Coated Platelet Levels Correlate With Bleed Volume in Patients With Spontaneous Intracerebral Hemorrhage

Calin I. Prodan, MD; Andrea S. Vincent, PhD; George L. Dale, PhD

Background and Purpose—Coated platelets are produced on dual agonist stimulation with collagen and thrombin. These highly procoagulant platelets are critical to normal hemostasis, and an earlier study demonstrated decreased coated platelet production in patients with spontaneous intracerebral hemorrhage. We have expanded this observation to investigate if coated platelet levels correlate with bleed volume in spontaneous intracerebral hemorrhage.

Methods—Coated platelet levels and bleed volume were determined in 45 patients with a diagnosis of spontaneous intracerebral hemorrhage.

Results—There was an inverse relationship between coated platelet levels and bleed volume ($r = -0.38$, $P = 0.01$).

Conclusions—These data support a link between decreased coated platelet synthesis and the severity of spontaneous intracerebral hemorrhage. (Stroke. 2010;41:1301-1303.)

Key Words: hemorrhage □ intracerebral stroke □ platelets

Coated platelets are a subpopulation of platelets observed on dual-agonist stimulation with thrombin and collagen. The biochemical characteristics of coated platelets include a robust prothrombinase activity, retention of several procoagulant proteins on the cell surface, and release of microparticles. The critical nature of coated platelets in hemostasis is revealed by a genetic disorder in dogs, in which deficiency of coated platelets results in spontaneous mucosal bleeding and postsurgical hemorrhage.

Previous studies in ischemic stroke indicated that coated platelet levels are elevated in patients with nonlacunar stroke as compared with control subjects, a finding in keeping with the thrombotic nature of these events. In contrast, coated platelet production in patients with spontaneous intracerebral hemorrhage (SICH) is decreased when compared with control subjects. We now sought to investigate if the level of coated platelets relates to the bleed volume observed in patients with SICH.

Materials and Methods

Forty-five consecutive patients, 15 women and 30 men, with a diagnosis of SICH were recruited from the Neurology Service at the University of Oklahoma Health Sciences Center and the Veterans Affairs Medical Center. All patients were diagnosed with SICH by a board-certified neurologist and underwent brain CT at presentation. A neuroradiologist provided a definitive reading for every scan obtained. We excluded patients with concurrent use of anticoagulants, antiplatelet agents (aspirin, clopidogrel), or HMG-CoA reductase inhibitors (statins), or diabetes; or hemorrhage secondary to trauma, tumor, hemorrhagic transformation of cerebral infarct, rupture of an aneurysm, or vascular malformation. The percentage of men in our study is a result of the composition of the US Armed Forces during the time these veterans served.

Bleed volume was determined on admission from the CT scan by using the ABC/2 formula in which $A$ is the greatest diameter on the largest hemorrhage slice, $B$ is the diameter perpendicular to $A$, and $C$ is the approximate number of axial slices with hemorrhage multiplied by the slice thickness.

After obtaining informed consent, coated platelet levels were determined as previously described. Reports are reported as percent of cells converted into coated platelets. Individuals performing the coated platelet assay were not aware of the clinical diagnosis corresponding to a specific sample and neurologists establishing the diagnosis of SICH were not aware of coated platelet levels.

Statistical analyses were performed using SAS (SAS System for Windows, Version 9.1; SAS Institute Inc, Cary, NC). Analyses included calculation of descriptive statistics, independent $t$ tests, bivariate and partial correlations for comparison of continuous variables, and $\chi^2$ or Fisher exact tests for comparison of categorical variables with significance set at $P \leq 0.05$.

Sample size was calculated to achieve 90% power to detect a potential linear correlation between bleed volume and coated platelet levels with $r \approx 0.4$ and statistical significance at $P < 0.05$ assuming that bleed volume will range between 1 and 60 mL and coated platelet levels will range between 5% and 40%.

Results

Demographic data; the use of medications that may influence coated platelet levels such as selective serotonin reuptake inhibitors, HMG-CoA reductase inhibitors (statins), or antiplatelet medication; and coated platelet levels for patients with SICH are listed in the Table. The mean coated platelet level was 28.6% (range, 5.5% to 62.2%), the mean bleed volume was 16.6 mL (range, 1 to 52 mL), and the mean time

Received February 8, 2010; accepted February 16, 2010.
From the Department of Neurology (C.I.P.), University of Oklahoma Health Sciences Center, and Veterans Affairs Medical Center, Oklahoma City, Okla; the Center for the Study of Human Operator Performance (A.S.V.), University of Oklahoma, Norman, Okla; and the Department of Medicine (G.L.D.), University of Oklahoma Health Sciences Center, Oklahoma City, Okla.
Correspondence to Calin I. Prodan, MD, 711 SL Young Boulevard, PPOB-215, Oklahoma City, OK 73104. E-mail calin-prodan@ouhsc.edu
© 2010 American Heart Association, Inc.

Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.110.581447
between onset of symptoms and coated platelet measurement was 14.4 hours (range, 5 to 24 hours).

A bivariate correlation was performed to examine the relationship between bleed volume and coated platelet levels; the results showed a significant inverse linear correlation ($r = -0.38; P = 0.01$; Figure). We then examined the partial correlation between bleed volume and coated platelet levels controlling for age; race; gender; smoking; and use of statins, selective serotonin reuptake inhibitors, and antiplatelets. The resulting partial correlation between bleed volume and coated platelet levels was almost identical to the bivariate correlation results ($r = -0.35; P = 0.03$).

Mortality recorded at 30 days was 22.2% (n = 10). Statistical analysis of coated platelet levels distributed as tertiles as a correlate of mortality at 30 days was performed using a 2-sided Pearson $\chi^2$ test. The results showed no difference ($P = 0.9$) among the lower (3 deaths), middle (4 deaths), and upper tertiles (3 deaths) of coated platelets. In addition, coated platelet levels were not different between the patients who died (n = 10) and those who survived (n = 35) at 30 days ($t(43) = 0.55, P = 0.59$, independent $t$ test).

### Table. Demographics, Coated Platelet Levels, and Relevant Medications of Patients With SICH

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Patients (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean±1 SD)</td>
<td>60.1±16.3</td>
</tr>
<tr>
<td>Gender, women/men</td>
<td>15/30</td>
</tr>
<tr>
<td>Race, black/white/other</td>
<td>12/30/3</td>
</tr>
<tr>
<td>Current smoking, smoker/nonsmoker</td>
<td>15/30</td>
</tr>
<tr>
<td>Pertinent medications</td>
<td></td>
</tr>
<tr>
<td>Statins, yes/no</td>
<td>10/35</td>
</tr>
<tr>
<td>SSRIs, yes/no</td>
<td>5/40</td>
</tr>
<tr>
<td>Antiplatelets, yes/no</td>
<td>13/32</td>
</tr>
<tr>
<td>Coated-platelet levels, % (mean±1 SD)</td>
<td>28.6±12.2</td>
</tr>
</tbody>
</table>

SSRI indicates selective serotonin reuptake inhibitor.

**Discussion**

Our initial investigation of coated platelet synthesis in SICH noted lower coated platelet levels shortly after the hemorrhagic event as compared with normal control subjects. This finding suggested that decreased coated platelet synthesis may be linked to the events leading to intracerebral hemorrhage. The current investigation indicates the existence of an inverse correlation between coated platelet levels and the bleed volume in SICH; lower levels of coated platelets correlate with larger bleed volume. These data are consistent with observations in experimental animals demonstrating that extremely low coated platelet production can result in a bleeding diathesis.

Recent studies by Naidech et al examining platelet reactivity in patients with intracerebral hemorrhage detected reduced platelet activity associated with early hematoma growth and worse functional outcome. Although it is tempting to correlate their findings with ours, the connection may be tenuous because of differences in methodology. Although Naidech et al estimated platelet reactivity with the Verify Now instrument, which measures platelet aggregation, our assays of coated platelet potential primarily measure the ability to generate prothrombinase activity. A recent publication reinforces the conclusion that aggregation and thrombin generation are distinct functions not measured by a common analytic technique. The relative importance of these different methodologies for quantitating events critical to SICH awaits additional studies.

We have not detected an association between mortality at 30 days and coated platelet levels. Potential explanations include the fact that our study was not powered to detect such an association and that bleed volume may not be the sole factor linked to death in SICH.

These current data together with our earlier study suggest that low levels of coated platelets are linked to the events leading to SICH and the severity of the hemorrhage. However, an inability to potentiate coated platelet production currently hinders an immediate extension of these findings to prevention and/or treatment studies. Further investigation of the role of coated platelets in SICH is warranted with the anticipation that a better understanding of the mechanisms involved will lead to more effective strategies for predicting risk for initial and recurrent events.

### Acknowledgments

We are indebted to Robert Cox and Paul Friese for their assistance with this project.

### Sources of Funding

This work was supported by the Department of Veterans Affairs and grants from the Oklahoma Center for Advancement of Science and Technology (C.I.P.) and the American Heart Association (G.L.D.).

### Disclosures

None.

### References


Coated Platelet Levels Correlate With Bleed Volume in Patients With Spontaneous Intracerebral Hemorrhage
Calin I. Prodan, Andrea S. Vincent and George L. Dale

Stroke. 2010;41:1301-1303; originally published online April 15, 2010; doi: 10.1161/STROKEAHA.110.581447

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/41/6/1301

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/