Background and aims It is well known that the interaction between platelets (PLTs), endothelial cells, and leukocytes contributes to thrombosis in patients with acute coronary syndrome. The aim of this study was to investigate the significance of PLTs and eosinophils (EOS) in coronary arterial thrombi.

Methods PLT count, mean PLT volume, PLT mass, EOS count, EOS percentage, and troponin I level in peripheral blood were determined in 81 patients with angina pectoris (AP) and 49 patients with acute myocardial infarction (AMI). A total of 12 thrombus specimens from AMI patients were submitted for histopathological analysis. EOS presence in thrombectomy specimens were checked by hematoxylin–eosin staining and confirmed by Luna staining.

Results Results showed that EOS were present in all 12 samples (100%). Cell count and percentage of EOS in peripheral blood of patients with AMI were lower than those in patients with AP (both P < 0.00001). A higher PLT count was observed in AMI patients (243 ± 70), especially among female patients or those who were older than 60 years, when compared with AP patients (216 ± 60; all P < 0.05).

According to the troponin I level, we divided AMI patients into groups I (≥20 ng/ml) and II (< 20 ng/ml). Group I had a lower EOS percentage compared with group II (P = 0.0496).

PLT count was also lower in group I with no statistical difference found (P = 0.1202). Moreover, there was an inverse correlation between the EOS percentage and the troponin I level (r = −0.434).

Conclusion In conclusion, patients with AMI presented with a decreased EOS percentage and an increased PLT count. The decreased EOS percentage suggested serious myocardial damage. The study indicated that EOS play an important role in thrombosis in patients with acute coronary syndrome. Coron Artery Dis 26:101–106 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

Keywords: acute coronary syndrome, eosinophils, platelets, thrombosis

Background Thrombosis is one of the main causes of acute coronary syndrome (ACS). Activated platelets (PLTs) play a critical role in the initial stages of blood coagulation and in the formation of a hemostatic plug. PLT reactivity is increased in acute thrombus events. Increasing evidence has demonstrated that PLTs are essential for coronary thrombosis [1–3]. The increased mean PLT volume (MPV), which was an indicator of elevated PLT activation, was reported to be associated with a greater risk for myocardial infarction and ischemia-reperfusion injury [4–6]. Several studies have shown that anti-PLT therapy significantly reduces the risk for cardiovascular events [7,8].

Besides PLTs, eosinophils (EOS) are also essential during thrombosis. Throughout the previous decades, the possible roles of EOS in blood coagulation and thrombosis were suggested. Numerous reports of thrombotic events in patients with EOS-related disorders confirmed that EOS were involved in the thrombus formation process. Patients who had hypereosinophilic syndrome (HES) were more prone to thrombosis [9,10]. It was estimated that one-quarter to nearly half of the HES patients exhibited thrombosis [1,11]. EOS release some cytokines to initiate coagulation, activate PLTs, and inhibit anticoagulant activity, which eventually culminates in thrombin generation and clot formation [12,13].

Sakai et al. [14] described the evolvement of EOS infiltration in the thrombi suction from infarct-related arteries of acute myocardial infarction (AMI) patients. They found EOS infiltration in 64% (106/165) of the samples. However, the association between EOS and PLTs in the peripheral blood of acute coronary syndrome patients, as well as their difference in angina pectoris (AP) and AMI patients remained unclear. In this study, we analyzed 12 samples of thrombim and found demonstrable infiltration.
of EOS and PLTs. Moreover, we investigated the changes in PLT count, MPV, EOS count and percentage, and the troponin I level in the peripheral blood of patients with AP (n = 81) or AMI (n = 49).

Reagents and methods
Clinical examination
A total of 130 patients, including 81 patients with AP and 49 patients with AMI, were enrolled in this study. All AMI patients underwent thrombus aspiration before balloon expansion during emergency percutaneous coronary intervention at Beijing Tiantan Hospital between 1 January 2012 and 31 December 2013. Tissue samples obtained from these patients were used in the present study. AMI was diagnosed according to ST-segment elevation of the ECG and an increase in the troponin I level. All patients underwent emergency percutaneous coronary intervention within 12 h from the onset of chest pain and/or oppression. The clinical characteristics of the patients are presented in Table 1.

