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Full Length Research Paper

Association between circulating levels of P-selectins and burden of thrombus formation in patients with ST-elevation acute myocardial infarction

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We tested the hypothesis that, in the acute phase of ST-segment elevation myocardial infarction (STEMI), the circulating level of P-selectin (PS) is predictive of angiographic morphologic features that indicate burden of thrombus formation in the infarct-related artery (IRA). One hundred and ninety-five consecutive patients with acute myocardial infarction (AMI) who underwent primary percutaneous coronary intervention (PCI) within 12 h of symptom onset were divided into the high-burden thrombus formation (HBTF) (N = 84) group and low-burden thrombus formation (LBTF) group (N = 111) based on cineangiography carried out during PCI. Blood samples for measurement of the circulating levels of high-sensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6), soluble intercellular cell adhesion molecule-1 (sICAM-1), PS and white blood cell (WBC) count were collected before PCI. The WBC count, neutrophil granulocyte count and PS level were significantly higher in the HBTF group than in the LBTF group (11.24 ± 3.62 vs 10.00 ± 3.35, p = 0.014; 8.84 ± 3.42 vs 7.66 ± 3.23, p = 0.015; 13.62 ± 8.13 vs 7.80 ± 4.17, p = 0.000, respectively). Patients in the HBTF group had a significantly lower prevalence of post-PCI thrombolysis in myocardial infarction (TIMI) grade-3 flow than that of the LBTF group (84.52% vs 93.69%, p = 0.037). Compared with the LBTF group, the HBTF group had higher peak values of the myocardial band (MB) fraction of creatine kinase (CK-MB). (308.52 ± 215.26 vs 213.79 ± 185.27, p = 0.005). By logistic regression analysis, PS (OR 1.259, 95%CI 1.125 - 1.408, p = 0.000) were independent predictors of thrombus formation. PS level was the strongest independent predictor of angiographic morphologic features that indicate HBTF in AMI.

Key words: Acute myocardial infarction, thrombus formation, P-selection.

INTRODUCTION

Platelets play a crucial part in the pathogenesis of acute coronary syndrome (ACS). Activated platelets adhere to the vessel wall at the site of a ruptured plaque and initiate formation of an arterial thrombus, which leads to ischemia or infarction (Théroux and Fuster, 1998; Gawaz, 2004; Yip et al., 2004). Increasing evidence suggests that markers of platelet activation can be used to identify disease activity (Kabbani et al., 2001; Yip et al., 2005). P-selectin (PS) is a cell-adhesion molecule produced by activated platelets and endothelial cells. PS is important in modulating the interaction of these cells with neutrophils and monocytes, In platelets, PS is rapidly translocated to the platelet surface after stimulation and is up-regulated in fresh thrombi, thereby contributing to thrombus formation (Hsu-Lin et al., 1984; Carlos and Harlan, 1998; Frenette et al., 1995; Gamble et al., 1990). Studies have indicated that PS mediates the accumulation of leukocytes, which in turn promotes fibril deposition (Palabrica et al., 1992). Other authors have shown that PS expression on platelets determines the aggregate size and stability of platelets (Merten and Thiagarajan, 2000).

Recent studies have demonstrated that the infarct-related

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artery (IRA) usually has a high burden of thrombus formation (HBTF) (Yip et al., 2001). A link between the angiographic morphologic features that indicate HBTF and the slow/no-reflow phenomenon has been demonstrated (Yip et al., 2002). Even if there is thrombolysis in myocardial infarction (TIMI) grade-3 flow in the IRA with HBTF, some patients may not achieve effective myocardial perfusion because of the micro-cardiovascular disease. Little is known about the predictors of thrombus formation in patients with acute myocardial infarction (AMI). Identifying these predictive factors is very important because it will help to pinpoint high-risk patients likely to benefit from intensive medical therapy.

We tested the hypothesis that, in AMI, the levels of white blood cells (WBCs) and PS are predictive of angiographic morphologic features that indicate HBTF in the IRA of patients with ST-elevation acute myocardial infarction (STEMI).

MATERIALS AND METHODS

Approval of the study protocol

This study was approved by the Ethics Committee of Xuanwu Hospital. All patients supplied written informed consent to be involved in the study.

