Reticulated Platelets in Acute Coronary Syndrome: A Marker of Platelet Activity

To the Editor: Mean platelet volume (MPV) is elevated in patients with acute coronary syndrome (ACS) (1) and is used as an independent predictor of recurrent myocardial infarction and cardiac death (2). Reticulated platelets (RP) are newly formed platelets. They are larger in size, have higher granule content, and selectively bind to thiazole orange dye (TO) that stains ribonucleic acid and enhances its fluorescence signal (3). We then performed this study to determine whether RP increase in ACS.

The study population consisted of 13 control subjects, 31 patients with unstable angina (UA), 25 patients with non–ST-segment elevation myocardial infarction (NSTEMI), and 23 patients with ST segment elevation myocardial infarction (STEMI). The Baylor College of Medicine Institutional Review Board approved the study.

Volumes of 5 cc of blood were drawn before institution of anticoagulation therapy into collection tubes containing ethylenediaminetetraacetic acid (1.5 mg/ml). Complete blood count and MPV were performed using an automated analyzer (Sysmex HST, Kobe, Japan) within 30 min of blood collection. The range of expected platelet volume in our laboratory is 7.4 to 10.4 fl for a platelet count of 150 to 400 k/dl.

Reticulated platelets were prepared and measured according to the previously described methods (4,5). To avoid nonspecific labeling of RP, RNase treatment was performed as a control for measurement of %RP. Platelet-rich plasma was prepared by centrifugation of collected blood in acid-citrate-dextrose (8.5/1.5, v/v) at 170 g for 10 min at 22°C. The platelet-rich plasma was fixed with 1% paraformaldehyde for 15 min divided into two aliquots. One was incubated with 20 µl/ml RNase (Ambion, Austin, Texas) for 30 min at 22°C. The second aliquot remained untreated with RNase and was used as a blank control. The specimen was processed as indicated for platelet labeling.

Flow cytometric analysis was performed using a FACScalibur (Becton Dickinson) flow cytometer. The GPlb-positive cells were gated in a TO (FL1) versus PE (FL2) dot plot. A positive range was set for platelets with fluorescence higher than 99% of the normal platelets below the line was established. The platelets were assumed to represent RP. To define the frequency of RP, a line with the same slope as the platelet cluster set to segregate 99% of the normal platelets below the line was established. The placement of this line is admittedly arbitrary; however, it permits a consistent and objective approach to the analysis of patients’ samples. In addition, expected platelet volume in our laboratory is 7.4 to 10.4 fl for a platelet count of 150 to 400 k/dl.

Before, although it has been suggested that assuming a Pra value of 5 mm Hg results in insignificant differences in CFI (5). In contrast, our findings show that substantial errors occur when right atrial pressure is assumed to be a fixed value. It is interesting to note that Seiler et al. (6) found a better correlation between Doppler- and pressure-derived CFI when a measurement of central venous pressure was included, as compared with those calculations where venous pressure was assumed to be 5 mm Hg. Given that almost one-sixth of patients in our study were assigned to the wrong CFI category when Pra was assumed to be negligible or a fixed value, we believe that measurement of right atrial pressure is imperative when calculating CFI.

The FFR and CFI were derived and validated with the inclusion of right atrial pressure. Ignoring Pra in everyday practice debases the fidelity of FFR and may lead to inappropriate therapy in some cases. Similarly, assuming a fixed arbitrary Pra value leads to the fidelity of CFI and may lead to inappropriate therapy in some cases. If the simplified index Pd/Pa is used, values between 0.70 and 0.80 mandate recalculation of true FFR, after measurement of Pra.

