Platelet microparticle number is associated with the extent of myocardial damage in acute myocardial infarction

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Abstract

Introduction: Activated platelets generate microparticles. Increased platelet microparticles occur in acute myocardial infarction (AMI) and contribute to intracoronary thrombosis and subsequent myocardial injury. This study aimed to investigate the impact of platelet microparticles on intracoronary thrombosis by assessing the relationship between platelet microparticles and the extent of myocardial damage in AMI.

Material and methods: This was a cross sectional study. The subjects were patients with acute coronary syndrome (ACS). Forty-one consecutive subjects with ACS admitted to intensive cardiovascular care unit were enrolled. The clinical spectrum of ACS comprised AMI (n = 26), both ST-elevation AMI (STEMI) and non-ST-elevation AMI (NSTEMI), and unstable angina (n = 15). Platelet microparticles were isolated from peripheral venous blood and detected with anti-CD42b-PE by the flow cytometry method. The extent of myocardial damage was determined by measuring the peak level of serial cardiac enzymes within 24 h of admission.

Results: Subjects with AMI had a significantly higher number of platelet microparticles than those with unstable angina (4855 ±4509/µl vs. 2181 ±1923/µl respectively; p = 0.036). Subjects with STEMI had the highest number of platelet microparticles, but no significant difference was detected as compared to those with NSTEMI (5775 ±5680/µl vs. 3601 ±1632/µl). The number of platelet microparticles in AMI was positively associated with the extent of myocardial damage (peak CK-MB: r = 0.408, p = 0.019 and peak GOT: r = 0.384, p = 0.026).

Conclusions: The number of platelet microparticles was increased in AMI as compared to unstable angina and associated with the extent of myocardial damage.

Key words: platelet microparticle, acute myocardial infarction, thrombosis, myocardial damage.

Introduction

Acute coronary syndrome (ACS) reflects ischaemic myocardium due to occluded coronary arteries. Depending on the quantity and the location of occluded arteries, myocardial infarction may follow. Acute myocardial infarction (AMI) occurs due to an ongoing necrotic myocardium in the distal part of the occlusions [1]. Coronary artery thrombosis is an

underlying pathology in the majority of AMI [1]. Disrupted atherosclerotic plaque which exposes the subendothelial milieu to circulating platelets and coagulant proteins is the major pathomechanism responsible for coronary artery thrombosis [2]. In particular, platelets have a major role in the initiation, perpetuation and progression of intracoronary thrombosis through several means of activation [3, 4].

Among many modes of activations, platelets are able to generate microparticles through the membrane shedding process [5]. These microparticles bear platelet membrane components that have been modified upon activation; thus they act similarly as to activated platelets [5]. Increased platelet microparticles are detected in patients with ACS and AMI, reflecting platelet activation [6, 7]. However, there are still contradictory results regarding the number and the implications of platelet microparticles in AMI [6, 8–10].

Procoagulant activity of circulating microparticles has been suggested to play a role in the progression of intracoronary thrombosis in AMI [11]. Platelet microparticles contain phosphatidylserine in the surface layer and express tissue factor that determines their capacities in promoting blood coagulation [5, 12–14]. Furthermore, increased thrombin generation by platelet microparticles is also a contributory factor in their procoagulant properties [14]. As a part of platelet activation, increased platelet microparticles in AMI may exaggerate intracoronary thrombosis, causing intracoronary occlusion due to their procoagulant properties. This, in turn, leads to the aggravation of distal myocardial injury and the progression to myocardial necrosis.

In this study, we sought to provide clinical evidence that increased platelet microparticles are associated with the amplification of intracoronary thrombosis by assessing the relation between the number of platelet microparticles and the infarct size of damaged myocardium following AMI.

Material and methods

Subjects

The study design was cross-sectional. Subjects were patients with ACS and hospitalized in the Intensive Cardiovascular Care Unit (ICCU) of Dr. Sardjito Hospital, Yogyakarta, Indonesia. Subjects were enrolled consecutively from April to July 2009. The inclusion criteria were: (1) patients with ACS with the onset of angina ≤ 48 h, (2) patients aged from 30 to 75 years, and (3) patients voluntarily participated in this study by signing an informed consent form. The exclusion criteria were: (1) patients with known history of chronic kidney disease (CKD) stage V, chronic heart failure (CHF), hepatic cirrhosis and valvular heart disease (VHD), (2) patients with concomitant acute stroke, acute infection, sepsis, chronic inflammatory diseases and other thromboembolic diseases, (3) patients with known history of malignancy and (4) patients undergoing primary percutaneous coronary intervention or thrombolysis in this episode of ACS.

