Cell-derived microparticles: composition and function

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P-selectin- and CD63-exposing platelet microparticles reflect platelet activation in peripheral arterial disease and myocardial infarction

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Abstract

**Background:** Platelet-derived microparticles are generally considered a marker of platelet activation in cardiovascular disease.

**Objectives:** We studied to which extent subpopulations of platelet-derived microparticles parallel platelet activation *in vitro* and *in vivo*.

**Methods:** Using flow cytometry, we analyzed platelet-derived microparticle subpopulations from resting and activated platelets *in vitro* (*n* = 6), as well as from plasma samples of patients with stable angina, peripheral arterial disease, myocardial infarction [MI; non-ST-elevation (NSTEMI) and ST-elevation (STEMI)], and older age- and sex-matched and young healthy individuals (*n* = 10 for all groups, except NSTEMI (*n* = 11)). Coagulation markers prothrombin fragment F$_{1+2}$ and thrombin-antithrombin complexes (TAT) were determined by ELISA. The microparticle-associated fraction of soluble (s)P-selectin was estimated by ELISA.

**Results:** *In vitro*, stimulation of platelets with thrombin receptor activating peptide (TRAP, 15 µM) or calcium ionophore A23187 (2.5 µM) increased fractions of both platelets and platelet-derived microparticles exposing P-selectin or CD63 (*P* < 0.001 for all). Whereas the number of platelet-derived microparticles released by A23187-stimulated platelets increased significantly (*P* < 0.001), the number of platelet-derived microparticles released from TRAP-stimulated platelets remained constant (*P* > 0.05). *Ex vivo*, numbers of circulating microparticles were comparable in all groups. Compared with young persons, P-selectin exposing platelet-derived microparticles were increased in older persons (*P* = 0.02) and were further increased in patients with NSTEMI (*P* = 0.007) and STEMI (*P* = 0.045). CD63 exposing platelet-derived microparticles were increased in patients with peripheral arterial disease (*P* = 0.041), NSTEMI (*P* = 0.001) and STEMI (*P* = 0.049). Subpopulations exposing P-selectin or CD63 correlated with each other (*r* = 0.581; *P* < 0.001), but neither correlated with the plasma concentrations of F$_{1+2}$ or TAT. The microparticle-associated fraction of sP-selectin constituted only 2.2 ± 4.7% (mean ± SD) of total sP-selectin.

**Conclusions:** Platelet-derived microparticle subpopulations reflect platelet activation status better than the total number of platelet-derived microparticles. Increased concentrations of circulating platelet-derived microparticle subpopulations are found in aging, and further increases are encountered in peripheral arterial disease and MI.
Platelet activation occurs during development of atherosclerosis as well as thrombus formation in acute manifestations of atherosclerotic disease [1]. Accurate determination of platelet activation status remains a challenge, but could provide a useful tool in identifying patients at risk for future cardiovascular events.

Current methods are based on the detection of reversible and irreversible platelet activation, which can be monitored by direct and indirect methods [2]. Direct methods include flow cytometric analysis of platelet membrane glycoproteins that, upon activation, either become exposed (e.g. P-selectin or CD63) or change their conformation (e.g. glycoprotein IIb-IIIa), as well as measurements of platelet secretion products in plasma or urine. Whereas platelet flow cytometry requires fresh samples, is labor-intensive, and is complicated by fixation procedures, secretion products are either not very specific for platelets [e.g. soluble (s)P-selectin] or are sensitive to sampling and handling artefacts (e.g. β-thromboglobulin).

Indirect methods are functional platelet assays, which evaluate the ex vivo platelet function thought to be dependent on the platelet activation status in vivo. In this way, both reversible (e.g. aggregation and adhesion) and irreversible platelet activation (i.e. the secretion response) can be studied. Although rapid platelet function assays have been developed, correlations with direct assays are poor, suggesting that such functional assays do not necessarily reflect the actual in vivo activation status [3].

In vitro, the release of microparticles from platelets has been associated with the secretion response [4]. Therefore, the numbers of circulating platelet-derived microparticles are considered to reflect the platelet activation status in vivo, for instance in patients with peripheral arterial disease [5], unstable angina [6], myocardial infarction (MI) [7], cerebrovascular accident [8,9] and diabetes [9]. Because analysis of platelet-derived microparticles does not require fixation and can be applied to stored plasma samples, batchwise analysis of the in vivo platelet activation status is feasible. However, although several studies have confirmed concomitant changes in exposure of platelet P-selectin and circulating numbers of platelet-derived microparticles in vivo [10,11], others found discrepancies between the two [6,7,12-14], which may call into question the relevance of platelet-derived microparticle numbers as a marker of platelet activation.