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REFERENCES

Figure 1. (A) Platelets stained with GPIb in phosphate-buffered saline (PBS). The M1 marker was set to represent autofluorescent staining. (B) Platelets stained with GPIb and thiazole orange. The M1 marker indicates platelets that stained positive for thiazole orange. (C) Platelets treated with RNase and subsequently stained with GPIb and thiazole orange. The M1 marker represents the reticulated platelets that are positive for thiazole orange.
Table 1. Baseline Characteristics of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 13)</th>
<th>UA (n = 31)</th>
<th>NSTEMI (n = 25)</th>
<th>STEMI (n = 23)</th>
<th>p Value</th>
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</thead>
<tbody>
<tr>
<td>Demographics</td>
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<tr>
<td>Age (yrs), mean ± SD</td>
<td>56.0 ± 11.3</td>
<td>59.1 ± 8.7</td>
<td>59.6 ± 9.8</td>
<td>55.6 ± 9.2</td>
<td>0.35</td>
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<tr>
<td>Male</td>
<td>9 (69.2)</td>
<td>24 (77.4)</td>
<td>17 (68)</td>
<td>17 (73.9)</td>
<td>0.86</td>
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<td>Risk factors</td>
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<tr>
<td>Diabetes</td>
<td>6 (46.2)</td>
<td>14 (45.2)</td>
<td>9 (36)</td>
<td>12 (52.2)</td>
<td>0.43</td>
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<tr>
<td>History of MI</td>
<td>3 (23)</td>
<td>3 (9.7)</td>
<td>7 (28)</td>
<td>9 (39.1)</td>
<td>0.01*</td>
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<tr>
<td>Hypertension</td>
<td>7 (53.8)</td>
<td>9 (29)</td>
<td>8 (32)</td>
<td>6 (26.1)</td>
<td>0.77</td>
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<tr>
<td>Dyslipidemia</td>
<td>8 (61)</td>
<td>12 (38.7)</td>
<td>10 (40)</td>
<td>14 (60.9)</td>
<td>0.79</td>
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<tr>
<td>Treatment</td>
<td></td>
<td></td>
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<tr>
<td>ASA</td>
<td>3 (23)</td>
<td>6 (19)</td>
<td>2 (8)</td>
<td>4 (17)</td>
<td>0.59</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>2 (15)</td>
<td>4 (13)</td>
<td>1 (4)</td>
<td>2 (9)</td>
<td>0.65</td>
</tr>
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<td>Cardiac enzymes (mean ± SD)</td>
<td></td>
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<tr>
<td>Creatine kinase</td>
<td>23 ± 5</td>
<td>103.3 ± 87.5</td>
<td>660.0 ± 550.1</td>
<td>2,403.3 ± 1,138.2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Troponin-1</td>
<td>0.10 ± 1.2</td>
<td>0.34 ± 1.6</td>
<td>29.55 ± 21.9</td>
<td>86.54 ± 68.1</td>
<td>&lt;0.001*</td>
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<tr>
<td>Hematologic variables (mean ± SD)</td>
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<tr>
<td>Platelet count (×10^3/µL)</td>
<td>224.4 ± 35.8</td>
<td>219.2 ± 32.5</td>
<td>201.5 ± 32.6</td>
<td>188.8 ± 35.9</td>
<td>0.002*</td>
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<tr>
<td>Mean platelet volume</td>
<td>9.36 ± 0.43</td>
<td>9.28 ± 0.91</td>
<td>10.13 ± 1.07</td>
<td>11.21 ± 1.22</td>
<td>&lt;0.0001*</td>
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</table>

Values in parentheses are percentages. *Statistically significant difference exists between study subgroups.

ASA = acetylsalicylic acid; MI = myocardial infarction; NSTEMI = non-ST-segment elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction; UA = unstable angina.

GPIb PE alone (Fig. 1A), GPIb PE and TO (Fig. 1B), and RNAsse-treated platelets stained with labeled GPIb PE and TO (Fig. 1C).

The data are expressed as mean ± SD. Comparisons between each of the four groups were analyzed by unpaired t test; comparisons among the four groups were also compared using one-way analysis of variance with adjustment for multiple comparisons. A p value <0.05 was considered statistically significant.

Baseline characteristics are displayed in Table 1. As shown, there were no differences between the four groups, except for history of myocardial infarction (p = 0.011). The mean platelet count and MPV increased among the groups (p < 0.0001).

Percent of TO-positive platelets was 3.64 ± 1.06 for control subjects, 10.53 ± 2.71 for patients with unstable angina, 15.99 ± 6.39 for patients with NSTEMI, and 18.94 ± 4.89 for STEMI subjects (p < 0.0001). Similarly RNAsse-treated TO-positive platelets displayed a similar pattern (p < 0.0001). The percentage of RP was 11.9 ± 4.57 in the STEMI subjects, 10.63 ± 4.94 in patients with NSTEMI, 8.39 ± 2.49 in patients with unstable angina, and 2.54 ± 0.88 in the control subjects (p < 0.0001).

Patients who presented with STEMI had significantly higher RP compared with the UA group in paired group analysis (p = 0.00026 for STEMI). The correlation coefficient between troponin and RP count was 0.42 (p = 0.05).

In this study, we demonstrated a significant increase in RP in patients with AMI (NSTEMI or STEMI) and UA compared with patients with stable angina. We also demonstrated an increase in RP in patients with AMI compared with UA. Previous studies have demonstrated similar findings in patients with cardio-embolic stroke (6). Our data reconfirmed increased MPV in patients with ACS, accompanied with a significant increase in platelet density as evidenced by nonspecific binding to TO in RNAsse-treated samples. Nonspecific TO binding has been shown to correlate with platelet granularity (7).

Reticulated platelet analysis using TO and flow cytometry is a convenient method for evaluating platelet kinetics in patients with ACS. Using this method, platelets' behavior is analyzed in their physiologic milieu without activation. Our study suggests a novel practical assay of platelet kinetics in ACS, which may have future implications for risk stratification and antiplatelet therapy.

REFERENCES