Comorbidities, i.e. hypertension, diabetes mellitus, previous ischaemic heart disease and smoking behaviour, were obtained from anamnesis. Hypertension was defined as subjects previously diagnosed with hypertension and/or had been taking antihypertensive drugs regularly or those with blood pressure > 140/90 mm Hg on admission and in the ICCU. Diabetes mellitus was defined as subjects previously diagnosed with diabetes mellitus and/or had been taking antidiabetic drugs regularly. Previous ischaemic heart disease (IHD) was defined as subjects previously admitted to the ICCU for an ACS episode or had a coronary stent placement. Current smoking was defined as subjects who smoked regularly and were still smoking within 3 months before this study.

The Medical and Health Research Ethics Committee of the Faculty of Medicine Universitas Gadjah Mada and Dr. Sardjito Hospital, Yogyakarta, Indonesia, approved the study protocol. The subjects participated voluntarily in the study and signed a written informed consent form prior to the study. The study protocol was prepared and performed according to a good clinical practice regulation.

Clinical spectrums of acute coronary syndrome

The diagnosis of ACS was based on the symptom of angina, the changes in ST segment and/or T waves on the electrocardiogram and the elevation of cardiac enzymes, i.e. troponin I and CK-MB. Acute coronary syndrome were divided into two distinct diagnoses: AMI and unstable angina. Acute myocardial infarction was further divided into two clinical spectrums, i.e. ST-elevation AMI (STEMI) and AMI without ST elevation (NSTEMI).

ST-elevation AMI was determined as angina lasting more than 20 min and electrocardiogram examination revealed ST segment elevation > 1 mm in two or more consecutive limb leads (I and aVL or II, III and aVF leads) and an elevation > 2 mm in two or more consecutive precordial leads (leads V1–V6). Non-ST-elevation AMI was determined as angina and elevated cardiac enzyme (troponin I ≥ 0.6 ng/ml or CK-MB dynamic elevation) but without ST segment elevation satisfied the criteria for STEMI. The electrocardiography finding in NSTEMI could be ST-segment depression, T wave inversion or normal electrocardiogram. Unstable angina was determined as angina, non-ST elevation

electrocardiogram changes and without elevated cardiac enzyme (troponin I < 0.6 ng/ml and no CK-MB dynamic elevation).

Laboratory examination

Peripheral venous blood was first collected on admission in the emergency room prior to the patients' transfer to the ICCU as a hospital procedure for routine haematology and biochemistry examination. Platelet indices were measured as platelet count, mean platelet volume (MPV) and platelet distribution width (PDW). The second blood sample collection was done on patients fasting approximately 8 h after admission to the ICCU for the measurement of lipid profile and platelet microparticles.

Platelet microparticle examination

Platelet microparticles were detected according to a previous description [15]. A blood sample was withdrawn within 24 h after hospital admission. The blood sample was withdrawn from antecubital veins in subject in a supine position with a BD-Vacutainer tube containing citrate anticoagulant (BD Diagnostic, Maryland, USA). The sample was immediately centrifuged at 160 g for 10 min to collect platelet-rich plasma (PRP). Subsequently, PRP was centrifuged at 6000 g for 1 min to collect platelet-poor plasma (PPP). 50 µl of PPP was incubated with antibody CD42b-PE (BD Bioscience, Maryland, USA) in a TruCount tube (BD Diagnostic, Maryland, USA) for 30 min to detect platelet microparticles. Then, 1 ml of saline buffer was added. The sample was then run and analysed with flow cytometry (FACSCalibur, Becton Dickinson, Maryland, USA). Platelet microparticles were determined as particles with diameter size < 1.5 µm and positivity of CD42b-PE [15]. The absolute number of platelet microparticles was calculated based on the formula of CD42b positivity within the threshold of 1.5 µm gated events (R2) multiplied by the total number of bead events (based on the datasheet it was 50 000) and normalized with the number of TruCount bead events (R1) and volume sample (i.e. 50 µl) [16].

Myocardial damage examination

The extent of myocardial damage was determined by measuring the peak level of serial serum cardiac enzymes. In our hospital, panel measurement of cardiac enzymes consists of glutamate oxaloacetate transaminase (GOT), lactate dehydrogenase (LDH) and creatine kinase MB (CKMB) in the blood. Consecutive blood samples were withdrawn every 6 h within 24 h after admission, and cardiac enzyme concentrations were measured. Peak level was determined as the highest value of each concentration among serial serum samples.

Statistical analysis

Statistics analysis was performed with SPSS16 (SPSS Inc., Chicago, Illinois, USA). A normality test was conducted for continuous data with the Shapiro-Wilk test ($p > 0.05$ indicated normal distribution) before each analysis. A logarithmic transformation was carried out if the original continuous data were non-normally distributed. For normally distributed data, Student's *t*-test was used to compare data between two groups (AMI and unstable angina), and for non-normally distributed data the Mann-Whitney test was performed. Comparison among groups (STEMI, NSTEMI and unstable angina) was performed with one-way ANOVA and post-hoc least significant difference (LSD) analysis. Correlation between the number of platelet microparticles and cardiac enzymes or haematology indices was performed using the Pearson correlation test or non-parametric Spearman test based on the normality distribution. A p-value < 0.05 was considered significant.

