hungary
[c] Department of Information Technology, Nikola Vaptsarov Naval Academy, Varna, Bulgaria
[d] Australian Maritime College, University of Tasmania, Launceston, Australia
[e] Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary
[f] 1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary

ARTICLE INFO

Article history:
Received 12 November 2014
Received in revised form 3 February 2015
Accepted 4 February 2015
Available online 9 February 2015

Keywords:
Myocardial infarction
Peripheral artery disease
Thrombus
Fibrin
Platelet

ABSTRACT

Introduction: Fibrin structure and cellular composition of thrombi profoundly affect the clinical outcomes in ischemic coronary and peripheral artery disease. Our study addressed the interrelations of structural features of thrombi and routinely measured laboratory parameters.
Materials and methods: Thrombi removed by thromboaspiration following acute myocardial infarction (n = 101) or thrombendarterectomy of peripheral arteries (n = 50) were processed by scanning electron microscopy and immunostaining for fibrin and platelet antigen GPIIb/IIIa to determine fibrin fibre diameter and relative occupancy by fibrin and cells. Correlations between the structural characteristics and selected clinical parameters (age, sex, vascular localization, blood cell counts, ECG findings, antiplatelet medication, accompanying diseases, smoking) were assessed.
Results: We observed significant differences in mean fibre diameter (122 vs. 135 nm), fibrin content (70.5% vs. 83.9%), fluorescent fibrin/platelet coverage ratio (0.18 vs. 1.06) between coronary and peripheral thrombi. Coronary thrombi from smokers contained more fibrin than non-smokers (78.1% vs. 62.2% mean occupancy). In the initial 24 h, fibrin content of coronary thrombi decreased with time, whereas in peripheral thrombi platelet content increased in the first 7 days. In coronaries, higher platelet content and smaller vessel diameter were associated with thinner fibrin fibres, whereas hematocrit higher than 0.35 correlated with larger intrathrombotic platelet occupancy. Smoking and dyslipidaemia strengthened the dependence of clot platelet content on systemic platelet count (the adjusted determination coefficient increased from 0.33 to 0.43 and 0.65, respectively).
Conclusion: Easily accessible clinical parameters could be identified as significant determinants of ultrastructure and composition of coronary and peripheral thrombi.

Introduction

Intravascular blood clots are highly heterogeneous in structure [1,2]. Macroscopically visible differences (e.g. the classical distinction of white and red thrombi) are tightly related to microscopic structural variations. Although these variations are strongly influenced by hemodynamic conditions and the general composition of blood, the effects of routinely registered clinical parameters on thrombus architecture are poorly elucidated. Conversely, it also remains to be clarified how structural properties affect the clinical picture and the course of disease. These correlations hold not only pathophysiological significance, but they may also provide new avenues for finding therapeutic targets. Both experimental [3] and theoretical [4] approaches evidence essential effects of flow dynamics and shear on thrombogenesis and on platelet deposition. Because of the known hemodynamic dissimilarities between coronary and peripheral arteries [5] evaluation of thrombus structure in these arteries could further the understanding of the role of flow conditions in thrombogenesis. Focusing on arterial thrombosis, the primary goal of the present study was to identify structural differences between clots formed at different sites of the arterial vasculature

* Corresponding author at: Semmelweis University, Department of Medical Biochemistry, 1094 Tűzoltó u. 37–47., Budapest, Hungary. Tel.: +36 1 4591500/60035; fax: +36 1 267 0031.
E-mail address: Krasimir.Kolev@eok.sote.hu (K. Kolev).
[1] These authors contributed equally to the study.

http://dx.doi.org/10.1016/j.thromres.2015.02.004
0049-3848/

and to compare these differences with available clinical data in order to identify potential association patterns.

