Patients With a Prolonged Bleeding Time and Normal Aggregation Tests May Have Storage Pool Deficiency: Studies on One Hundred Six Patients

By H. Karel Nieuwenhuis, Jan-Willem N. Akkerman, and Jan J. Sixma

One hundred six patients with storage pool deficiency (SPD) were studied with respect to platelet count, bleeding time, total platelet ATP and ADP, platelet serotonin, and in vitro aggregation. The diagnosis of SPD was made on basis of a prolonged bleeding time, a decreased total platelet ADP, and a diminished level of serotonin. Fifty-one patients of a prolonged bleeding time. a decreased total platelet ATP and ADP, and a diminished level of serotonin. Fifty-one patients with a prolonged bleeding time and normal aggregation tests may have hereditary or acquired SPD.

Patients was 18%, about one-half the frequency of von Willebrand's disease (vWd). Twenty-three percent of all patients had normal aggregation responses to ADP, epinephrine, and collagen; 33% had aggregation tracings typical for a secretion defect; and 44% had miscellaneous aggregation abnormalities. These findings indicate that SPD is common, heterogeneous, and not necessarily associated with in vitro aggregation abnormalities.

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NORMAL AGGREGATION TESTS IN SPD

RESULTS

Patients. The diagnosis of SPD was made in 106 patients (62 women, 44 men) on the basis of a prolonged bleeding time, a decreased level of total ADP, and decreased platelet serotonin (Fig 1). Fifty-one patients (30 women, 21 men) from 34 unrelated families had congenital SPD. In 7 patients (2 families), the defect was part of the Hermansky-Pudlak syndrome. In one family, the defect was associated with another congenital abnormality: SPD was found in a boy with severe hemophilia B and in his sister who was a carrier for hemophilia B. In 9 patients (6 families), congenital SPD was associated with mild thrombocytopenia (100,000 to 140,000 platelets/μL). In the other patients, the platelet disorder was the sole abnormality.

Fifty-five patients had acquired SPD. The diagnoses of the associated clinical disorders are given in Table 1.

Aggregation tests. ADP 5 μmol/L, epinephrine 1.0 μmol/L, and collagen 1.0 μg/mL induced irreversible aggregation tracings in 19 of 20 control subjects, with a maximal change in light transmission ranging from 50% to 100%. The pattern of aggregation in these 19 subjects was the reference for the classification of category A. If the patients’ platelets did not respond to low concentrations of the agonists, higher concentrations of collagen (4 μg/mL) and epinephrine (5 μmol/L) were also tested.

At first presentation, 13 patients with congenital SPD and 10 patients with acquired SPD had normal aggregation responses to ADP, epinephrine, and collagen (category A). Seventeen patients with congenital SPD and 17 patients with acquired SPD had a typical pattern of a secretion disorder (category B). Twenty-one patients with congenital and 24 patients with acquired SPD had atypical aggregation tracings (category C). All kinds of patterns were found in this category, eg, normal ADP aggregation with decreased epinephrine- and collagen-induced aggregation, abnormal ADP- and epinephrine-induced aggregation with normal response to low-dose collagen, decreased epinephrine-induced aggregation with normal ADP- and collagen-induced aggregation, or abnormal aggregation in response to a low dose of collagen with a normal response to ADP. The overall frequency of the different aggregation types at first presentation in 102 patients studied was: category A, 23%; category B, 33%, and group C, 44%.

To study the variability of the aggregation tests in congenital SPD, we compared the tracings of the first and second investigations. A consistent pattern of aggregation on repeated study was found in 11 of 13 patients (85%) in category A and in 11 of 17 patients (65%) in category B. Inconsistency among the aggregation studies was commonly found in category C. Only 9 of 21 patients (43%) were again classified in category C.

The parameters used in this study to diagnose SPD were also variable on repeated examinations. The bleeding time was performed 154 times in 51 patients with congenital SPD. A normal bleeding time was found 14 times. Total ADP and serotonin, both measured 117 times in these patients, were in the normal range 6 times and 20 times, respectively.

Normal aggregation tracings. The first patient with normal aggregation tests and SPD was found in 1980. She had a lifelong bleeding tendency and a prolonged bleeding time of 22 minutes. She was studied eight times over a period of 5 years, and the normal aggregation tracings and platelet abnormalities (platelet nucleotides, serotonin, bleeding time) were consistent. Deficiency of the dense granules in this patient was further confirmed by direct measurement of ATP and ADP in the granule compartment by digitonin-induced cell lysis as described previously.6

In two unrelated families, two members of each family had the combination of SPD, prolonged bleeding time, and normal aggregation studies. All 17 patients of category A on whom the tests were performed had a normal response to arachidonic acid and ionophore A23187.

