

Myocardial Infarction

Circulating Monocyte-Platelet Aggregates Are an Early Marker of Acute Myocardial Infarction

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OBJECTIVES	We investigated whether elevated levels of circulating monocyte-platelet aggregates (MPA) can be used to identify patients with acute myocardial infarction (AMI).
BACKGROUND	Commonly used blood markers of AMI reflect myocardial cell death, but do not reflect the earlier pathophysiologic processes of plaque rupture, platelet activation and resultant thrombus formation. Circulating MPA form after platelet activation.
METHODS	In a single center between October 1998 and November 1999, we measured circulating MPA in a blinded fashion by whole blood flow cytometry in 211 consecutive patients who presented to the emergency department (ED) with chest pain and were admitted to rule out AMI. Acute myocardial infarction was diagnosed by a CK-MB fraction greater than three times control.
RESULTS	Patients with AMI (n = 61), as compared with those without AMI (n = 150), had significantly higher numbers of circulating MPA (11.6 ± 11.4 vs. 6.4 ± 3.6 , mean \pm SD, $p < 0.0001$). After controlling for age, the adjusted odds of developing AMI for patients in the 2nd, 3rd and 4th quartiles of MPA, in comparison with patients in the lowest quartile (odds ratio = 1.0), were 2.1 (95% confidence interval [CI]: 0.7, 6.8), 4.4 (95% CI: 1.5, 13.1) and 10.8 (95% CI: 3.6, 32.0), respectively. The number of circulating MPA in patients with AMI presenting within 4 h of symptom onset (14.4) was significantly greater than those presenting after 4 h (9.4) and after 8 h (7.0), ($p < 0.001$). Of the 61 patients with AMI, 35 (57%) had a normal creatine kinase isoenzyme ratio at the time of presentation to the ED, but had high levels of circulating MPA (13.3).
CONCLUSIONS	Circulating MPA are an early marker of AMI. (J Am Coll Cardiol 2001;38:1002-6) © 2001 by the American College of Cardiology

In the U.S., approximately 1,100,000 individuals per year have a new or recurrent acute myocardial infarction (AMI). Of these patients, 25% of men and 38% of women die within one year of diagnosis (1). Early treatment intervention significantly reduces morbidity and mortality in AMI (2). New ST-segment elevation on the surface electrocardiogram is highly suggestive of AMI, but the prevalence of AMI may be as low as 20% among patients with new or dynamic ST-segment depression or T-wave inversion (3). The diagnosis of AMI in patients presenting without electrocardiographic evidence of ST-segment elevation relies on the use of markers of myocardial necrosis. The MB isoform of creatine kinase (CK-MB) and cardiac troponins are the most common markers of myocardial necrosis used in the diagnosis of AMI, but they do not appear in the peripheral circulation until at least 4 h after onset of injury (2). Furthermore, these markers do not measure the pathophysiologic processes that result in myocardial necrosis, namely coronary plaque erosion or rupture, leukocyte and platelet activation and coronary artery thrombosis (4).

Upon activation, platelets degranulate and adhere to monocytes, a process mediated by the platelet surface expression of P-selectin (CD62P) (5,6), which binds to the constitutively expressed P-selectin glycoprotein ligand-1 (PSGL-1) on monocytes (7). Firm attachment is subsequently mediated by monocyte CD11b/CD18-dependent platelet interactions (8). The measurement of monocyte-platelet aggregates (MPA) may be a more robust signal of platelet activation than the detection of surface P-selectin on individual platelets because degranulated platelets rapidly lose surface P-selectin *in vivo* yet continue to circulate (9). Indeed, the presence of increased circulating leukocyte-platelet aggregates has been demonstrated in patients with stable angina (10), unstable angina (11), AMI (12) and in those undergoing percutaneous coronary interventions (13).

In this observational study, we, therefore, used whole blood flow cytometry (14) to investigate whether the presence of elevated levels of circulating MPA is an early marker of AMI in patients presenting to an emergency department (ED) with a chief complaint of chest pain.

METHODS

Study population. The protocol was approved by the Committee for the Protection of Human Subjects in Research at the University of Massachusetts Medical School.

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Abbreviations and Acronyms

AMI	= acute myocardial infarction
CI	= confidence interval
CK-MB	= creatine kinase isoenzyme
ED	= emergency department
FITC	= fluorescein isothiocyanate
MPA	= monocyte-platelet aggregates
PSGL-1	= P-selectin glycoprotein ligand-1

The study population consisted of 211 consecutive patients presenting with a chief complaint of chest pain to the ED at UMass Memorial Medical Center during the period October 1998 to November 1999. Peripheral blood samples were drawn in the ED at the time of hospital presentation in a standardized manner by trained technicians.