Study population and inclusion criteria

All patients in the Cardiology unit of the Department of Xuanwu Hospital (Capital Medical University, Beijing, China) with STEMI of duration <12 h were considered eligible for primary percutaneous coronary intervention (PCI). The angiographic morphologic features of the IRA that indicate HBTF (Yip et al., 2002) are defined as: (i) An angiographic thrombus with a greatest linear dimension that is more than three fold the reference lumen diameter (RLD); (ii) a cutoff pattern (that is, lesion morphology with an abrupt cutoff without tapering before the occlusion); (iii) an accumulated thrombus (> 5 mm of linear dimension) proximal to the occlusion; (iv) a floating thrombus proximal to the occlusion; (v) persistent dye stasis distal to the obstruction; and (vi) RLD of the IRA ≥ 4.0 mm. It was defined as low burden of thrombus formation (LBTF) without angiographic morphologic features of the IRA above.

We drew-up specific exclusion criteria to avoid other variables that could influence the circulating levels of markers of inflammation and blood coagulation. We excluded patients: (i) Who had surgery or trauma within the preceding 2 months; (ii) with renal insufficiency (creatinine > 1.5 mg/dL), malignancy or liver cirrhosis, febrile disorders, acute or chronic inflammatory disease on study entry or a recent history of infection; (iii) with AMI onset ≥ 12 h; who had used antiplatelet agents for >3 days before AMI.

From May 2007 to April 2008, 195 patients (84 patients with HBTF and 111 patients with LBTF) were enrolled in the study.

Definitions and data collection

AMI was defined as typical chest pain lasting for >30 min with ST segment elevation (ST-SE) >1 mm in two or more consecutive precordial or inferior leads. Procedural success was defined as successful primary PCI with residual stenosis of the index lesions <20% after stent deployment and a final TIMI flow of grade 3 in the IRA. Detailed in-hospital and follow-up data (age, sex, coronary risk factors, serial levels of MB fraction of creatine kinase (CK-MB), WBC count, platelet count, creatinine level, echocardiography, angiographic findings) were collected prospectively and entered into a computerized database.

Procedure and protocol

A transradial artery approach using a 6-F arterial sheath is routinely used for treatment of AMI in our hospital unless the Allen's test for both hands is positive. A 6-F Kimny guiding catheter (Boston Scientific Scimed Incorporated, Maple Grove, MN, USA) was used for diagnosis and primary PCI. Clopidogrel (preoperative loading dose of 300 mg, then 75 mg/day) was given for at least one year to patients who underwent primary stenting. Aspirin (preoperative loading dose of 300 mg, then 100 mg/day) was given to each patient

Analyses of blood samples

Blood samples were obtained by venous puncture as soon as diagnosis of STEMI was made in the Emergency Department of Xuanwu Hospital. WBC count and platelet count were determined by standard laboratory methods. The concentration of highly sensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6), intercellular adhesion molecule (ICAM) and PS in plasma was determined in duplicate using a standard enzyme-linked immunosorbent assay (ELISA) and a commercial kit (R&D Systems, Minneapolis, MN, USA) according to manufacturer's instructions. Intra-individual variation of these levels was assessed in the study patients, the mean intra-assay coefficient of variation was <7%.

Clinical prognosis

Clinical information regarding major adverse events, including any-cause death, re-infarction, heart failure requiring hospitalization, or stroke, was obtained during the hospitalization. Heart failure was defined as a hospital readmission for which heart failure was the primary reason. Stroke was defined as a new focal neurological deficit of sudden onset lasting ≥24 h that was caused by hemorrhage or ischemia with corroborative imaging evidence by computed tomography (CT) or magnetic resonance imaging (MRI).

Statistical analysis

Results are number (%) or the mean ± SD. Comparisons between groups were done using Student's *t*-test for continuous variables and the chi-square test for categorical variables. $p < 0.05$ was considered significant. Multivariate logistic analysis was done to identify independent predictors for thrombi. All variables with $p < 0.05$ in the univariate analysis were tested. All calculations were carried out using the statistical package for the social sciences (SPSS) 13.0 for Windows statistical program (SPSS, Incorporated, Chicago, IL, USA).

