Time course of various cell origin circulating microparticles in ST-segment elevation myocardial infarction patients undergoing percutaneous transluminal coronary intervention

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Abstract. The present study aimed to investigate the time course of changes in microparticles (MPs) in patients with ST-segment elevation myocardial infarction (STEMI) that underwent percutaneous transluminal coronary intervention (PCI). A total of 24 STEMI patients undergoing primary PCI were enrolled, and circulating MPs were detected immediately prior to and after PCI, and at 4, 24 and 48 h post-PCI. Standard Megamix beads, based measurement protocols, were employed to measure MPs of different cell origin, including endothelial MPs (EMPs), platelet MPs (PMPs) and leukocyte-derived MPs (LMPs), which were identified by CD144, CD41 and CD45, respectively. The results indicated that PMP levels were evidently elevated immediately after PCI, and reached a maximum level at 48 h. In addition, LMP and EMP levels were significantly decreased immediately after the PCI, and then increased gradually with time. The total quantity of the three aforementioned MP types increased gradually at 48 h following PCI. Furthermore, coronary angiographic Gensini scores were significantly positively correlated with the level of PMPs (r^2=0.42; P=0.0006). Log-normalized high sensitivity-C-reactive-protein was also significantly correlated with LMPs (r^2=0.86; P<0.01). In conclusion, the time course of the changes in circulating MPs of different cell origin, provided information on possible functions of different MPs in STEMI.

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Introduction

Circulating microparticles (MPs) released by various cells upon activation or apoptosis have been reported to be associated with cardiovascular events, which are characterized by endothelial dysfunction, abnormal hemostasis/thrombosis and/or a pro-inflammatory state (1). It has previously been reported that increased circulating MP levels indicated a poor prognosis in cardiovascular patients (2). Thus, MPs are emerging as novel biomarkers of acute myocardial infarction (AMI); however, further detailed evidence is required (2).

Inflammation in the coronary artery and inflammatory cytokines, such as C-reactive protein (CRP), have an important role in the pathogenesis of AMI (3). CRP has previously been identified as an important prognostic marker of unstable angina and myocardial infarction (4,5). MPs are important cytokine transporters (6), and their association with CRP has been investigated in several studies (7,8). However, the association between CRP and leukocyte-derived MPs (LMPs) has yet to be elucidated.

ST-segment elevation myocardial infarction (STEMI) results from ischemic injury due to rupture of unstable atherosclerotic plaque in a coronary artery (9). It has previously been observed that the number of total circulating MPs in STEMI patients was significantly higher than in patients with stable angina and controls (10). Upon reperfusion following percutaneous transluminal coronary intervention (PCI), large amounts of plaque-derived MPs were released from plaque in the crime vessel, and thus may influence the composition of circulating MPs. However, the time course of changes in the composition of MPs originating from different cells has yet to be determined.

In the present study, the concentrations of MPs of differing cell origin were measured in the circulation of STEMI patients undergoing PCI. Subsequently, the present study assessed whether there was a correlation between LMP levels and traditional serum markers for acute myocardial infarction, including cardiac troponin T (TnT) and high-sensitivity (hs)-CRP. Furthermore, the correlation between PMPs and coronary angiographic Gensini scores was examined.

Materials and methods

Study population. In total, 24 patients diagnosed with STEMI in the Department of Cardiology, Peking University Third Hospital (Beijing, China) between 1st of January 2012 and 1st of June 2013 were recruited into the present study. STEMI was diagnosed and treated according to the 2004 American College of Cardiology/American Heart Association guidelines (11). All patients underwent primary PCI within 12 h after the onset of symptoms, and the Thrombolysis In Myocardial Infarction (TIMI) flow grade (12) was ≥2 subsequent to PCI. Exclusion criteria included patients with an age of >80 years, cardiogenic shock at admission, TIMI flow grade <2 following PCI, previous history of myocardial infarction, significant valvular heart disease, peripheral vascular disease, chronic heart failure, chronic inflammatory diseases, significant kidney or hepatic diseases, cancer and administration of glycoprotein IIb/IIIa inhibitors. The present study was approved by the ethics review boards of Peking University Health Science Center (Beijing, China). All patients provided written informed consent for participation in the study. The Gensini Score, identifying the severity of coronary lesions, was calculated based on the angiographic results, according to a previously described method (13). A higher Gensini Score indicates more severe coronary lesions.