Blood sampling
Blood sample collection was always performed on admission to casualty, before any drug administration. Serum glucose, urea, and troponin levels were measured using an automated chemical analyzer (Olympus AU-600; Olympus, Tokyo, Japan), with reagents from the same manufacturer. Whole-blood PLT counts (reference range: 150–400×10^9/l), MPV (reference range: 7.2–11.1 fl), and EOS counts (reference range: 1.0–6.0%), expressed as a percentage of the white blood cell count (reference range: 4.6–10.2×10^9/l), were determined on a CELL DYN 4000 Abbott analyzer (Abbott Diagnostics, Santa Clara, California, USA), which was calibrated daily. The Department of Clinical Chemistry of the Beijing Tiantan Hospital participated in a quality assurance program.

Thrombus aspiration
Thrombus aspiration therapy was performed in all patients who showed an image of a large thrombus burden after coronary angiography, before any interventional devices other than guidewires and thrombectomy devices were used. All patients were administered heparin by injection (10 000 IU), and none received thrombolysis therapy or glycoprotein IIb/IIIa inhibitors before thrombus aspiration. Thrombus aspiration therapy was carried out with the Export XT Aspiration Catheter (Medtronic, Inc., Danvers, Massachusetts, USA).

Section staining
Tissue samples were placed in 10% buffered formalin immediately after aspiration. After embedding the samples in paraffin, they were cut to maximize the cross-sectional area along the long axis. Sections were stained with hematoxylin–eosin (HE) stain. Luna staining was performed as described previously [15]. In brief, slides were incubated for 5 min in 0.9 volumes of Weigert iron hematoxylin (0.005% acid hematoxylin and 0.6% ferric chloride in 2% HCl) with 0.1 volumes of 1% Biebrich scarlet and 0.1% acid fuchsin in 1% acetic acid. After differentiation in 1% acetic alcohol, slides were washed in water. Final color development was performed with 0.5% lithium carbonate.

Ethics statement
This study was approved by the ethical committee of the Beijing Tiantan Hospital, and written informed consent was obtained from the participants.

Statistics
Analyses were carried out using SPSS 12.0.1 (SPSS Inc., Chicago, Illinois, USA). Values were expressed as mean±SD. P-values were two-sided, and P less than 0.05 was considered statistically significant. Differences among groups were assessed using the unpaired t-test (parametric values) or by one-way analysis of variance (more than two groups). Correlation was assessed by deriving Pearson’s correlation coefficient.

Results
Eosinophils and platelets infiltrated into thrombi
A total of 12 thrombectomy specimens were submitted for histopathological analysis, including two from right coronary arteries, seven from left anterior descending arteries, and three from left circumflex arteries. Pathologically, eight samples (75%) were of mixed thrombus type (white thrombus and red thrombus). White thrombi were composed predominantly of PLT and fibrin, whereas red thrombi were composed of red blood cells and fibrin. EOS were observed in all 12 samples (100%). HE staining revealed eosinophilic granules. The location of EOS within thrombi is shown in Fig. 1. To confirm the presence of EOS, we performed Luna staining, on which eosinophilic granules appeared red on a blue background (Fig. 1e), in contrast to a pink colored background on HE staining (Fig. 1a and b). Furthermore, immunohistochemical staining of EOS peroxidase (EPX-mAb) was used to assess EOS

| Table 1 Baseline clinical characteristics of patients |
|-----------------|-----------------|-----------------|
| AMI | AP |
| Age (mean±SD) (years) | 61±11 (n = 25) | 62±12 (n = 32) |
| Male sex [n (%)] | 34 (69) | 47 (58) |
| Female sex [n (%)] | 15 (31) | 34 (42) |
| Body weight (mean±SD) (kg) | 71±10 | 73±11 |
| WBC | 12.4±4.5 | 6.9±1.5 |
| EOS | 0.05±0.05 | 0.13±0.09 |
| EOS% | 0.55±0.62 | 1.93±1.14 |
| PLT | 243±70 | 216±60 |
| MPV | 9.8±1.2 | 9.5±1.2 |
| PDW | 12.6±1.4 | 12.4±1.6 |
| Troponin I | 17.8±10.8 | All <0.05 |

AMI, acute myocardial infarction; AP, angina pectoris; EOS, eosinophil; MPV, mean platelet volume; PDW, platelet distribution width; PLT, platelet; WBC, white blood cell.
accumulation/degranulation [16]. Evidence of EOS degranulation includes extracellular release of EPX, enucleated EOS (i.e. cytoplasmic fragments), and/or the presence of free EOS granules. Assessments of degranulation following EPX-mAb immunohistochemical analysis revealed few areas exhibiting granular protein release (indicated by asterisk, Fig. 2, lower panel; arrows indicate EOS).

