CONCISE REPORT

Quantitation of Platelet-Associated IgG by Radial Immunodiffusion

By Bernard S. Morse, Dennis Giuliani, and Murray Nussbaum

Platelet-associated IgG (PAIgG) was measured by a simple radial immunodiffusion technique using washed solubilized platelets and commercially available immunoplates. Subjects with normal platelet counts had PAIgG levels of 1.5-7.0 fg/platelet. Subjects with idiopathic immune thrombocytopenic purpura (ITP) had levels ranging from 5.7 to 70.5 fg/platelet. All patients with recurrent ITP and 85% of patients with acute ITP had elevated PAIgG.

CURRENT methods of measuring platelet-associated IgG (PAIgG) are complex and limited in their availability to a few research centers. We have simplified the quantitation of PAIgG by using a radial immunodiffusion technique with commercially available immunoplates and have determined the levels in a variety of disorders in which platelet antibodies were suspected. The results obtained by this technique are comparable to those of the other more complex methods.

MATERIALS AND METHODS

After obtaining informed consent, 10-30 ml of blood were collected in EDTA or ACD tubes from 20 normal subjects and 72 patients with thrombocytopenias of varying etiology. Samples were processed within 24 hr of collection. A platelet button was obtained by first centrifuging the blood specimen at 20°C and 207 g for 10 min, removing the platelet-rich plasma, and then centrifuging it at 20°C and 2000 g for 5 min. The platelet button was resuspended in 1 ml of aqueous 1% ammonium oxalate for 10 min; 0.1 ml of aqueous saturated sucrose solution was added and the remaining plasma was removed by applying the entire mixture to a 1 x 10 cm column of sepharose 2B, equilibrated with phosphate-buffered saline (PBS). Platelets and contaminating leukocytes were found in the turbid-appearing void volume. The total number of platelets and leukocytes in the effluent was counted by electronic particle counting (Coulter Thrombocounter and D2, respectively) in an aliquot of the suspension. Specimens containing platelets of less than 25,000/μl were recounted using a 20-μl aliquot. Because contaminating leukocytes were less than 1%, a 200-μl aliquot was routinely used for the leukocyte count. Specimens containing more than 4% leukocytes were discarded. Alternatively, plasma contamination could be removed by washing the platelet button 5 times at 20°C in 16 x 95 mm polystyrene tubes with phosphate-buffered saline (PBS) containing 0.01 M EDTA. Specimens were divided into aliquots containing 10⁶ platelets. In severely thrombocytopenic patients, as few as 0.25 x 10⁶ platelets could be used.

After resuspension in PBS, the platelets were again centrifuged at 20°C and 2000 g for 5 min and the supernate discarded. The tubes were inverted and allowed to drain for several minutes. The interior of each tube was then carefully dried with cotton swabs. At this point, the platelet button could be analyzed for IgG or stored at -20°C.

When ready for analysis, the platelet button was solubilized by the addition of 10 μl of 1% Triton X-100 in PBS and by gentle sonication for several minutes. The entire specimen was then transferred to a well of a low level IgG immunoplate (LC-Partigen, Calbiochem-Behring). It was determined that 90% of the solubilized platelet material was thus transferred to the well when this procedure was tested with added 125I serum albumin. Appropriate IgG standards were added to additional wells, and diffusion was then allowed to proceed for 60 hr at room temperature. The diameters of the immunodiffusion rings were measured with a micrometer eyepiece. Calibration curves were constructed relating the standard IgG concentration to the area of the immunoprecipitate ring. Serum IgG levels were measured by a nephelometric method.

RESULTS

Initially, specimens were analyzed in duplicate on separate immunoplates, resulting in an insignificant mean percentage difference in ring diameters of 3.4%. No differences were detected using blood collected in EDTA or ACD if platelets were stored or analyzed within 24 hr of collection. Since the low-level IgG plates are standardized to quantitate 160-2600 ng of IgG, the lowest level of detection is 1.6 fg IgG/platelet for 10⁶ platelets, 3.2 fg for 0.5 x 10⁶ platelets, and 6.4 for 0.25 x 10⁶ platelets.