We hypothesized that measuring platelet-derived microparticle-associated P-selectin or CD63 is a feasible and reliable method of assessing platelet activation. Because of the intricacy of adequately assessing the in vivo platelet activation status, as discussed before, first we evaluated the exposure of P-selectin and CD63 on both platelets and platelet-derived microparticles under controlled in vitro conditions. To substantiate the relevance of our in vitro findings, we also determined the exposure of P-selectin and CD63 on circulating platelet-derived microparticles in groups of individuals with various degrees of atherosclerotic disease. Finally, we measured the coagulation activation markers prothrombin fragment F1+2 and thrombin-antithrombin complexes (TAT), which have been associated with platelet activation and platelet-derived microparticle release [15].
Methods

Reagents and assays
For detection of microparticle origin, we used phycoerythrin (PE)-labeled anti-glycophorin A (JC159, IgG₁) and fluorescein isothiocyanate (FITC)-labeled CD61 (Y2/51, IgG₁) from Dako A/S (Glostrup, Denmark); CD4-PE (CLB-T4/2,6D10, IgG₁) and CD66E-PE (CLB-gran/10, IH4Fc, IgG₁) from Sanquin (Amsterdam, the Netherlands); CD8-PE (SK1, IgG₁), CD14-PE (MØP9, IgG₂b), CD20-PE (L27, IgG₁), IgG₁-FITC (X40), and IgG₁-PE (X40) from Becton, Dickinson and Company Immunocytometry Systems (BD; San José, CA, USA); CD62E-PE (HAE-1f, IgG₁) from Ancell Corp. (Bayport, MN, USA); CD54-PE (84H10, IgG₁) and allophycocyanin (APC)-labeled annexin V from Caltag (Burlingame, CA, USA); IgG₂b-PE (MCG2b) from Immuno Quality Products (Groningen, the Netherlands); CD63-PE (CLB-gran12, IgG₁) and anti-P-selectin-PE (CD62P-PE, CLB-Thromb/6, IgG₁) from Coulter/Immunotech (Marseilles, France). We had previously demonstrated that the anti-P-selectin antibody used does not cross-react with (endothelial) E-selectin [16]. Plasma concentrations of sP-selectin, F₁+₂ and TAT were determined by ELISA as described by the manufacturer (Parameter human P-Selectin Immunoassay by R&D Systems, MN, USA, and Enzygnost F₁+₂ micro and TAT by Dade Behring GmbH, Marburg, Germany, respectively).

In vitro platelet activation and flow cytometric analysis
Venous blood was obtained from six healthy individuals who had not taken any medication during the previous 10 days and had given their informed consent. The blood was collected into 1/10th volume of 10.9 mmol/L trisodium citrate, and centrifuged at 180 g for 15 min at 20°C to obtain platelet-rich plasma (PRP). PRP (3.5-4.5 mL) was acidified with 1/6th volume of acid citrate dextrose (ACD; 71 mmol/L citric acid, 85 mmol/L trisodium citrate and 110 mmol/L D-glucose, pH ~4.4). The acidified PRP from each healthy individual was divided into two aliquots. To one of the aliquots dibutyryl cAMP (dbcAMP) was added (final concentration 2 mmol/L), to the other nothing, and the PRP was incubated for 20 min at 37°C. Platelets were then pelleted by centrifugation at 700 × g for 20 min at 20°C, then washed once with a buffer containing 137 mmol/L NaCl, 2.6 mmol/L KCl, 1.0 mmol/L MgCl₂, 11.9 mmol/L NaHCO₃, 5.6 mmol/L D-glucose, 1 mmol/L EDTA (buffer A; pH 6.5). For the platelet suspensions pretreated with dbcAMP, this buffer also contained 2 mmol/L dbcAMP. Finally, platelets were resuspended in buffer A without EDTA or dbcAMP (4.5 mL, pH 7.35). Platelet counts were determined with a CELL-DYN 4000 hematology analyzer (Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL, USA).