Coronary thrombi that are formed on ruptured atherosclerotic plaques of coronary arteries thereby causing acute myocardial infarction (AMI) are now routinely treated by percutaneous coronary intervention (PCI) with or without thromboaspiration [6,7], whereas thrombi or emboli of the peripheral large arteries are usually removed via open surgery. Though both techniques offer a great opportunity for microscopic evaluation of the removed specimen, pathological examination of thrombi is not part of routine diagnostics, perhaps due to the lack of definite evidence on its prognostic value for the final resolution of clot remnants at the sites of culprit lesion or distal embolization. However, there is abundant experimental evidence for the impact of fibrin structure on the mechanical and lytic stability of clots [2,8,9], which on its own affects the disease evolution and clinical outcome. In addition, data on the correlations of routinely measured clinical parameters and the structural characteristics of arterial thrombi could provide clues to estimate the fibrinolytic susceptibility of patients, for whom thrombolysis is the therapy of choice, e.g. in acute ischemic stroke according to the current guidelines [10].

Materials and Methods

Patient Characteristics

Fifty consecutive peripheral artery thrombosis patients and 101 consecutive AMI patients of various age and sex were recruited over 22 months. Coronary patients were referred to primary PCI after developing symptoms of STEMI or NSTEMI (inclusion criteria: definite diagnosis of AMI, eligibility for PCI, no previous thrombolytic therapeutic approach and TIMI thrombus grade ≥ 3). Angiography and thromboaspiration were performed from radial or femoral access with QuickCat (Spectranetics Int., Leusden, Netherlands), Export (Medtronic Inc, Minneapolis, MN) or Eliminate (Terumo, Gifu, Japan) aspiration catheters. All patients were pre-treated immediately before revascularization with aspirin (500 mg), intravenous bolus of unfractionated heparin (5000 IU), abciximab (unless an absolute contraindication was present) and clopidogrel (600 mg). Patients already taking any antiplatelet agents on a regular basis were given a reduced dose. Peripheral artery thrombosis patients presenting with acute symptoms were treated by thrombendarterectomy by Fogarty catheter and semi-closed endarterectomy by Vollmar ring stripper in local or general anaesthesia (inclusion criteria: definite radiographic and clinical diagnosis of arterial occlusion, eligibility for surgery). Thrombi formed in bypass-grafts from previous interventions in peripheral artery disease or emboli stemming from lesions proximal to the site of occlusion were not handled separately in this study. During vascular reconstruction systemic heparin (150 IU/kg) was administered before clamping. Thirty-nine of the thrombendarterectomized patients received antiplatelet drugs (aspirin, clopidogrel or their combination) preoperatively. Eight patients were on oral anticoagulant therapy.

Patient characteristics are summarized in Table 1. In a few cases, the number of evaluated data (shown in parentheses) is lower than the total number of patients due to incomplete clinical documentation (e.g. aspirin and clopidogrel premedication was taken into account only when adequate documentation was available). All AMI patients, and those peripheral patients with macroscopically visible atherosclerotic plaques were considered positive for atherosclerosis. Patients already taking antihyperlipidaemic agents or showing with abnormal cholesterol or triglyceride levels at screening were classified as dyslipidaemic and all of them were on standard statin treatment at the time of blood sampling. Blood was drawn by standard venipuncture within 24 h prior PCI or thrombendarterectomy, and collected in Vacutainer tubes (Beckton Dickinson) containing K_2-EDTA for blood count. Written informed consent was obtained from all patients and the study protocol was approved by the institutional and regional ethical board.

Table 1
Selected clinical data of patients and measured structural characteristics of thrombi.