Results

Subject characteristics

During the study period, 68 patients with ACS were admitted to the ICCU. Fourteen patients did not match the inclusion criteria, i.e. 6 patients had onset of anginal pain > 48 h and 8 patients had age > 75 years. Among 54 patients included, 13 patients were excluded (1 patient had CKD stage V, 5 patients had CHF, 2 had concomitant acute stroke, 4 patients received primary percutaneous coronary intervention or thrombolysis, and 1 patient did not provide a blood sample). Therefore, 41 subjects were enrolled in this study.

Twenty-six subjects were diagnosed with AMI and 15 with unstable angina. Among AMI, 11 were NSTEMI and 15 were STEMI. The characteristics of subjects are shown in Table I. The age, gender and comorbidities among subjects did not significantly differ. Clinical presentation did not differ either. A significantly higher white blood cell count was detected in AMI, especially in STEMI, as compared to unstable angina. Peak cardiac enzymes were also significantly higher in AMI than in unstable angina, with STEMI having the highest peak levels among groups. With respect to medical treatments, there were no significant differences among groups.

Platelet microparticles based on ACS spectrums

The number of platelet microparticles was significantly higher in AMI as compared to unstable angina (mean ± SD: 4855 ±4509/µl vs. 2181 ±1923/µl, $p = 0.036$). Based on the clinical spec-

Table I. Characteristics of subjects

Characteristics	Unstable angina *n* = 15	AMI *n* = 26	*P*-value*	NSTEMI *n* = 11	STEMI *n* = 15	*P*-value**
Age, mean ± SD [years]	58.5 ±10.5	62.2 ±12.4	0.328	67.9 ±10.1	58.1 ±12.5	0.062
Gender, *n* (%):						
Male	13 (86.7)	20 (76.9)	0.448	8 (72.7)	12 (80.0)	0.674
Female	2 (13.3)	6 (23.1)		3 (27.3)	3 (20.0)	
Comorbidity, *n* (%):						
Hypertension	10 (66.7)	13 (50.0)	0.300	8 (72.7)	5 (33.3)	0.079
Diabetes mellitus	3 (20.0)	2 (7.7)	0.249	1 (9.1)	1 (6.7)	0.501
Previous IHD	8 (53.3)	7 (26.9)	0.091	4 (36.4)	3 (20.0)	0.166
Current smoking	4 (26.7)	9 (34.6)	0.434	1 (9.1)	8 (53.3)	0.059
Clinical presentation, mean ± SD:						
Systolic blood pressure [mm Hg]	136.3 ±27.8	129.6 ±27.9	0.462	133.2 ±27.6	127.0 ±28.9	0.658
Diastolic blood pressure [mm Hg]	76.0 ±17.3	76.4 ±21.6	0.958	76.4 ±16.2	76.3 ±25.4	0.999
Heart rate [x/min]	80.3 ±14.9	81.2 ±21.6	0.888	78.1 ±13.5	83.5 ±26.3	0.777
Haematology, mean ± SD:						
Haemoglobin [g/dl]	14.3 ±1.7	13.7 ±1.8	0.293	13.5 ±1.9	13.8 ±1.6	0.538
White blood cells [× 10^3/mm^3]	10.0 ±3.6	12.8 ±4.4	0.041	10.6 ±3.8	14.4 ±4.2***	0.007
Platelet [× 10^3/mm^3]	228.1 ±83.4	271.9 ±81.5	0.109	288.1 ±99.9	260.0 ±66.3	0.195
Mean platelet volume [fl]	9.2 ±1.4	9.1 ±1.7	0.906	9.2 ±1.3	9.1 ±1.9	0.993
Platelet distribution width (%)	12.7 ±2.5	13.6 ±2.7	0.286	13.0 ±2.4	14.1 ±2.9	0.336
Lipid profiles, mean ± SD:						
Total cholesterol [mg/dl]	183.7 ±35.9	179.5 ±45.1	0.800	187.0 ±47.8	173.9 ±44.1	0.763
LDL cholesterol [mg/dl]	132.5 ±28.5	116.6 ±37.0	0.344	123.7 ±33.6	111.3 ±39.9	0.476
HDL cholesterol [mg/dl	38.5 ±7.9	41.4 ±9.7	0.449	43.8 ±6.9	39.6 ±11.3	0.449
Triglyceride [mg/dl]	147.8 ±40.8	116.1 ±62.8	0.158	103.8 ±61.1	125.3 ±65.2	0.262
Peak cardiac enzyme, mean ± SD:						
GOT [U/l]	33.3 ±11.3	223.5 ±201.6	< 0.001	72.1 ±47.1	334.5 ±199.5***	< 0.001
LDH [U/l]	451.2 ±138.3	2101.4 ±3626.1	0.029	614.8 ±193.5	3191.5 ±4521.4***	0.018
CK-MB [U/l]	6.9 ±3.0	74.8 ±66.1	< 0.001	32.9 ±23.9	105.5 ±70.8***	< 0.001
Medical treatment, *n* (%):						
Heparin/fondaparinux	15 (100)	26 (100)	N/A	11 (100)	15 (100)	N/A
Nitrate	15 (100)	26 (100)	N/A	11 (100)	14 (93.3)	0.411
Aspirin	15 (100)	26 (100)	N/A	11 (100)	15 (100)	N/A
Clopidogrel	15 (100)	26 (100)	N/A	11 (100)	15 (100)	N/A
Statin	15 (100)	26 (100)	N/A	11 (100)	15 (100)	N/A
ACE inhibitor	7 (46.7)	10 (38.5)	0.607	6 (54.5)	4 (26.7)	0.317
β-Blocker	4 (26.7)	2 (7.7)	0.117	2 (18.2)	0 (0)	0.110
Furosemide	3 (20.0)	8 (30.8)	0.356	3 (27.3)	5 (33.3)	0.712