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Table 1. Patients With SPD

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital</td>
<td>51</td>
</tr>
<tr>
<td>Acquired</td>
<td>55</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>5</td>
</tr>
<tr>
<td>Acute nonlymphocytic leukemia</td>
<td>5</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>8</td>
</tr>
<tr>
<td>Chronic granulocytic leukemia</td>
<td>7</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia</td>
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</tr>
<tr>
<td>Polycythemia vera</td>
<td>4</td>
</tr>
<tr>
<td>Essential thrombocythemia</td>
<td>6</td>
</tr>
<tr>
<td>Myelofibrosis</td>
<td>2</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>2</td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>1</td>
</tr>
<tr>
<td>Waldenstrom's macroglobulinemia</td>
<td>1</td>
</tr>
<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>2</td>
</tr>
<tr>
<td>Erythropoietic protoporphyra</td>
<td>1</td>
</tr>
<tr>
<td>Systemic lupus erythematosus and SLE-like disease</td>
<td>7</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>1</td>
</tr>
<tr>
<td>Disseminated intravascular coagulation</td>
<td>4</td>
</tr>
</tbody>
</table>

Abbreviation: SLE, systemic lupus erythematosus.

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Fig 1. Bleeding time (expressed in minutes), total ADP (in μmol/10⁹ platelets) and serotonin (in nmol/10⁹ platelets) in 106 patients with storage pool deficiency. Shaded areas are the normal ranges (mean ± 2 SD), which for bleeding time is 5.6 ± 2.6 (n = 61), for total ADP 4.0 ± 2.0 (n = 63), and for serotonin 370 ± 170 (n = 15). Patients with normal aggregation tests (O).
After 1980, we determined platelet nucleotides and serotonin in every patient with an unexplained prolonged bleeding time independent of the results of the aggregation tests. Excluding the results of patients diagnosed before 1980, we calculated that the frequency of normal aggregation tests in patients with congenital SPD is 31% (13 of 41 patients); in patients with acquired SPD is 20% (10 of 50 patients).

**Relative frequency of congenital SPD.** To define the relative frequency of congenital SPD, we reviewed the medical records of all 390 patients referred to our outpatient clinic for a bleeding tendency between January 1982 and December 1985. None of the patients had ingested drugs, nor did they have another disorder. A prolonged bleeding time associated with a normal platelet count was observed in 145 patients. In this group of 145 patients, vWd was diagnosed in 52 patients (36%), congenital SPD was diagnosed in 27 (18%), defect of thromboxane synthesis was diagnosed in 4 (3%), and platelet function disorders with miscellaneous aggregation abnormalities (secretion defect, defect of response to thromboxane A2, or other poorly defined aggregation abnormalities) was diagnosed in 23 (16%). We found no explanation for the bleeding tendency associated with a prolonged bleeding time in 39 patients (27%).

**DISCUSSION**

Patients with congenital or acquired SPD have a mild bleeding disorder associated with a prolonged bleeding time; their platelets are deficient in the nonmetabolic pool of ADP, which is stored in the dense bodies. We studied 106 patients with SPD; 23% of the patients had normal aggregation tracings.

These data provide a perspective on aggregation studies in SPD that differs completely from that of many previous reports and reviews. It is widely held that SPD platelets show a characteristic pattern of aggregation on stimulation in vitro: absence of second wave of aggregation with ADP and epinephrine and impaired response with collagen. In contrast, we found this pattern in only 33% of patients. The other patients had either normal aggregation responses or biphasic responses to at least one of the agonists. The high frequency of normal aggregation tests in this study may be a consequence of our approach to patients with a bleeding tendency and a prolonged bleeding time; assays for platelet nucleotides and serotonin were performed independently of the results of the aggregation tests.

In this study, we defined the pattern of the irreversible aggregation tracing with a change of light transmission of >50% as "normal." This definition implies that one can sometimes observe "abnormal" aggregation tests in a normal population; in this study, one of 20 subjects, and in a previous study, 8 of 80 controls had reversible tracings. If one were also to classify these patterns as normal, even more SPD patients may be classified as normal aggregation tests.

To evaluate the aggregation tests in a well-defined group of patients, we required abnormalities of all three parameters (bleeding time, total ADP, and serotonin) on the first examination for the diagnosis of SPD. The variability of the laboratory findings in the patients with congenital SPD indicates, however, that more patients with a mild bleeding disorder may have SPD. Repeated testing may be required to establish the diagnosis SPD in these subjects. The same problem was described by Abildgaard and colleagues in the diagnosis of vWd.

Normal aggregation tracings or minimal aggregation abnormalities have been described previously in a few patients with congenital SPD, but not in acquired SPD. Lages and Weiss reported two patients with SPD in whom biphasic aggregation responses to ADP and epinephrine consistently were observed. Platelets from these patients secreted greater quantities of ADP after stimulation by epinephrine than did SPD platelets with comparable ADP deficiencies and absent second-phase aggregation. In addition, their platelets were extremely sensitive to stimulation with ADP. These two mechanisms and endoperoxide- or thromboxane-mediated aggregation could contribute to the biphasic aggregation.

In the present study, we observed normal aggregation tracings in patients with excessively long bleeding times and manifest bleeding symptoms, indicating that in many patients no correlation exists between in vitro aggregation and the in vivo situation. We found no correlation between the categories A, B, and C and clinical manifestations of bleeding.

We conclude that SPD is common, is heterogeneous, and is not necessarily associated with in vitro aggregation abnormalities. Thus, SPD cannot be excluded with simple aggregation studies. These findings will change the approach to the bleeding patient with a prolonged bleeding time.

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