Diagnostic criteria. A diagnosis of AMI was made when the serum CK-MB level was greater than three times the upper limit of normal at our hospital laboratory.

Monoclonal antibodies. Y2/51 (DAKO, Carpinteria, California) is directed against CD61 (GPIIIa, i.e., it is platelet-specific) and was purchased conjugated to fluorescein isothiocyanate (FITC). MøP9 (Becton Dickinson, Rutherford, New Jersey) is directed against CD14 on the monocyte surface and was purchased conjugated to phycoerythrin. Y2/51 and MøP9 are murine IgG monoclonal antibodies. Murine IgG-FITC (DAKO) was used as a nonspecific binding isotypic control.

MPA. There were no centrifugation, gel filtration, vortexing or stirring steps that could artifactually activate platelets or monocytes. The first 2 ml of drawn blood was discarded. Blood was then drawn into a 3.8% sodium citrate Vacutainer (Becton Dickinson, San Jose, California) and immediately (within 10 min) fixed at 4°C for between 10 min and 2 h at a final concentration of 1% formalin (Polysciences, Warrington, Pennsylvania) and 1.5X Hanks Balanced Saline solution concentrate (GIBCO, Grand Island, New York). This duration of fixation does not result in artifactual platelet activation. Once fixed, the samples are stable for up to 48 h (data not shown). The samples were then incubated at 22°C for 10 min with saturating concentrations of the monoclonal antibodies Y2/51-FITC (GPIIIa-specific) and MøP9-PE (CD14-specific) and then diluted fivefold with distilled H₂O, vortexed and incubated at 22°C for 10 min (to lyse the red blood cells). Two volumes of phosphate-buffered saline, pH 7.4, were added to bring the final blood dilution to 1:33 (to reduce monocyte/platelet coincidence). The samples were placed on ice until analysis within 4 h by flow cytometry (with low-flow setting) in a FACSCalibur flow cytometer (Becton Dickinson) equipped with a 488 nm argon ion laser, standard three-color filter configuration and CELLQuest cell analysis software (Becton Dickinson). Fluorescence output was standardized daily using SPHERO Rainbow Calibration Particles (Spherotech, Libertyville, Illinois). Monocytes were identified by their brightly positive CD14 expression on a two-parameter dot plot display-

ing linear orthogonal light scatter vs. MøP9-PE (FL2). The threshold was set on forward angle light scatter to include all monocytes and exclude debris and uncomplexed platelets. The percentage of MPA was identified in single parameter histograms of Y2/51-FITC (FL1) fluorescence displaying events from the monocyte gate. The positive analysis region was determined using an IgG-FITC conjugated isotypic control. A minimum of 2,000 monocytes was counted per test.

Statistical analysis. Differences in patient demographic and medical history characteristics between those with AMI and those with chest pain not attributable to AMI were compared using chi-square and Student *t* tests of statistical significance for categorical and continuous variables, respectively. Analysis of variance was used to perform multiple data comparisons using Bonferroni's method of correction of average number of MPA as well as to test for differences in MPA between the AMI and non-AMI comparison groups. To control for the potentially confounding influence of age and other covariates (including past medical history and concomitant medications), we carried out a logistic regression analysis to examine the association between the occurrence of AMI and MPA. A Pearson correlation coefficient was used to measure the relationship between the duration of acute coronary symptoms and the level of MPA in AMI patients.

RESULTS

Baseline characteristics. There were no significant differences between patients with (*n* = 61) and without (*n* = 150) confirmed AMI with respect to age, gender, history of hypertension, stable angina, unstable angina, AMI, diabetes, hyperlipidemia and use of tobacco, beta-adrenergic blocking agents, calcium antagonists and angiotensin-converting enzyme inhibitors (Table 1).

Circulating MPA. Of patients presenting to the ED with chest pain, those with AMI (*n* = 61), as compared with those without AMI (*n* = 150), had significantly higher numbers of circulating MPA (11.6 ± 11.4 vs. 6.4 ± 3.6, mean ± SD, *p* < 0.0001) (Fig 1). The median number (with 25th/75th percentiles) of circulating MPA in patients with AMI as compared with those without AMI was 8.7 (6.3/13.1) vs. 5.4 (4.3/7.1). Moreover, patients with AMI had significantly higher numbers of circulating MPA than patients discharged with a diagnosis of unstable angina (*n* = 39) (11.6 ± 11.4 vs. 5.5 ± 2.6, mean ± SD, *p* < 0.001). The median number (with 25th/75th percentiles) of circulating MPA in patients with AMI as compared with those with a discharge diagnosis of unstable angina was 8.7 (6.3/13.1) vs. 4.8 (4.1/6.1).