**P-value was calculated comparing unstable angina vs. AMI and analysed with χ^2 test or Fisher exact test for categorical data and Student's t-test for continuous data; **P-value was calculated comparing unstable angina, STEMI and NSTEMI and analysed with χ^2 test for categorical data and ANOVA test for continuous data. Post-hoc LSD was applied if ANOVA test was statistically significant (p < 0.05). ***Post-hoc LSD, vs. NSTEMI p < 0.05, vs. unstable angina p < 0.05.*

trum, STEMI subjects had the highest number of platelet microparticles (5775 ±5680/µl) among groups. NSTEMI, reflecting a less severe spectrum of ACS, had a lower amount of platelet microparticles than subjects with STEMI (3601 ±1632/µl), but this difference was not statistically significant (post-hoc LSD: p = 0.178). Unstable angina, the mildest spectrum of ACS, had the lowest platelet microparticles among groups (2181 ±1923/µl; post-hoc LSD: p = 0.012 vs. STEMI and p = 0.344

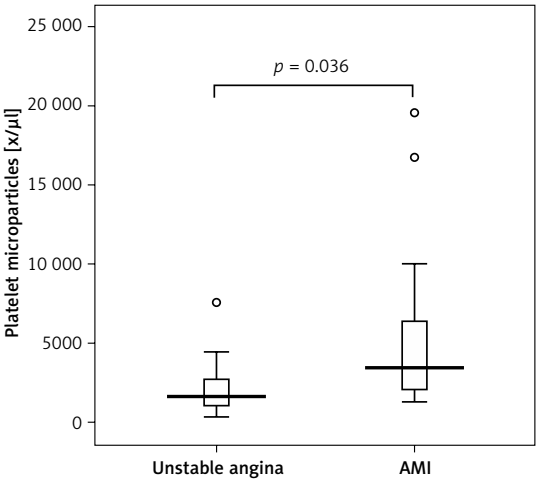

Figure 1. The number of platelet microparticles was significantly higher in subjects with AMI as compared to those with unstable angina (Student's *t*-test: $p = 0.036$)

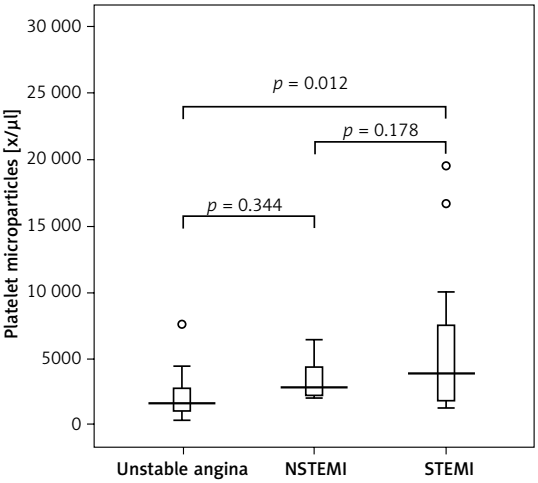

Figure 2. The number of platelet microparticles according to clinical spectrums of ACS. STEMI had the highest number of platelet microparticles (ANOVA test: $p = 0.040$), but it did not differ significantly with NSTEMI (post-hoc LSD: $p = 0.178$), whereas it differed significantly with unstable angina (post-hoc LSD: $p = 0.012$), which had the lowest amount of platelet microparticles

vs. NSTEMI). A box plot of platelet microparticles among groups and clinical spectrums are shown in Figures 1 and 2. A representative dot plot is shown in Figure 3.