To 1 mL aliquots of the platelet suspensions treated with dbcAMP nothing was added (non-activated platelets). To 1 mL aliquots of the platelet suspensions not treated with
dbcAMP, CaCl$_2$ (final concentration 2.5 mmol/L) was added alone as a control, or in combination with either thrombin receptor activating peptide (TRAP, SFLLRN, from Bachem AG, Bubendorf, Switzerland, final concentration 15 μmol/L) or calcium ionophore A23187 (Calbiochem, San Diego, CA, USA; final concentration 2.5 μmol/L). The mixtures were incubated at 37°C for 30 min, without stirring to avoid platelet aggregation. Activation was stopped by the addition of 2.5 mmol/L final concentration of EDTA, and platelet counts were determined.

For flow cytometric analysis, 5 μL aliquots were taken from each tube and diluted in 35 μL of HEPES buffer (137 mmol/L NaCl, 2.7 mmol/L KCl, 1 mmol/L MgCl$_2$, 20 mmol/L HEPES, 3.3 mmol/L Na$_2$PO$_4$, 1 g/L bovine serum albumin, 5.6 mmol/L D-glucose; pH 7.4). CD61-FITC, and a PE-labeled anti-P-selectinor CD63 monoclonal antibody (5 μL each) were added. The mixtures were incubated in the dark for 30 min at 20°C, after which the platelets were fixed by addition of 1.5 mL of 0.3% (w/v) paraformaldehyde, and analyzed on a FACSCalibur flow cytometer with CellQuest Pro 4.0.2 software (BD). Forward scatter (FSC), side scatter (SSC) and fluorescence data were obtained with gain settings in the logarithmic mode. Platelets and platelet-derived microparticles were selected based on their platelet marker positivity and FSC/SSC characteristics [17,18]. For each sample, 10000 platelet marker (CD61) positive events were acquired. To identify marker positive events, thresholds were set based on samples incubated with similar concentrations of isotype-matched control antibodies.

**Patient and healthy subject groups**

Blood samples were obtained from (i) patients with stable angina pectoris (Canadian Society of Cardiology class II) having at least one significant (> 50%) coronary stenosis ($n$ = 10); (ii) patients with ST-elevation MI [19] (STEMI; $n$ = 10); (iii) patients with non-ST-elevation MI [19] (NSTEMI; $n$ = 11); (iv) patients with angiographically confirmed peripheral arterial disease ($n$ = 10). Blood samples from the cardiac patients were collected at the Department of Cardiology of the Academic Medical Center (AMC, Amsterdam, the Netherlands) before they underwent catheterization and/or percutaneous coronary intervention. Blood samples from patients with peripheral arterial disease were obtained at the Department of Vascular Surgery (AMC) before the patients underwent elective bypass surgery, and patients were advised to refrain from strenuous exercise at least 24 hours before sampling. All NSTEMI patients were sampled within 12 hours, and all STEMI patients within 6 hours after onset of symptoms. All patients received medication according to standard clinical practice. None received coumarin derivatives, thrombolytic therapy, or anti-platelet medication other than aspirin. Patients with stable angina pectoris received aspirin therapy, whereas those with peripheral arterial disease were advised to refrain from aspirin use at least 7 days before elective surgery and received prophylactic low-molecular weight heparin, and MI patients had received aspirin and low-molecular weight heparin upon admission. Blood samples were also obtained from healthy individuals, age- and sex-
matched to the patient groups \( (n = 10) \), as well as from healthy individuals less than 50 years of age \( (n = 10) \). The study was approved by the local medical ethics committee of the AMC.

Venous blood was collected from the cubital vein into 1/10th volume of 10.9 mmol/L trisodium citrate (BD) with minimal venous occlusion. All blood samples were collected by a single clinician (P.M.v.d.Z.) and processed within 30 min. Blood samples were centrifuged \( (20 \text{ min at } 1550 \times g) \) at room temperature. Only the upper 2/3 of the apparent plasma fractions were collected and briefly mixed in a plastic 10 mL tube. Aliquots of 250 \( \mu L \) were immediately snap frozen in liquid nitrogen for at least 15 min, to finally be stored at \(-80^\circ C\) until analysis.