	Coronary (101)	Peripheral (50)	All (151)
Demographic data			
Male	62.4% (63/101)	60% (30/50)	61.6% (93/151)
Female	37.6% (38/101)	40% (20/50)	38.2% (58/151)
Age at operation	62.9 ± 13.9 (100)	67.1 ± 12.4 (50)	64.2 ± 13.6 (150)
Accompanying diseases			
Atherosclerosis	100% (101/101)	92% (46/50)	97.4% (147/151)
Diabetes	15.8% (12/76)	34% (17/50)	23% (29/126)
Hypertension	67.1% (51/76)	96% (48/50)	78.6% (99/126)
Dyslipidaemia	38.2% (29/76)	58% (29/50)	46% (58/126)
Uraemia	1.3% (1/76)	6% (3/50)	3.2% (4/126)
Thrombophilia	0% (0/76)	6% (3/50)	2.4% (3/126)
Tumor	1.3% (1/76)	12% (6/50)	5.6% (7/126)
Smoking			
Current or former	56.6% (43/76)	74% (37/50)	63.5% (80/126)
Never	43.4% (33/76)	26% (13/50)	36.5% (46/126)
Laboratory findings			
WBC (10^3/μL)	11.9 ± 4.3 (101)	9.6 ± 4.8 (49)	11.1 ± 4.6 (150)
Plt (10^3/μL)	241.1 ± 63.9 (101)	267.9 ± 107.6 (49)	249.8 ± 81.4 (150)
RBC (10^6/μL)	4.5 ± 0.6 (101)	4.5 ± 0.6 (49)	4.5 ± 0.6 (150)
Hgb (g/dL)	13.4 ± 1.8 (101)	13.7 ± 2.2 (49)	13.5 ± 1.9 (150)
Ht (%)	40.3 ± 5.3 (101)	40.5 ± 6.0 (49)	40.4 ± 5.5 (150)
CRP (mg/L)	16.1 ± 26.2 (81)	62.7 ± 63.2 (11)	21.7 ± 35.7 (92)
ECG findings			
STEMI	80.8% (80/99)		
NSTEMI	19.2% (19/99)		
Inferolateral MI	3.5% (3/85)		
Inferior MI	51.8% (44/85)		
Anterior MI	42.4% (36/85)		
Anteroseptal MI	1.2% (1/85)		
Posterior MI	1.2% (1/85)		
Localization of thrombi			
Venous		6% (3/50)	
Branch		4% (2/50)	
Axillo-femoral		2% (1/50)	
Aorto-femoral		4% (2/50)	
Ilio-femoral		44% (22/50)	
Femoro-popliteal		26% (13/50)	
AAA		14% (7/50)	
CX	14% (14/100)		
LAD	41% (41/100)		
RCA	41% (41/100)		
LAD/RCA	1% (1/100)		
Arteficial	3% (3/100)		
Antithrombotic medication prior to intervention			
Aspirin	100% (86/86)	64% (32/50)	86.8% (118/136)
Clopidogrel	96.6% (82/88)	14% (7/50)	64.5% (89/138)
Clinical data of thrombi			
Thrombus age (h)	14.6 ± 37.9 (93)	174.8 ± 241.1 (40)	62.8 ± 153.7 (133)
Vessel diameter (mm)	3.2 ± 0.4 (87)	14.9 ± 17.7 (50)	7.5 ± 12.0 (137)

Listed values are either mean ± SD or median [IQR]. The number of available samples or data is shown in parentheses. Abbreviations: WBC - white blood cells; Plt - platelets; RBC - red blood cells; Hgb - hemoglobin; Ht - hematocrit; CRP - C-reactive protein; MI - myocardial infarction; STEMI - ST-elevation myocardial infarction; NSTEMI - non-ST-elevation myocardial infarction; AAA - abdominal aorta aneurysm; CX - circumflex coronary artery; LAD - left anterior descending coronary artery; RCA - right coronary artery.

Scanning Electron Microscope (SEM) Imaging of Thrombi

Immediately after the intervention thrombus samples were fixed and processed as previously described [2]. Images were taken with scanning electron microscope EVO40 (Carl Zeiss GmbH, Oberkochen, Germany) and morphometrically analysed in two ways: 1) Images were divided in 432 squares (8.4 μm^2 each) using Photoshop 7.0 (Adobe, San José, CA) and these areas were classified as fibrin, red blood cells (RBC), platelet, white blood cells (WBC) or their