Association between platelet microparticles and myocardial damage

Since in subjects with AMI the number of platelet microparticles was increased, we investigated whether they were associated with myocardial damage following AMI. The extent of myocardial damage, as reflected by peak serum levels of cardiac enzymes, was positively and significantly correlated with increased platelet microparticles, both among subjects with ACS and those with AMI. Correlation data are shown in Table II and Figure 4.

Association between platelet microparticles and platelet indices

Platelet microparticles did not significantly correlate with platelet indices, such as platelet count, PDW and MPV, in subjects with ACS or in those with AMI. Furthermore, platelet microparticles did not correlate with haemoglobin and white blood cell count. Correlation data are shown in Table II.

Discussion

Our study showed that patients with AMI had a significantly higher number of platelet microparticles detected in peripheral blood as compared to patients with unstable angina. This finding clinically signifies that the number of platelet microparticles is associated with coronary artery thrombosis. Increased platelet microparticles perpetuate coronary artery thrombosis, which causes myocardial necrosis. This study also indicated that, among AMI patients, a greater number of platelet microparticles is detected in STEMI than that in NSTEMI. However, the difference is not

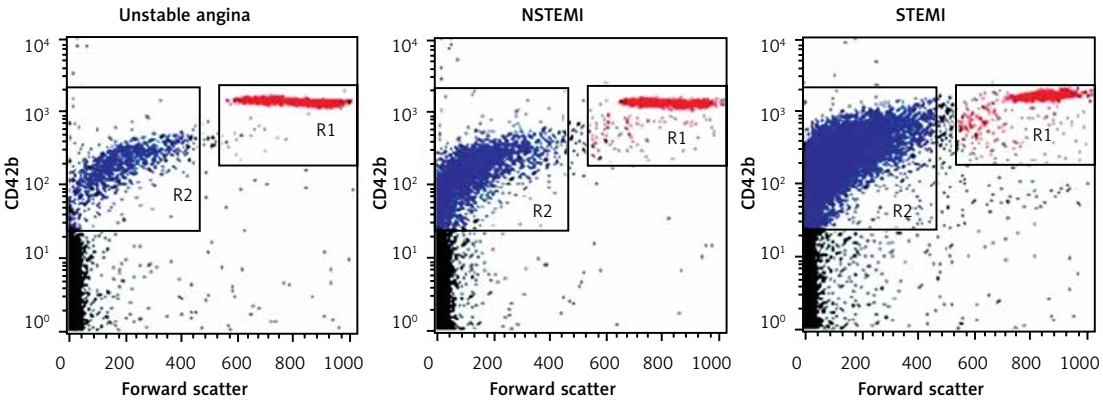

Figure 3. Representative dot-plot of CD42b positivity of platelet microparticles from flow cytometry analysis according to clinical spectrums of ACS. R2 was gated for CD42b+ and the threshold of 1.5 µm, indicating platelet microparticle. R1 was gated for collected TruCount bead events

Table II. Correlation of platelet microparticles with myocardial damage and haematology indices

Parameters	ACS (n = 41)		AMI (n = 26)	
	r	P-value	r	P-value
Myocardial damage:				
Peak GOT	0.372	0.017*	0.384	0.026
Peak LDH	0.297	0.059*	0.177	0.194*
Peak CKMB	0.482	0.001	0.408	0.019
Haematology and platelet indices:				
Platelet count	0.179	0.132	0.226	0.134
Platelet distribution width	–0.075	0.322	–0.188	0.183
Mean platelet volume	–0.164	0.153	–0.148	0.236
Haemoglobin	–0.045	0.391	–0.108	0.300
White blood cell count	0.033	0.418	0.108	0.300