Eosinophil levels in the peripheral blood decreased in patients with acute myocardial infarction
The AMI group had significantly lower EOS counts and percentages (0.05±0.05 vs. 0.13±0.09, *P*<0.00001 and 0.55±0.62 vs. 1.93±1.14, *P*<0.00001, respectively) and higher PLT counts (*P*<0.02; Table 1) compared with the AP group. Male patients and patients with age less than 60 years in the AMI group had a significantly lower EOS count and percentage compared with patients in the AP group (both *P*<0.00001, one-way analysis of variance; Table 2). Among AMI patients, those with troponin I level at least 20 ng/ml had a significantly lower EOS percentage than those with troponin level below 20 ng/ml (0.3±0.33 vs. 0.11±0.1, *P*=0.0496; Table 2). An increase in the serum C-reactive protein level, which was prone to increase under stress, was detected in both AMI and AP patients. Data showed no difference between the two groups (11.8±6 vs. 8.1±16, *P*=0.21).

Platelet activity decreased in patients with acute myocardial infarction
A higher PLT count was seen in AMI (243±70) patients, especially among female patients and among patients 60 years or older (253±63 and 261±80, respectively; Table 2), when compared with AP patients (216±60). PLT count showed a significant difference between the AMI and AP groups (*P*=0.02; Table 1). In the AMI group, patients with a troponin I level of at least 20 ng/ml had a lower PLT count than those with troponin levels below 20 ng/ml (3.09±0.84 vs. 3.72±1.11, *P*=0.1202; Table 2). There was no difference in MPV between the AMI and AP groups (9.8±1.2 vs. 9.5±1.2, *P*=0.18; Table 1). The PLT count was significantly correlated with platelet distribution width in all groups (*r*=0.917, *P*<0.001, Pearson’s correlation).

Low eosinophil and platelet counts indicated severe coronary artery disease
There was an inverse correlation between EOS percentage and troponin I level (*r*=-0.434, *P*=0.03, Pearson’s correlation). Patients with troponin I levels below 20 ng/ml had higher EOS and PLT counts compared with those with troponin levels of at least 20 ng/ml; however, these differences were not significant in the AMI group (Table 2). No correlation was found between EOS count and hospitalization days (*r*=0.149). AMI patients
Fig. 3a showed obviously decreased EOS percentages compared with AP patients (Fig. 3b), which is shown in Fig. 3.

Discussion

Histopathologic findings from some studies suggest that PLTs play a pivotal role in the formation of thrombi. Thrombosis begins with PLTs adhering to the damaged vascular walls. In addition, EOS are a key factor during this process [2,3]. EOS could promote thrombi formation by activating PLTs [12]. Furthermore, the EOS granule proteins, particularly major basic proteins, potentially contribute toward the hypercoagulation seen in some patients with HES [17]. The relationship between EOS and thrombus formation was confirmed in HES patients. It is estimated that more than one-quarter of HES patients show thromboembolic events [1,10,12]. The best described thromboembolic events that are closely related to HES are cases of intracardiac thrombi, as well as pulmonary and arterial emboli [18]. Cardiovascular complications are a major cause of morbidity and mortality among HES patients. Whereas earlier studies have reported that up to 84% of HES patients show signs and symptoms of cardiac disease, more recent reports have suggested that the frequency is closer to 40–50% [19]. Other cases, including intra-abdominal, cerebral, and cutaneous thromboses, have also been reported. Some of these involved cardioembolic diseases involving multiple organs, and occasionally the origin of the thrombi appeared to be multifocal [20].

In the present study, EOS were observed in all 12 coronary thrombi samples. The results indicate that EOS play a more important role than has been considered previously. It is very interesting that EOS counts and percentages in peripheral blood decreased markedly in AMI patients compared with AP patients. What has happened to those ‘lost’ EOS? Were all of them deposited in the thrombi or in other places, or were they destroyed or degraded? These questions need further investigation.