Treatment and procedures. STEMI patients were treated with a loading dose of aspirin (300 mg; 100 mg daily; Bayer AG, Leverkusen, Germany) and clopidogrel (600 mg; 75 mg daily; Sanofi, Paris, France) at admission, and a bolus of 100 IU/kg heparin (Sanofi) prior to PCI. The PCI procedure was performed according to ACC/AHA/SCAI guidelines (14), and involved the implantation of drug-eluted stents. Following PCI, the patients received standard therapy including aspirin (Bayer), clopidogrel, statins (Pfizer, Inc., New York, NY, USA), β-blockers (Astra-Zeneca, London, UK) and angiotensin-converting enzyme inhibitors (Astra-Zeneca, London, UK)/angiotensin II receptor blockers (if there were no contraindications; Sanofi). Serum TnT, creatine kinase-MB and hs-CRP levels were detected in blood samples immediately prior to PCI, immediately after PCI, and at 4, 24 and 48 h post-PCI, by the Department of Clinical Laboratory of Peking University Third Hospital. The measurement methods were as previous described (15).

MP separation. Blood samples were collected at multiple time points prior to and after PCI: Immediately prior to PCI, immediately after PCI, and at 4, 24 and 48 h post-PCI. The blood samples (3 ml) were collected from the right radial artery during PCI for the first two time-points, while samples from subsequent time-points were collected by standard vein puncture by trained nurses. Vacuum blood collection tubes with sodium citrate as a anticoagulation agent (BD Vacutainer Citrate tubes; Becton Dickinson, Franklin Lakes, NJ, USA) were used. Platelet-free plasma was immediately separated by 1409 x g centrifugation for 15 min, followed by 13,000 x g centrifugation for 2 min at room temperature. Platelet-free plasma was stored at -80°C for MP detection.

MP detection. For the detection of MPs, a Beckman Coulter Gallios flow cytometer (Beckman Coulter, Inc. Brea, CA, USA) was used to ensure the accurate enumeration and characterization of MPs of different origin. Megamix beads (0.5, 0.9 and 3 μm) were purchased from Biocytex (Marseille, France) and were used according to the manufacturer's instructions and as previously described (16). Endothelial MPs (EMPs) were identified using mouse monoclonal Phycoerythrin-CD144 antibodies (1:50; 358505; Biolegend, London, UK), platelet MPs (PMPs) were identified using mouse monoclonal fluorescein isothiocyanate-CD41 antibodies (1:100; ab19708; Abcam, Cambridge, UK), and LMPs were identified using mouse monoclonal PerCP/CY5.5-CD45 antibodies (1:50; 368503; Biolegend). EMPs were identified by dual staining using Phycoerythrin CD144 and Annexin V, PMPs were identified by dual staining using fluorescein isothiocyanate CD41 and Annexin V, and LMPs were identified by dual staining using PerCP/CY5.5 CD45 and Annexin V. All fluorescence stains were purchased from Nanjing KeyGen Biotech. Co., Ltd. (Nanjing, China) and staining was performed according to the manufacturer's instructions.

Table I. Patients' characteristics and therapies administered.

Characteristic	Value
Age, years	75-40 (58±4)
Male, %	62.5
Smoking, %	37.5
Diabetes, %	70.8
Hypertension, %	66.7
Body-Mass Index, kg/m^2	18.4-29.4 (23.7±1.5)
Total cholesterol, mmol/l	3.3-5.8 (4.6±0.3)
HDL-C, mmol/l	0.6-1.1 (0.8±0.04)
LDL-C, mmol/l	1.9-4.6 (3.2±0.3)
Medication	
Statin, %[a]	100
ACEI/ARB, %[a]	79.2
β-blocker, %[a]	100
Asprin+clopidogrel, %[b]	100

[a]Dosage varies between patients. [b]Aspirin and clopidogrel both administered 100 mg/day. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blockers.