**Non-parametric Spearman correlation test was applied because one parameter of the data was not normally distributed.*

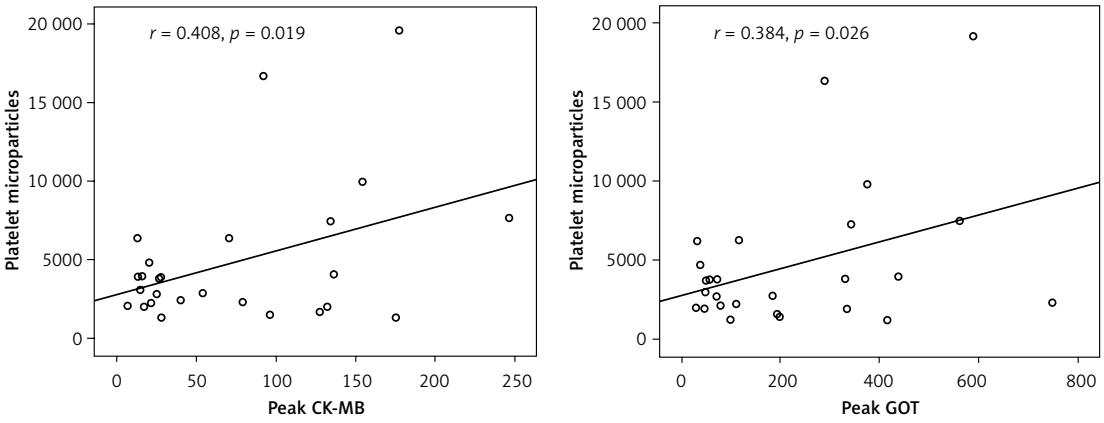

Figure 4. Positive and significant correlation between an increased number of platelet microparticles and a peak level of GOT and CK-MB, indicating the extent of myocardial damage, in subjects with AMI

statistically significant. This observation suggests that, in addition to thrombosis perpetuation, increased platelet microparticles may also contribute to thrombosis progression that turns into intracoronary thrombus occlusion. Furthermore, our result demonstrated that the increased number of platelet microparticles was associated with the extent of myocardial damage following myocardial infarction.

Platelet activation is the main contributing factor in acute coronary syndrome [17]. One of the determinants for the extent of thrombus occlusion in the culprit artery and the subsequent distal myocardial infarction is the degree of platelet activation [18, 19]. Platelet microparticles are released from membrane vesicular shedding of activated platelets [5, 10]. Several surface markers for platelet microparticles have been investigated, including CD42b or GPIb. GPIb connects with GPIX and GPV in the formation of complex receptors for von Willebrand factors, thrombin and several coagulation factors which mediate thrombus formation [19]. GPIb also facilitates platelet binding through P-selectin of endothelia and Mac-1 of leukocytes, which promotes platelet microparticles' ability to enhance endothelial activation and promote inflammation [19, 20].

Using the CD42b marker in detecting platelet microparticles, we confirmed that the number of platelet microparticles bearing CD42b was higher in AMI as compared to unstable angina. We suggest that increased CD42b(+)-platelet microparticles in AMI contributes to the greater occlusion of coronary arteries with subsequent necrosis of distal myocardium, while this does not occur in unstable angina. Perpetuation of coronary thrombosis may involve CD42b(+)-platelet microparticles through thrombin-mediated fibrin generation [14, 19]. However, a coronary angiography study should be performed in these patients to confirm this suggestion.

A previous study reported a similar finding [21]. It showed that the level of CD42(+)-platelet microparticles was higher in AMI as compared to other spectrums of coronary artery diseases such as unstable angina and stable coronary disease [21]. Similar to our study, it measured platelet microparticles from the blood of the subjects within 72 h of the onset of chest pain. Nevertheless,

the study did not discriminate acute myocardial infarction into STEMI and NSTEMI. Biasucci *et al.* reported a significant elevation of CD42b(+)-platelet microparticles in both STEMI and NSTEMI as compared to patients with stable angina [6]. This study measured 0.5–1.0 µm microparticles and found that CD42b(+)-platelet microparticles were increased sharply at 24 h after admission, whereas on admission and at 48 h, the amounts were comparable among STEMI, NSTEMI and stable angina [6]. Similar to our result, this study found a non significant difference of platelet microparticles between STEMI and NSTEMI. In another study, using a solid-phase capture assay, Min *et al.* reported that CD42b(+)-platelet microparticles were significantly higher in STEMI, both detected in the culprit coronary circulation and peripheral blood, as compared with normal subjects [9]. Using this assay, both large and small-size platelet microparticles were detected [22]. Unlike our study, neither study included subjects with unstable angina. Unstable angina constitutes a lower degree of myocardial ischemia, mostly due to non-critical coronary thrombus occlusion or coronary stenosis without thrombus formation. Thus our study confirms the previous finding that a higher amount of platelet microparticles, particularly with CD42b positivity, is detected in both STEMI and NSTEMI as compared with the lighter spectrum of ACS, i.e. unstable angina.

However, another study showed a different result in which it revealed that CD42b(+)-platelet microparticles did not increase in AMI (both STEMI and NSTEMI) as compared to normal control and stable coronary artery disease [8]. In this study, the size of platelet microparticles was limited to those < 0.5 µm, dubbed small-size platelet microparticles. Small-size platelet microparticles and large-size platelet microparticles, detected by our methods, are distinctively regulated in various diseases [22]. Unlike large-size microparticles, the functionality of small-size platelet microparticles is unclear, as to whether they represent a prothrombotic or procoagulant predisposition. This study did not measure small-size CD42b(+)-platelet microparticles in unstable angina; therefore whether small-size platelet microparticles also contributed in the development of myocardial necrosis following coronary occlusion cannot be concluded.