**Microparticle isolation and flow cytometric analysis**

Microparticles were isolated as described previously [17,18]. Briefly, snap frozen plasma samples \( (250 \mu L) \) were centrifuged at \( 20^\circ C \) for 30 min at \( 17570 \times g \). The upper 225 \( \mu L \) were discarded. The remaining plasma was diluted with 225 \( \mu L \) phosphate-buffered saline (PBS; 154 mmol/L NaCl, 1.4 mmol/L phosphate, pH 7.4) containing 10.9 mmol/L trisodium citrate (PBS/citrate). Microparticles were resuspended and centrifuged again \( (30 \text{ min, } 17570 \times g, 20^\circ C) \). After removal of the supernatant \( (225 \mu L) \), microparticles were resuspended, and diluted 4-fold in PBS/citrate. Five \( \mu L \) aliquots of this suspension were used for flow cytometric analysis.

Samples were analyzed in a FACSCalibur flow cytometer with CellQuest software (BD) [18]. Microparticles from six patients in each group were double-stained with annexin V and a cell-specific monoclonal antibody, and microparticles from 10 patients in each group were triple-stained with annexin V, CD61, and either anti-P-selectin or CD63. Microparticles were analyzed for 1 minute, and identified by their characteristic FSC and SSC, and by their ability to bind annexin V and a cell-specific monoclonal antibody [17,18,20]. From the number of events \( (N) \) in the upper right (marker and annexin V positive) quadrant of the flow cytometric analysis (FL-1 versus FL-2, corrected for isotype control antibody binding and autofluorescence), the number of microparticles per liter of plasma was calculated as: number/L=\( N \times \frac{100}{5} \times \frac{955}{60} \times \frac{10^6}{250} \), in which 5 \( (\mu L) \) is the volume of microparticle suspension, 100 \( (\mu L) \) is the total volume of washed microparticle suspension, 955 \( (\mu L) \) is the total volume in the tube before analysis, 60 \( (\mu L) \) is the sample volume analyzed, \( 10^6 \) is the number of microliters per liter, and 250 \( (\mu L) \) is the original volume of plasma.

**Statistical Analysis**

All not normally distributed data are presented as median (interquartile range); other data are presented as mean \( \pm \) SD. For comparisons of normally and not normally distributed data, t tests and Mann-Whitney U tests were used, respectively. The \( \chi^2 \) test was used for
comparison of dichotomous variables. For multiple comparisons, the Kruskal-Wallis test or one-way analysis of variance (ANOVA) for matched samples followed by Bonferroni’s multiple comparison test was used. Correlations were calculated in patients and healthy persons together, using Spearman’s rank correlation test. $P < 0.05$ was regarded as statistically significant.

**Results**

**Platelet-derived microparticles in vitro**

Washed human platelets ($n = 6$) were stimulated with either TRAP (15 μM) or the calcium ionophore A23187 (2.5 μM). As shown in Figure 1A, compared with non-activated (i.e. dbcAMP-treated) platelets, the fractions of platelets exposing P-selectin (CD62P) or CD63 strongly increased in response to both TRAP and A23187 ($P < 0.001$ for both P-selectin and CD63, using either agonist). Platelet disappearance during stimulation was negligible ($P > 0.05$ compared with non-activated platelets; data not shown).

The fractions of platelet-derived microparticles exposing P-selectin or CD63 in response to TRAP or A23187 are shown in Figure 1B. Compared with non-activated platelets, the fractions of platelet-derived microparticles exposing P-selectin or CD63 increased upon incubation with TRAP or A23187 ($P < 0.001$ for both P-selectin and CD63, using either agonist).

Numbers of platelet-derived microparticles released in response to stimulation with TRAP were not different when compared with non-activated platelets (Figure 1C). Only stimulation with A23187 increased platelet-derived microparticle numbers ($P < 0.001$).

**Platelet-derived microparticles in vivo**

The baseline characteristics of patients and controls are shown in Table 1. There were no significant differences in age and sex between the older healthy persons and the other groups [young healthy subjects (except for age), patients with stable angina pectoris, peripheral arterial disease, NSTEMI, STEMI]. Patients with stable angina had a higher body mass index than the older healthy persons (27.8 vs. 23.3 kg/m²; $P = 0.012$). None of the healthy individuals had clinically manifest cardiovascular disease.

In each group of subjects studied, the majority of microparticles originated from platelets (Figure 2A). The overall fraction of CD61 exposing microparticles was 96% (93-98%), and the numbers did not differ among groups ($P = 0.954$, Kruskal-Wallis test). The other microparticles (Figure 2B) originated from erythrocytes [positive for glycophorin A, 4% (1-7%)] and to a lesser extent (≤2%) from T helper cells (CD4), T cytotoxic/suppressor cells (CD8), monocytes (CD14), B cells (CD20), endothelial cells (CD62E) and granulocytes (CD66E). Because no differences among groups were observed regarding
numbers and cellular origin of the non-platelet microparticles, data are summarized for all groups in Figure 2B.