After controlling for age and nitrate use, patients with AMI still had a significantly higher number of circulating MPA than patients without AMI (*p* < 0.001). After controlling for age, the adjusted odds of developing AMI for the 2nd, 3rd and 4th quartiles of MPA, in

Table 1. Baseline Characteristics

	AMI (n = 61)	No AMI (n = 150)	p Value
Age (mean)	56	58	NS
Men	67%	67%	NS
Prior stable angina	14%	12%	NS
Prior unstable angina	33%	25%	NS
Prior AMI	29%	27%	NS
Prior CHF	3%	10%	NS
Valvular heart disease	0%	7%	NS
Hypertension	60%	63%	NS
Hypercholesterolemia	33%	22%	NS
Current tobacco use	11%	21%	NS
Family history of CAD	11%	24%	NS
Diabetes	23	15%	NS
Current medications			
Aspirin	9%	17%	NS
Beta-blocker	10%	18%	NS
Nitrates	55%	15%	< 0.05
Calcium antagonists	5%	11%	NS
ACE inhibitor	11%	12%	NS

ACE = angiotensin-converting enzyme; AMI = acute myocardial infarction; CAD = coronary artery disease; CHF = congestive heart failure.

comparison with patients in the lowest quartile (odds ratio = 1.0), were 2.1 (95% confidence interval [CI]: 0.7, 6.8), 4.4 (95% CI: 1.5, 13.1) and 10.8 (95% CI: 3.6, 32.0), respectively (Table 2).

To assess whether MPA are an early marker of myocardial infarction, we compared the levels of MPA in patients with confirmed AMI presenting within 4 h of symptom onset with those presenting after 4 h and after 8 h of chest pain. The number of circulating MPA in patients with AMI presenting within 4 h of acute coronary symptoms (14.4 ± 16.6) was significantly higher than those presenting after 4 h (9.4 ± 4.5 , $p < 0.001$) and after 8 h (7.0 ± 3.6 , $p < 0.001$) (Fig. 2).

Of the 61 patients with AMI, 35 (57%) had a normal CK-MB ratio (0 to 2.3) at the time of presentation to the ED but high levels of circulating MPA (13.3 ± 14.6) (Fig. 3).

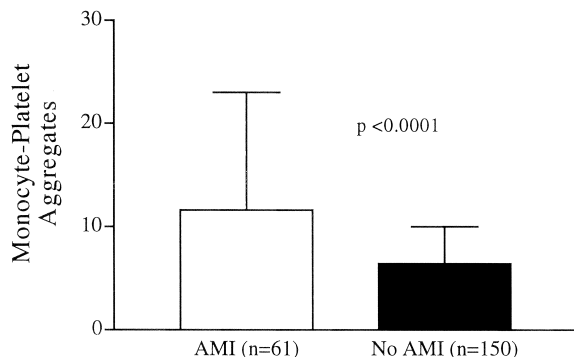


Figure 1. Monocyte-platelet aggregates in the peripheral circulation of patients presenting to an emergency department with chest pain. Data are mean \pm SD. AMI = acute myocardial infarction.

Table 2. Adjusted Odds of Developing AMI According to Levels of Circulating Monocyte-Platelet Aggregates

Quartile	Odds Ratio	95% CI
1	1	—
2	2.1	0.7, 6.8
3	4.4	1.5, 13.1
4	10.8	3.6, 32

AMI = acute myocardial infarction; CI = confidence interval.

DISCUSSION

Our study demonstrates that, of patients presenting to an ED with chest pain, those with AMI have higher numbers of circulating MPA than those without AMI. Furthermore, MPA appear in the peripheral circulation earlier than routine markers of myocardial necrosis, such as CK-MB.

Markers of myocardial cell death and MPA in AMI. A rise and fall in serum cardiac enzymatic markers in patients presenting with chest pain satisfies one of the criteria set by the World Health Organization for the diagnosis of AMI (15). After myocardial cell injury, myoglobin may appear in the peripheral circulation as soon as 1 h, creatine kinase and its MB-isoform within 4 h to 6 h and cardiac troponins within 4 h to 12 h (2). In this study, elevated levels of circulating MPA appeared in the blood before CK-MB. Indeed, the levels of circulating MPA were highest in patients with AMI who did not yet have elevated levels of CK-MB at the time of presentation.