RESULTS

Baseline characteristics

The baseline data for the study population are shown in Table 1. Significant differences between the groups with respect to mean age, sex, levels of creatinine, platelet, hs-CRP, IL-6, soluble intercellular adhesion molecule

Table 1. Baseline characteristics of patients.

Characteristic	HBTF group (n = 84)	LBTF group (n = 111)	P
Age, (y)	59.9 ± 12.8	62.1 ± 12.4	0.812
Males, n (%)	69 (82.1%)	87 (78.4%)	0.515
Hypertension, n (%)	50 (59.5%)	69 (62.2%)	0.230
Diabetes mellitus, n (%)	21 (25.0%)	22 (19.8%)	0.388
Current smokers, n (%)	56 (66.7%)	73 (65.7%)	0.895
Glucose (mmol/L)	9.50 ± 4.50	8.53 ± 3.94	0.106
WBC (10^9/L)	11.24 ± 3.62	10.00 ± 3.35	0.014
Neu (10^9/L)	8.84 ± 3.42	7.66 ± 3.23	0.015
Platelets (10^9/L)	256.46 ± 63.27	253.18 ± 73.31	0.743
hsCRP (mg/L)	6.50 ± 5.18	5.68 ± 4.98	0.452
IL-6 (pg/mL)	10.32 ± 13.10	8.74 ± 12.91	0.472
sICAM (ng/mL)	19.28 ± 9.50	19.44 ± 8.51	0.911
PS (ng/mL)	13.62 ± 8.13	7.80 ± 4.17	0.000
Time of door to balloon (h)	4.08 ± 2.50	4.55 ± 2.72	0.237
LVEF (%)	54.31 ± 10.85	56.34 ± 10.85	0.232
E/A	1.15 ± 0.56	1.06 ± 0.49	0.334
Peak CK-MB (ng/mL)	308.52 ± 215.26	213.79 ± 185.27	0.005
Post-PCI TIMI-3 flow, n (%)	71 (84.52%)	104 (93.69%)	0.037
MACE, n (%)	1 (1.2%)	1 (0.9%)	0.842

WBC: White blood cell; Neu: neutrophil granulocyte; hs-CRP: highly sensitive C-reactive protein; IL-6: interleukin-6; sICAM: soluble intercellular adhesion molecule; PS: P-selection; MACE: major adverse events; LVEF: left-ventricular ejection fraction; E/A: ratio of early (E) and atrial (A) transmitral maximal flow velocities; CK-MB: serial MB fraction of creatine kinase; TIMI: thrombolysis in myocardial infarction; PCI: percutaneous coronary intervention.

(sICAM) and frequencies of coronary risk factors (history of diabetes mellitus and hypertension and smoking) were not observed ($p > 0.05$).

WBC count, neutrophil granulocyte count (Neu) and PS level were significantly higher in the HBTF group than in the LBTF group (11.24 ± 3.62 vs 10.00 ± 3.35, $p = 0.014$; 8.84 ± 3.42 vs 7.66 ± 3.23, $p = 0.015$; 13.62 ± 8.13 vs 7.80 ± 4.17, $p = 0.000$) (Table 1).

Angioplasty results and prognosis

Patients in the HBTF group had a significantly lower rate of post-PCI TIMI grade-3 flow than that of patients in the LBTF group (84.52% vs 93.69%, $p = 0.037$). Compared with the LBTF group, the HBTF group had higher peak creatine kinase-myocardial band fraction (CK-MB) values (308.52 ± 215.26 vs 213.79 ± 185.27, $p = 0.005$), but the two groups had the same prevalence of major adverse events (Table 1).

Prediction of HBTF

Logistic regression analysis identified only the circulating level of PS (odds ratio (OR) 1.259, 95% confidence interval (CI) 1.125 - 1.408, $p = 0.000$) as significant independent predictors of HBTF on angiography. HBTF was also related to the peak level of CK-MB (Table 2).