Figure 3 shows representative dot plots of CD61 exposing microparticles, as detected in an older healthy person (Figure 3A) and a NSTEMI patient (Figure 3B). Staining with anti-P-selectin revealed that the NSTEMI patient had a relatively large subpopulation of platelet-derived microparticles that exposed P-selectin (Figure 3D, upper right quadrant) when compared with the healthy control (Figure 3C). As is also evident in Figure 3, all P-selectin exposing microparticles are platelet-derived microparticles, i.e. the upper left quadrants of Figures 3C and 3D do not show any events.

Figures 4A and 4B show the fractions of platelet-derived microparticles that expose P-selectin (Figure 4A) or CD63 (Figure 4B). Comparison among groups (Kruskal-Wallis test) showed significant differences for both P-selectin and CD63 ($P < 0.001$ and $P = 0.007$, respectively). Compared with the young healthy group, the P-selectin exposing subpopulations were increased in older individuals ($P = 0.028$), and were further increased

![Figure 1](image-url)

**Figure 1.** A. Percentage of platelets exposing P-selectin or CD63. B. Percentage of platelet-derived microparticles exposing P-selectin or CD63. C. Numbers of microparticles released per $10^3$ platelets. Data are presented as mean and SD. One-way ANOVA for matched samples was performed, followed by Bonferroni’s multiple comparison test between the non-activated (dibutyryl cAMP-treated) versus the other samples. N.S., not significant ($P > 0.05$); ***$P < 0.001$. TRAP, thrombin receptor activating peptide.
Table 1. Baseline characteristics of patients and healthy subjects included in the study.

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\(^1\) Documented MI or CAD in parents or siblings before the age of 60. \(^2\) Daily use of aspirin during the 7 days prior to blood collection. *Compared with older healthy subjects \( P < 0.05 \). ***Compared with older healthy subjects \( P < 0.001 \).

ACE, angiotensin-converting enzyme; BMI, body mass index; CAD, coronary artery disease; CVA, cerebrovascular accident; MI, myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; PAD, peripheral arterial disease; PCI, percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction; TIA, transient ischemic attack.
in NSTEMI ($P = 0.007$) and STEMI ($P = 0.045$) patients. CD63 exposing subpopulations were increased in patients with peripheral arterial disease ($P = 0.041$), NSTEMI ($P = 0.001$) and STEMI ($P = 0.049$). Prior use of aspirin in NSTEMI (6 of 11 patients) and STEMI patients (4 of 10 patients) did not affect the exposure of P-selectin or CD63 (data not shown). The numbers of platelet-derived microparticles exposing P-selectin or CD63 correlated strongly ($r = 0.581; P < 0.001$).

![Figure 2. Plasma microparticle numbers. A. Numbers of CD61 positive microparticles in patients and healthy subjects. B. Numbers of erythrocyte- (glycophorin A), T helper cell- (CD4), T cytotoxic/suppressor cell- (CD8), monocyte- (CD14), B cell- (CD20), endothelial cell- (CD62E), and granulocyte-derived (CD66E) microparticles in all patients and healthy subjects. Data are presented as median and interquartile range. NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.](image-url)
Figure 3. Origin of P-selectin exposing microparticles. A. Microparticles of a representative healthy individual and B. microparticles of a representative NSTEMI patient, stained for annexin V and CD61. C. Microparticles from a representative healthy individual and D. microparticles from a representative NSTEMI patient, stained for annexin V, CD61 and P-selectin.

The concentrations of TAT and F1+2 are shown in Table 2. There were no differences in TAT and F1+2 concentrations among groups (Kruskal-Wallis test), and there were no correlations between subpopulations of platelet-derived microparticles exposing P-selectin or CD63 and concentrations of TAT ($r = 0.086$, $P = 0.512$; $r = 0.055$, $P = 0.677$ respectively) or F1+2 ($r = -0.131$, $P = 0.314$; $r = -0.166$, $P = 0.209$, respectively).