Table 2 Differences in measured values between the studied groups

|           | AMI       |               |               |               | AP        |               |               |               |
|-----------|-----------|---------------|---------------|---------------|-----------|---------------|---------------|---------------|
|           | EOS       | EOS%          | PLT           | MPV           | cTnI      | EOS           | EOS%          | PLT           |
| **Sex**   |           |               |               |               |           |               |               |               |
| Male      | 0.04 ± 0.03* | 0.47 ± 0.43** | 239 ± 73      | 9.9 ± 1.2     | 18.3 ± 10.1 | 0.14 ± 0.12   | 2.04 ± 1.3    | 214 ± 54      |
| Female    | 0.06 ± 0.07 | 0.73 ± 0.91   | 253 ± 63$     | 9.7 ± 1.4     | 16.2 ± 13.6 | 0.11 ± 0.05   | 1.78 ± 0.86   | 219 ± 67      |
| **Age**   |           |               |               |               |           |               |               |               |
| <60       | 0.04 ± 0.03$ | 0.39 ± 0.34*  | 228 ± 57      | 10.1 ± 1.2    | 179 ± 11   | 0.15 ± 0.12   | 2.16 ± 1.17   | 212 ± 55      |
| ≥60       | 0.06 ± 0.06 | 0.73 ± 0.81   | 261 ± 80**    | 9.5 ± 1.3     | 17.7 ± 11  | 0.1 ± 0.07    | 1.76 ± 1.09   | 220 ± 63      |
| **cTnI**  |           |               |               |               |           |               |               |               |
| <20       | 0.03 ± 0.04 | 0.3 ± 0.33    | 3.72 ± 1.11   | 10.7 ± 0.7    |           | 0.15 ± 0.12   | 2.16 ± 1.17   | 212 ± 55      |
| ≥20       | 0.01 ± 0.01 | 0.11 ± 0.09$  | 3.09 ± 0.84   | 10.7 ± 0.5    |           | 0.1 ± 0.07    | 1.76 ± 1.09   | 220 ± 63      |

AMI, acute myocardial infarction; ANOVA, analysis of variance; AP, angina pectoris; cTn I, cardiac troponin I; EOS, eosinophil; MPV, mean platelet volume; PLT, platelet; WBC, white blood cell.

*p** = p ≤ 0.05, compared with the other corresponding groups, one-way ANOVA.

$P = 0.0496$, compared with the other corresponding groups, t-test.
Among AMI patients, those with troponin-I levels of at least 20 ng/ml presented with a significant lower EOS percentage than those with troponin I levels below 20 ng/ml. It is well known that the higher the troponin level, the worse the prognosis of the patient [21]. It appears that a decrease in the EOS percentage indicates serious myocardial damage, induced by extensive thrombi formation.

The interaction between EOS, PLTs, and the endothelium is a key step in thrombosis. Activated PLTs are associated with EOS in the pathology of several diseases, including asthma and HES [22]. PLTs are activated by EOS granules, major basic proteins and EPX. It has been reported that larger and hyper-reactive PLTs would accelerate the formation of thrombi. An increase in MPV, as an indicator of large and reactive PLTs, may therefore represent a risk factor for overall vascular mortality, including myocardial infarction. In this study, MPV in AMI patients did not show signs of reduction. In contrast, Keçoğlu et al. [23] reported that MPV increased in patients who had thrombus formation compared with those who did not have thrombus formation. To explain the correlation between MPV and EOS in AMI patients, a large number of cases would be necessary. We strongly suggested that more cases be included to address the real correlation between these factors. Ten years ago, Turakhia et al. reported that higher PLT counts were independently associated with the presence of residual thrombi in infarct-related arteries after administration of thrombolytic therapy [24]. Later, researchers found that increased PLT counts were an independent predictor of death and reinfarction in AMI patients [25,26]. In this study, PLT counts were found to be significantly higher in AMI patients compared with AP patients. The result is in accordance with previous reports.

In summary, our findings showed that EOS infiltration is frequently observed in coronary arterial thrombi. Patients with AMI had significantly lower EOS counts and percentages compared with AP patients. Further investigation suggested that the decreased EOS percentage indicated serious myocardial damage, which was induced by obvious thrombi formation.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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