Statistical analysis. Results are presented as the mean ± standard error of the mean. MP levels at different time points were compared using Student's t-tests, while binary logistic regression analysis was performed to identify an interaction between the Gensini score and MP levels. Two-tailed tests of significance are reported. For all comparisons, $P<0.05$ was considered to indicate a statistically significant difference. Statistical analysis was performed using SPSS version 19.0 (IBM SPSS, Armonk, NY, USA).

Results

Patient characteristics. A total of 24 patients diagnosed with STEMI were recruited. MP subpopulations in the blood

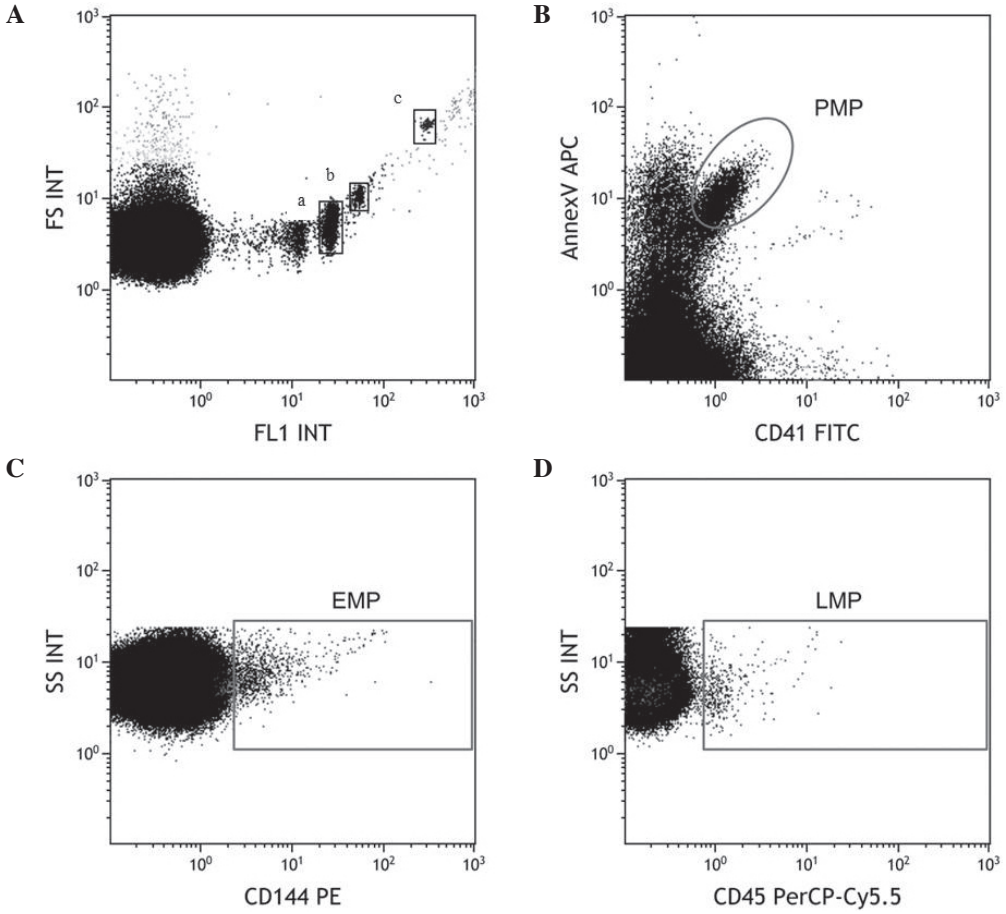

Figure 1. Identification of MPs and their subpopulations using flow cytometry. (A) Megamix beads containing 0.5, 0.9 and 3 μm beads (marked as a, b and c, respectively) were used to determine the gate to measure MPs. Various markers were used to detect the MPs originating from different cells: (B) PMPs were detected by CD41 and Annexin V; (C) LMPs were identified by PerCP/CY5.5-CD45; and (D) EMPs were identified by phycoerythrin-CD144 markers. MP, microparticle; PMP, platelet MPs; EMP, endothelial MPs; LMP, leukocyte-derived MPs.