DISCUSSION

The results of the present study have several striking clinical implications. Firstly, the circulating level of PS, WBC count and neutrophil granulocyte count were significantly higher in patients with HBTF than that in patients with LBTF. Secondly, levels of circulating PS were independently predictive of HBTF in the IRA. Thirdly, patients with HBTF in the IRA had a higher level of peak CK-MB and lower rate of TIMI-3 flow after PCI than that in patients with LBTF. The level of peak CK-MB was related to thrombus formation. These findings were corroborated by the finding that inflammatory mediators are frequently obtained from thrombi in human coronary arteries after AMI (Hoshiba et al., 2006).

Few studies have evaluated the role of leukocyte subtypes in determining cardiovascular risk and the results are conflicting. One study demonstrated that monocytes have the highest predictive value (Sweetnam et al., 1997), whereas other studies showed that neutrophils are the best predictors (Huang et al., 2003; Kawaguchi et al., 1996). Recent studies with animal models showed direct visualization of neutrophilic invasion of atherosclerotic

Table 2. Stepwise logistic regression analysis of independent predictors of thrombus formation.

Predictors	Odds ratio	95% confidence interval	P
WBC (10^9/L)	0.807	0.516 - 1.261	0.346
Neu (10^9/L)	1.356	0.851 - 2.161	0.201
PS (ng/mL)	1.259	1.125 - 1.408	0.000
Post-PCI TIMI-3 flow	0.598	0.152 - 2.341	0.460
Peak CK-MB (ng/mL)	1.003	1.000 - 1.005	0.038

WBC: White blood cell; Neu: neutrophil granulocyte; PS: P-selectin; TIMI: thrombolysis in myocardial infarction; PCI: percutaneous coronary intervention; CK-MB: serial MB fraction of creatine kinase.

plaques. Neutrophils may make plaques rupture more easily through the release of proteolytic enzymes, derivatives of arachidonic acid and superoxide radicals (Naruko et al., 2002; Li et al., 2009). The higher neutrophil count may not only mirror the exacerbated inflammatory conditions found in patients with atherosclerotic disease, but may also reflect the association of neutrophils with the instability of atherosclerotic plaques. The current study showed a substantially increased WBC count and neutrophil count in patients with thrombus formation compared with patients without thrombus formation. The suggestions presented here, which are based on clinical observation and laboratory findings, are supported by a study demonstrating that leukocyte accumulation promotes fibrin deposition (Palabrica et al., 1992). Two distinct mechanisms may explain this observation: (i) Neutrophilia as a reflection of systemic inflammatory status (and consequently increased cardiovascular risk) and (ii) lymphopenia as a reflection of the acute stress presented by acute coronary syndrome (Zazula et al., 2008). Platelet–neutrophil aggregates retrieved from ruptured plaques may be associated with an impaired coronary microcirculation and resultant myocardial necrosis/dysfunction. These findings underscore the importance of the interaction between thrombosis and inflammation in AMI pathogenesis (Arakawa et al., 2009). Accordingly, we suggest that sequestration of WBCs in the IRA may have had a key role in HBTF patients. Our findings therefore highlight the important role of the WBC count in assessment of the increased risk of poor coronary blood flow in the IRA after AMI (Schönbeck and Libby, 2001; Barron et al., 2000).

Although animal and *in-vitro* studies have stressed the important role of PS in facilitating the development of atherosclerosis lesions (Burger and Wagner, 2003) mediating endothelial–leukocyte–platelet interactions (Hsu-Lin et al., 1984; Carlos and Harlan, 1998; Frenette et al., 1995; Gamble et al., 1990) and stabilizing platelet aggregation (Merten and Thiagarajan, 2000), data on the role and circulating level of soluble P-selectin (sPS) after AMI remains limited (Shimomura et al., 1998; Gurbel et al., 2001). Yip et al. (2006) found that, among AMI patients, the serum level of sPS was markedly higher in the IRA than in the systemic circulation. An increased level of sPS in the IRA was significantly associated with increased atherothrombotic burden in the IRA. This suggests that sPS may play a crucial part in the formation and organized maintenance of the thrombus in the IRA. This is supported by an experimental study demonstrating that sPS facilitates fibrin deposition within the thrombus (Palabrica et al., 1992.