An interesting finding in our study was that the increased number of platelet microparticles was correlated with more extensive myocardial necrosis. Two mechanisms may be invoked to explain this finding. First, it was associated with total thrombus occlusion in the culprit vessel with subsequent acute transmural necrosis in the distal myocardium. Second, increased platelet microparticles caused microemboli and generation of microthrombi that give rise to additional distal microvascular occlusions and subsequent myocardial necrosis, thus increasing the magnitude of damage. The first occurred in the clinical spectrum of STEMI and the second one occurred in both STEMI and NSTEMI, which had a significantly higher number of platelet microparticles than unstable angina. Porto *et al.* observed that the intracoronary and systemic level of CD42(+)-platelet microparticles was associated with a higher coronary thrombus burden in the culprit vessel of patients presenting with STEMI [23]. Furthermore, an increased level of intracoronary CD42(+)-platelet microparticles was found in patients with a greater degree of coronary microvascular obstruction, suggesting the release of platelet microparticles from the culprit vessel and generation of microthrombi in the distal coronary microcirculation [23]. Since our study did not find a significant difference in the amount of platelet microparticles between STEMI and NSTEMI, we suggest that both mechanisms prevailed.

Primary revascularisation, either primary percutaneous coronary intervention (pPCI) or medical thrombolysis, for STEMI reduces the extent of myocardial destruction following total coronary occlusion and preserves viable myocardium. Post-pPCI and thrombolysis are associated with a reduced level of platelet microparticles detected both in coronary and systemic circulation [9, 24]. Our study excluded subjects with STEMI undergoing primary revascularisation; therefore the affected myocardium and levels of platelet microparticles were comparable between NSTEMI and non-primary revascularised STEMI. Antiplatelet treatments with aspirin and clopidogrel are associated with reduced platelet microparticle release. There was an inverse correlation between serum level of clopidogrel and the number of platelet microparticles [25]. A loading dose of aspirin and clopidogrel in the early treatment of ACS leads to peak serum levels of both drugs being achieved shortly, which is sufficient to hinder platelet activation and microparticle release [25]. The majority of research involving ACS and AMI patients measured platelet microparticles after a loading dose of antiplatelets; therefore some pathways of platelet microparticle generation were blocked. Despite antiplatelet loading, those with ACS and AMI had higher levels of platelet microparticles as compared to stable coronary diseases without antiplatelet loading. Since all subjects in our study were given loading of aspirin and clopidogrel, the antiplatelet influence in platelet microparticles was comparable among clinical spectrums.

Platelet activation itself predicts myocardial damage following acute myocardial infarction [18]. In particular, the level of CD42(+)-platelet microparticles correlated with the degree of myocardial ischaemic burden in STEMI, but not with

the infarct size [26]. Jung *et al.* concluded that the level of circulating platelet microparticles reflect the ischaemic coronary vascular bed, which bears stimulating factors for microparticle release such as vascular inflammation, endothelial dysfunction and increasing shear stress [26]. In the context of ACS, the increased level of platelet microparticles by the ischaemic coronary vessel may cause an increased thrombus burden once plaque rupture occurs, which results in total thrombus occlusion. Despite the lack of correlation between platelet microparticles and infarct size measured with cardiac magnetic resonance imaging, an ongoing myocardial necrosis detected with troponin T was significantly correlated with CD42(+)-platelet microparticles [26]. It indicates that increased platelet microparticles influence a dynamic phase of myocardial injury and necrosis during acute infarction. Our result extends this study by showing a positive correlation between platelet microparticles with myocardial damage measured with peak CKMB in AMI patients.

The results of our study may have a clinical implication in that the number of platelet microparticles is a potential biomarker for coronary artery thrombosis in ACS. An increased number of platelet microparticles in patients with ACS indicates the amplification of coronary artery thrombosis and thrombus burden. Another clinical implication is that an increased number of platelet microparticles may modify treatments, especially antiplatelets or anticoagulants, in patients with AMI.

Several limitations of our study must be addressed. The first is the small sample size of groups, which reduces the power to detect significant differences among groups. The second is the cross sectional design, which is insufficient to draw conclusions on the causal relationship and the proposed mechanisms. The third is that data of coronary angiography for assessing the coronary thrombosis were not available in the study. Therefore, further research is necessary to corroborate our study findings.