Microparticle-associated P-selectin constituted only $2.2 \pm 4.7\%$ of the total concentrations of sP-selectin in plasma, as assessed from the difference between concentrations of P-selectin in microparticle-containing plasma and in microparticle-depleted plasma after high-speed centrifugation (30 min at $17570 \times g$ and $20^\circ C$). No correlations were present between the subpopulations of P-selectin exposing platelet-derived microparticles and either the total concentrations of sP-selectin ($r = 0.153$, $P = 0.240$) or the calculated microparticle-associated fractions of sP-selectin ($r = -0.003$, $P = 0.991$).
Figure 4. Subpopulations of platelet-derived microparticles exposing P-selectin or CD63. Shown are the percentages of platelet-derived microparticles (i.e. events positive for CD61 and annexin V) exposing A. P-selectin or B. CD63.

NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction.

Discussion

The present study shows that subpopulations of platelet-derived microparticles exposing P-selectin or CD63 reflect platelet activation in vitro more closely than overall numbers of platelet-derived microparticles. Because platelet activation increases with age and in atherosclerotic disease [21,22], we studied the presence of platelet-derived microparticles and platelet-derived microparticle subpopulations in aging and in atherosclerotic disease in vivo. In our study, the majority of circulating microparticles originated from platelets in all individuals. Platelet-derived microparticle subpopulations but not the total numbers of platelet-derived microparticles increased with age and in patients with peripheral arterial disease and MI.
Table 2. Markers of inflammation and coagulation.

<table>
<thead>
<tr>
<th></th>
<th>Young healthy subjects</th>
<th>Older healthy subjects</th>
<th>Stable angina</th>
<th>PAD</th>
<th>NSTEMI</th>
<th>STEMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 11</td>
<td>n = 10</td>
</tr>
<tr>
<td>TAT (μg/L)</td>
<td>3.28 (2.41 – 4.68)</td>
<td>3.65 (2.16 – 4.27)</td>
<td>3.02 (2.34 – 3.43)</td>
<td>3.82</td>
<td>3.85</td>
<td>3.14</td>
</tr>
<tr>
<td>F1+2 (nmol/L)</td>
<td>0.68 (0.60 – 0.94)</td>
<td>0.89 (0.80 – 1.11)</td>
<td>0.85 (0.58 – 1.12)</td>
<td>0.91</td>
<td>0.82</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range). There were no differences between groups regarding levels of TAT and F1+2 (Kruskal-Wallis test).

MI, myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; PAD, peripheral arterial disease; STEMI, ST-elevation myocardial infarction; F1+2, prothrombin fragment F1+2; TAT, thrombin-antithrombin complexes.

Gawaz et al. reported that in the early stage of MI the fraction of P-selectin exposing platelets was increased but not the numbers of platelet-derived microparticles. This early stage was followed after 8 hours by an increase in the numbers of platelet-derived microparticles and by a concomitant decrease in platelet counts [23]. These data indicated that the total numbers of platelet-derived microparticles are not an early and sensitive marker of platelet activation in vivo. Rather, the late increase in platelet-derived microparticles and decrease in platelet count may be a consequence of altered thrombopoiesis in response to the inflammatory reaction during MI [24]. Thus, discrepancies occur between exposure of P-selectin on platelets and the numbers of platelet-derived microparticles, which we have found in vitro and others in vivo [6,7,12-14]. These discrepancies may be attributed, at least in part, to the persistent release of microparticles by megakaryocytes. Such microparticles may be erroneously identified as platelet-derived microparticles, because of similar antigenic profiles [25].

Our results thus indicate that subpopulations of platelet-derived microparticles may constitute a novel (plasma) marker for in vivo platelet activation. This marker not only reflects platelet activation better than the total numbers of circulating platelet-derived microparticles, but is also more feasible than measurement of the exposure of P-selectin on platelets. As the major fraction of sP-selectin in plasma is not associated with microparticles, direct determination of the microparticle-associated fraction of sP-selectin by ELISA is impossible. We are currently developing an ELISA capture assay for both CD61 and P-selectin.

Determination of subpopulations of platelet-derived microparticles may also be used in monitoring the risk-reducing effects of anti-platelet drugs. With regard to the present study, we found ongoing platelet activation despite prior use of aspirin. This is in line with reports of increased platelet activation after coronary stent implantation under aspirin use [26].
Thus, the risk-reducing properties of aspirin may be related, at least in part, to mechanisms other than attenuation of platelet activation.

In conclusion, our in vitro data show that platelet-derived microparticle subpopulations reflect the platelet activation status. Increased concentrations of circulating platelet-derived microparticle subpopulations are found in aging, and further increases are encountered in peripheral arterial disease and MI.

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References