The present study suggested that the level of sPS is higher in patients with HBTF than that in patients with LBTF, and that this effect is an independent predictor of thrombus formation. Studies demonstrated that PS is rapidly expressed on the surface of activated platelets and endothelial cells after plaque rupture and thrombus formation in the coronary artery (Carlos and Harlan, 1998).

Glycoprotein P-selectin, a membrane component of cell-storage granules (Berman et al., 1986), is rapidly translocated from the a-granules of platelets and the Weibel–Palade bodies of endothelial cells to the cell surface after an inflammatory process or other stimulation (Hsu-Lin et al., 1984; McEver et al., 1989). Consequently, enzymatic cleavage of expressed PS and alternative splicing of the messenger ribonucleic acid of PS occur quickly, giving rise to sPS in the peripheral blood that can be detected (Ishiwata et al., 1994; Johnston et al., 1990; Michelson et al., 1996). The link between endothelial–platelet–leukocyte interaction and thrombus formation is well recognized (Carlos and Harlan, 1998; Palabrica et al., 1992; Berger et al., 1998). PS expression on platelets in the determination of the ability and size of platelet aggregation has been shown *in vitro* (Merten and Thiagarajan, 2000). HBTF is usually maintained and no re-flow phenomena occur more frequently during late reperfusion time (> 240 min ≤ 3 days) (Yip et al., 2002; Yip et al., 2004). Studies in animal models have demonstrated that the infarction size of the myocardium can be reduced if monoclonal antibodies against PS are administered (Weyrich et al., 1993; Ueyama et al., 1997). An animal-model study demonstrated that accumulation of leukocytes in the thrombus (which is mediated by PS)

promotes fibrin deposition (Palabrica et al., 1992). Another study suggested that sPS may be involved in modulating leukocyte recruitment or thrombus growth (Ueyama et al., 1997).

The benefits of direct PCI are limited by a prevalence of combined slow flow (TIMI grade 2) of 5 - 20% and no-reflow (≤TIMI grade 1) phenomena (Kaul et al., 1999). The slow-flow and no-reflow are associated with more extensive myocardial necrosis and consequential left-ventricular dilatation, with poor regional and global contractile function and with a poor prognosis (Kern et al., 1996; Fu et al., 2007). The putative mechanisms for failure to achieve normal coronary flow in the IRA include distal embolization of the thrombus and of debris, microvascular damage or edema, reperfusion injury and microvascular dysfunction resulting from the intervention-induced release of lipid pool-like plaque contents (Tanaka et al., 2002). The present study suggests that no-reflow/slow flow of the IRA during PCI was associated with thrombus formation and that the peak level of CK-MB was higher in patients with thrombus formation than without thrombus formation. This supports the hypothesis that thrombus formation in the IRA is the most important factor leading to no-reflow or slow flow, increased myocardial necrosis and increased mortality (Yip et al., 2002).

There were several limitations to our study. First, this was a small retrospective study of patients who met strict entry criteria; but we believe that these criteria enabled us to demonstrate our results more clearly. Second, this study did not provide additional information with respect to the effect of the circulating level of these blood markers on the long-term outcomes of the patients.

Conclusion

The circulating level of PS, WBC count and neutrophil granulocyte count were higher and PS was an independent predictor of the angiographic morphologic features that indicate HBTF in AMI.

Abbreviations

STEMI, ST-Segment elevation myocardial infarction; **PS,** P-selectin; **IRA,** infarct-related artery; **AMI,** acute myocardial infarction; **PCI,** percutaneous coronary intervention; **HBTF,** high-burden thrombus formation; **LBTF,** low-burden thrombus formation; **hs-CRP,** high-sensitive C-reactive protein; **IL-6,** interleukin-6; **sICAM-1,** soluble intercellular cell adhesion molecule-1, **WBC,** white blood cell; **TIMI,** thrombolysis in myocardial infarction; **ACS,** acute coronary syndrome; **RLD,** reference lumen diameter; **ELISA,** enzyme-linked immunosorbent assay; **sPS,** soluble P-selectin; **CK-MB,** creatine kinase-myocardial band fraction.

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