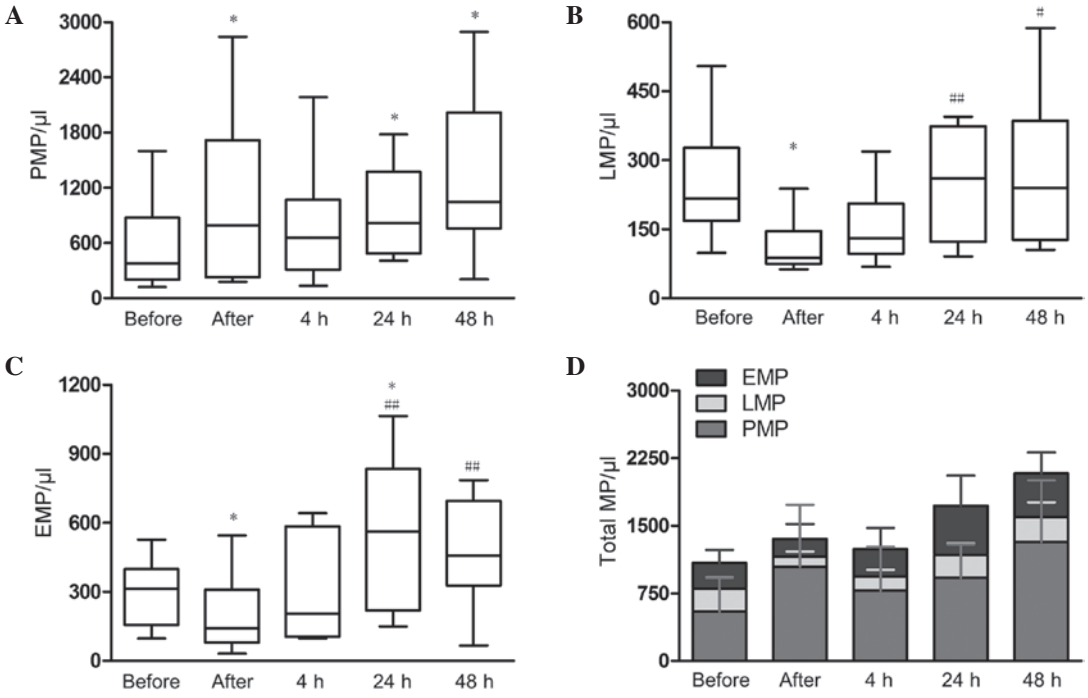

Figure 2. Time course of (A) PMPs, (B) LMPs, (C) EMPs and (D) total MPs during PCI as determined by flow cytometry. The levels of PMPs, LMPs, EMPs and total MPs at different time points after PCI were compared with levels immediately before and immediately after PCI. *P<0.05 vs. levels immediately before PCI; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. levels immediately after PCI. MP, microparticle; PMP, platelet MP; LMP; leukocyte-derived MP; EMP, endothelial MP; PCI, percutaneous transluminal coronary intervention.