In conclusion, we demonstrated that the number of platelet microparticles was significantly increased in AMI as compared to unstable angina. The number of platelet microparticles was correlated with the extent of myocardial damage following AMI.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Brener SJ. Insights into the pathophysiology of ST-elevation myocardial infarction. Am Heart J 2006; 151: S4-10.
2. Fuster V, Badimon L, Badimon JJ, et al. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992; 326: 242-50.
3. Davi G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med 2007; 357: 2482-94.
4. Freynhofer MK, Bruno V, Wojta J, et al. The role of platelets in athero-thrombotic events. Curr Pharm Des 2012; 18: 5197-214.
5. Nomura S, Ozaki Y, Ikeda Y. Function and role of microparticles in various clinical settings. Thromb Res 2008; 123: 8-23.
6. Biasucci LM, Porto I, Vito LD, et al. Differences in microparticle release in patients with acute coronary syndrome and stable angina. Circ J 2012; 76: 2174-82.
7. Matsumoto N, Nomura S, Kamihata H, et al. Association of platelet-derived microparticles with C-C chemokines on vascular complication in patients with acute myocardial infarction. Clin Appl Thromb Hemost 2002; 8: 279-86.
8. Montoro-García S, Shantsila E, Tapp LD, et al. Small-size circulating microparticles in acute coronary syndromes: relevance to fibrinolytic status, reparative markers and outcomes. Atherosclerosis 2013; 227: 313-22.
9. Min PK, Kim JY, Chung KH, et al. Local increase in microparticles from the aspirate of culprit coronary arteries in patients with ST-segment elevation myocardial infarction. Atherosclerosis 2013; 227: 323-8.
10. VanWijk MJ, VanBavel E, Sturk A, et al. Microparticles in cardiovascular diseases. Cardiovasc Res 2003; 59: 277-87.
11. Mallat Z, Benamer H, Hugel B, et al. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation 2000; 101: 841-3.
12. Muller I, Klocke A, Alex M, et al. Intravascular tissue factor initiates coagulation via circulating microvesicles and platelets. FASEB J 2003; 17: 476-8.
13. Biro E, Sturk-Maquelin KN, Vogel GMT, et al. Human cell-derived microparticles promote thrombus formation in vivo in a tissue factor-dependent manner. J Thromb Haemost 2003; 1: 2561-8.
14. Trappenburg MC, van Schilfgaarde M, Marchetti M, et al. Elevated procoagulant microparticles expressing endothelial and platelet markers in essential thrombocythemia. Haematologica 2009; 94: 911-8.
15. Koga H, Sugiyama S, Kugiyama K, et al. Elevated levels of remnant lipoproteins are associated with plasma platelet microparticles in patients with type-2 diabetes mellitus without obstructive coronary artery disease. Eur Heart J 2006; 27: 817-23.
16. Tramontano AF, O'Leary J, Black AD, et al. Statin decreases endothelial microparticle release from human coronary artery endothelial cells: implication for the Rho-kinase pathway. Biochem Biophys Res Com 2004; 320: 34-8.

17. Ferroni P, Riondino S, Vazzana N, et al. Biomarkers of platelet activation in acute coronary syndromes. Thromb Haemost 2012; 108: 1109-23.
18. Frossard M, Fuchs I, Leitner JM, et al. Platelet function predicts myocardial damage in patients with acute myocardial infarction. Circulation 2004; 110: 1392-7.
19. Rivera J, Lozano ML, Navarro-Núñez L, et al. Platelet receptors and signaling in the dynamics of thrombus formation. Haematologica 2009; 94: 700-11.
20. Viera AJ, Mooberry M, Key NS. Microparticles in cardiovascular disease pathophysiology and outcomes. J Am Soc Hypertens 2012; 6: 243-52.
21. Bernal-Mizrachi L, Jy W, Jimenez JJ, et al. High levels of circulating endothelial microparticles in patients with acute coronary syndromes. Am Heart J 2003; 145: 962-70.
22. Strasser EF, Happ S, Weiss DR, et al. Microparticle detection in platelet products by three different methods. Transfusion 2013; 53: 156-66.
23. Porto I, Biasucci LM, De Maria GL, et al. Intracoronary microparticles and microvascular obstruction in patients with ST elevation myocardial infarction undergoing primary percutaneous intervention. Eur Heart J 2012; 33: 2928-38.
24. Empana JP, Boulanger CM, Tafflet M, et al. Microparticles and sudden cardiac death due to coronary occlusion. The TIDE (Thrombus and Inflammation in sudden DEath) study. Eur Heart J Acute Cardiovasc Care 2015; 4: 28-36.
25. França CN, Pinheiro LFM, Izar MCO, et al. Endothelial progenitor cell mobilization and platelet microparticle release are influenced by clopidogrel plasma levels in stable coronary artery disease. Circ J 2012; 76: 729-36.
26. Jung C, Sörensson P, Saleh N, et al. Circulating endothelial and platelet derived microparticles reflect the size of myocardium at risk in patients with ST-elevation myocardial infarction. Atherosclerosis 2012; 221: 226-31.