Although serum cardiac markers reflect evidence of myocardial necrosis, they provide no information about the pathophysiologic state within the coronary artery. In contrast, circulating P-selectin/PSGL-1-dependent MPA may reflect platelet activation caused by intracoronary plaque disruption, fissuring or erosion. Thus, MPA may be a marker of plaque instability and provide information on the intracoronary dynamics of thrombosis and inflammation. Plaque rupture and thrombus formation are characteristic of the acute coronary artery syndromes of unstable angina, non-Q-wave myocardial infarction and Q-wave myocardial

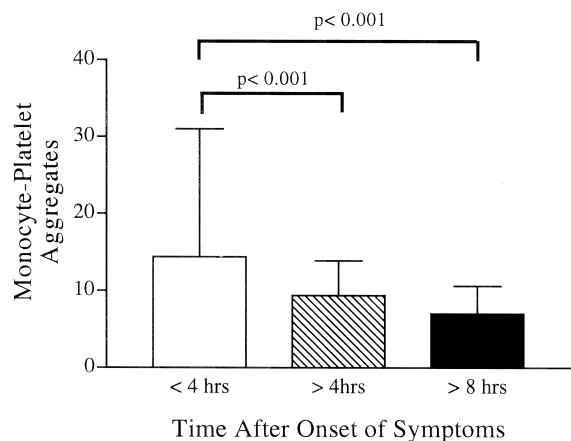


Figure 2. Monocyte-platelet aggregates in the peripheral circulation of patients with acute myocardial infarction with respect to the duration of symptoms. Data are mean \pm SD.

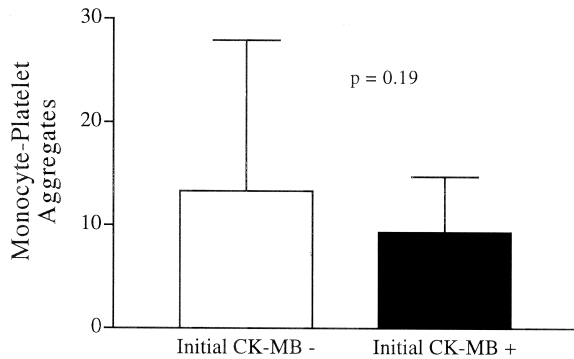


Figure 3. Monocyte-platelet aggregates in the peripheral circulation of patients with acute myocardial infarction with respect to initial creatine kinase isoenzyme (CK-MB). Data are mean \pm SD.

infarction (4). However, the degree of thrombus formation is greater and the composition of the thrombus different in patients with myocardial infarction than it is in those with unstable angina (16). Indeed, in our study, patients with AMI had significantly higher numbers of circulating MPA than patients with unstable angina.

While the formation of MPA is platelet P-selectin-dependent (5,6), the detection of platelet surface P-selectin may not be the most sensitive marker of platelet activation, because degranulated platelets rapidly lose surface P-selectin in vivo yet continue to circulate (9). Therefore, detection of circulating MPA may be a more robust signal of platelet activation than platelet surface P-selectin.

Pathophysiologic relevance of MPA. Platelet adhesion to monocytes is mediated by the platelet surface expression of P-selectin (5,6), which binds to the constitutively expressed PSGL-1 on monocytes (7,17,18). Because platelets are involved in both inflammation and thrombosis (19-23), the formation of MPA may represent targeting of both cell types to specific inflammatory or thrombotic sites (22,24,25). Moreover, adherence of platelets to monocytes has been shown to regulate various monocyte actions. For example, platelets supply cholesterol to monocytes (26) that may then mature into lipid-laden macrophages characteristic of coronary atherosclerosis (27). Additionally, platelet surface P-selectin induces the expression of tissue factor on monocytes (28) and promotes fibrin deposition (24) within a growing thrombus at sites of vascular injury. Furthermore, the binding of thrombin-stimulated platelets induces monocyte cytokine expression (12), and monocyte chemokine synthesis can be regulated by platelet surface P-selectin in concert with the platelet chemokine RANTES (29). It has been recently shown that activated platelets express CD40 ligand (CD40L, CD154) on their surface (30). Binding of CD40L to CD40 on monocytes leads to monocyte activation and production of cytokines including interleukin-6 (31), which is associated with unstable angina (32) and often parallels elevations in C-reactive protein (33). Elevation of C-reactive protein is, in turn, associated with acute coronary syndromes including AMI (34).

Study limitations. This study was performed at a single center with expertise in the use of flow cytometry to study

platelet function. Appropriate caution needs to be exercised in the interpretation of the data given because: 1) there was a relatively small number of patients studied; 2) there were difficulties in accurately identifying the exact time of onset of acute coronary symptoms; and 3) different results could be found in a sample population with a different prevalence of AMI. A large, multicenter study is, therefore, needed to confirm that high numbers of circulating MPA are an early marker of AMI. The detection of circulating MPA currently requires the use of expensive equipment and skilled technical staff. However, the development of a point-of-care device for the measurement of circulating MPA is feasible and would be advantageous. Future studies should investigate if there is any relationship between infarct size and the number of circulating MPA.

Conclusions. Circulating MPA are an early marker of AMI.

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