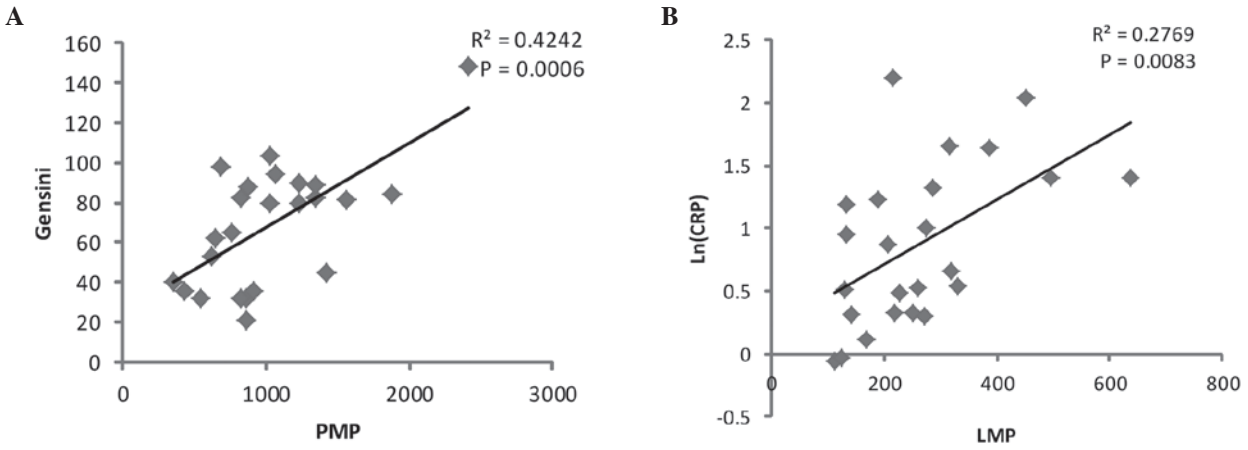

Figure 3. (A) Linear regression analysis results for Gensini score with PMPs; (B) Linear regression analysis results for log-normalized hs-CRP with LMPs. PMP, platelet microparticles; LMP; leukocyte-derived microparticles.

samples from all 24 patients were obtained. The clinical characteristics and medication administered to the participants of the present study are displayed in Table I, and were comparable with the patient population recruited in previous studies (17,18).

MP detection. In the present study, a Beckman Coulter Gallios flow cytometer (Beckman Coulter, Inc.) was used, which is a high-sensitivity cytometer with superior reproducibility for MP measurement (19,20). Megamix containing 0.5, 0.9 and 3 μm fluorescent beads was applied to ensure accurate identification of MPs in the flow cytometer (Fig. 1A). PMPs were identified as $CD41^+$/Annexin V^+. EMPs and LMPs were characterized by $CD144^+$ and $CD45^+$, respectively (Fig. 1B-D).

Time course of MPs. To the best of our knowledge, no previous study has examined the time course of MPs originating from different cells STEMI patients during PCI. In the present study, the levels of PMPs, EMPs and LMPs were measured at five time-points: Immediately prior to PCI, immediately after PCI, and 4, 24 and 48 h post-PCI (Fig. 2). It was revealed that the level of PMPs was evidently elevated immediately after PCI (1045±895/μl; P<0.05), and reached a maximum level at 48 h post-PCI (1325±882/μl; Fig. 2A). In addition, the levels of LMPs and EMPs decreased significantly immediately after PCI (LMPs: 250±126/μl vs. 114±59/μl, P=0.01; EMPs: 289±143/μl vs. 198±165/μl, P=0.04), and then increased gradually with time (Fig. 2B and C). EMPs reached peak levels at 24 h post-PCI, which is significantly higher compared with baseline levels (546±330/μl vs. 289±143, respectively; P=0.04). LMPs reached peak levels 48 h post-PCI. However, there was no significant difference compared with the baseline level (272±164 vs. 250±126, respectively; P=0.63). The total amount of MPs increased gradually 48 h after PCI (Fig. 2D).

Correlation between PMP levels and Gensini scores. PMPs have previously been reported to increase in patients with acute coronary syndrome (21), and may act as a marker of coagulation (22). Thus, the present study aimed to identify whether PMPs were correlated with the severity of coronary disease. Linear regression between coronary angiographic Gensini scores and PMP level prior to PCI was performed. The results identified that the Gensini score was significantly positively correlated with the level of PMPs prior to PCI (r^2=0.42; P=0.0006; Fig. 3A). However, no significant correlation was detected between EMPs and LMPs with the Gensini score (data not shown).

Correlation between LMP levels and CRP. LMPs have previously been identified to play an important role in atherosclerosis by promoting inflammation (23). CRP is also a well-established inflammatory marker, often used in patients with STEMI (5). Thus, linear regression analysis was performed between log-normalized hs-CRP [ln (hs-CRP)] and LMP levels prior to PCI, and a significant correlation was identified (r^2=0.86, P<0.01, Fig. 3B). However, PMPs and EMPs displayed no statistical correlation with hs-CRP (data not shown). In addition, no significant correlation was observed between MPs and TnT, or between MPs and CK-MB.

Discussion

Previous studies have investigated the changes in MPs of different cell origin in STEMI patients and alterations in MP levels at early (immediately after PCI) or late time-points (24 and 48 h after PCI) (7,17,21). However, to the best of our knowledge, there are no reports in the literature examining the detailed time course changes in MPs of different cell origin during PCI.

In the present study, different cell origin MPs were identified using flow cytometry, and the dynamic changes in MPs of different cell origin were elucidated. It was identified that LMP and EMP circulating levels decreased following successful reperfusion, which may result from the recovery of pump function and effective clearance of MPs. In the present study, circulating procoagulant PMPs increased significantly following the surgery, possibly due to the reperfusion of the occluded coronary artery containing procoagulant substance, as well as a marked amount of PMPs, which enter the circulation immediately after PCI (24). A second possible explanation may be the direct injury of vessels caused by PCI, which may result in the accumulation of PMPs. Subsequent to PCI, LMP

and EMP levels also increased. Recently, EMPs and LMPs have been reported to be a cause of fibrinolysis (25,26). Thus, the increase of EMPs and LMPs following PCI may act as an antagonistic response to the elevation of procoagulant PMPs.

Clinical trails concerning MPs have shown a great variation in results throughout the literature (27-29). A previous study indicated that PMPs only experience a slight increase following PCI surgery (7). This observation may be a result of the use of a different MP detection method. Compared with ELISA, flow cytometry is a more commonly used high-throughput technique for the enumeration and characterization of the cellular origin of MPs (20). Robert *et al* (16) established a standard flow cytometry protocol for the measurement of MPs based on Megamix beads and the results were reported in a multicenter study (19). The aforementioned protocol was utilized in the present study, along with the Beckman Coulter Gallios flow cytometer, which is a recent flow cytometer model that provides more accurate results.

The association between MPs and coronary heart disease has attracted increasing attention. Based on angiographic results, the Gensini score is a well established scoring system used to evaluate the severity of coronary disease (30). The association between Gensini score and serum procoagulant factors, including fibrinogen and glycoproteins, has been reported in previous studies (31,32). The present study revealed that Gensini score is significantly positively correlated with PMP levels prior to PCI, and thus procoagulant PMPs may reflect the high plaque burden in patients with higher Gensini scores. Furthermore, the present study examined the time course of LMPs during PCI surgery for the first time and determined the association between LMPs and hs-CRP. Previous studies have revealed the association between PMPs and hs-CRP; however, only a weak correlation was observed (7,8). Since hs-CRP is predominately a symbol of acute inflammation, the present study observed that LMPs, but not PMPs, correlated significantly with hs-CRP. In addition, it is noteworthy that the distribution of hs-CRP was not found to be a normal distribution (33), but a log-normal distribution (34). In the present study, an improved correlation was observed subsequent to logarithmic transformations of hs-CRP data.

In conclusion, the present study aimed to provide a detailed description of the time course of changes in circulating MPs of different cell origin in STEMI patients who underwent PCI, and provided information regarding the possible functions of different MPs in STEMI. Future research may focus on the functional importance of MPs of different cell origin in STEMI patients.

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