Figure 1 illustrates the values obtained from the 20 normal subjects with normal platelet counts and from the 71 patients with various illnesses. The normal subjects with normal platelet counts had PAIgG levels ranging from 1.5 to 7.0 fg/platelet (mean 3.8 ± 1.9). Twenty-seven patients with immune thrombocytopenia had PAIgG levels ranging from 5.7 to 70.5 fg/platelet, with 17% of patients having levels greater than 70.5 fg/platelet. Elevated PAIgG was also found in 17% of patients with recovered ITP, 40% of patients with SLE and thrombocytopenia, 57% of patients with thrombocytopenia occurring during the course of septicemia, and 100% of patients with IgG myeloma in whom the serum IgG level was clearly elevated, regardless of the platelet count. The results are similar to reports that used more complex techniques.

Supported in part by a grant from the American Heart Association, New Jersey Affiliate.

Submitted November 20, 1980; accepted December 11, 1980.


Address reprint request to Dr. Bernard S. Morse, Monte-Scaigione Memorial Laboratory, Division of Hematology, College of Medicine and Dentistry of New Jersey, the New Jersey Medical School, Newark, N.J.

© 1981 by Grune & Stratton, Inc.
0006-4971/81/5704-0029$01.00/0
platelet (mean 20.3 ± 15.7). PAIgG levels were elevated in 11 of 13 patients with acute ITP and all 14 patients with chronic or recurrent ITP. The means for acute ITP and recurrent or chronic ITP were 21.2 and 19.4, respectively. The platelet counts and PAIgG levels before and after treatment of 10 patients with various forms of immune thrombocytopenia are summarized in Table 1. All 10 patients, regardless of the form of therapy, showed a drop of PAIgG as platelet counts improved. Only 1 of 6 patients (17%) with recovered ITP showed an elevated value of 10.7 fg/platelet. None of these patients had received therapy for at least 6 mo. Twelve patients with nonimmune thrombocytopenia of varying etiologies showed PAIgG levels ranging from 1.6 to 16.7 fg/platelet (mean 6.2 ± 5.3). This group included 5 patients with acute leukemia, 2 patients with megaloblastic anemias (1 due to folate deficiency, the other had pernicious...

Table 1. Platelet IgG Levels in Immune Thrombocytopenia: Response to Treatment

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Platelet Count (cu mm)</th>
<th>Platelet IgG (fg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Acute ITP</td>
<td>Prednisone</td>
<td>20,000</td>
<td>252,000</td>
</tr>
<tr>
<td>Acute ITP</td>
<td></td>
<td>15,000</td>
<td>280,000</td>
</tr>
<tr>
<td>Recurrent ITP</td>
<td>Prednisone</td>
<td>50,000</td>
<td>190,000</td>
</tr>
<tr>
<td>Recurrent ITP</td>
<td></td>
<td>100,000</td>
<td>240,000</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>Prednisone</td>
<td>10,000</td>
<td>360,000</td>
</tr>
<tr>
<td>SLE</td>
<td>Prednisone</td>
<td>100,000</td>
<td>250,000</td>
</tr>
<tr>
<td>Acute ITP</td>
<td>Splenectomy</td>
<td>39,000</td>
<td>345,000</td>
</tr>
<tr>
<td>Recurrent ITP</td>
<td>None</td>
<td>80,000</td>
<td>140,000</td>
</tr>
<tr>
<td>Heparin</td>
<td>D/C heparin</td>
<td>112,000</td>
<td>400,000</td>
</tr>
<tr>
<td>Tolazamide</td>
<td>Prednisone</td>
<td>5,000</td>
<td>50,000</td>
</tr>
<tr>
<td></td>
<td>D/C tolazamide</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
anemia), 2 patients with cirrhosis of the liver, and 3 patients with myeloproliferative disorders (1 polycythemia and 2 with myelofibrosis). Elevated PAIgG levels were found in 1 of the patients with acute leukemia and in the 2 patients with megakaryoblastic anemias. The PAIgG levels of the ITP patients were significantly different when compared to normals and the patients with nonimmune thrombocytopenia ($p < 0.001$). No significant difference was noted between PAIgG levels of normals and the levels of the nonimmune thrombocytopenia group ($p = 0.4$). Serum IgG levels were normal in the above 3 groups.

Two of 5 (40%) of patients with SLE and thrombocytopenia and 1 of 3 (33%) of SLE patients with normal platelet counts had elevated PAIgG. Seven myeloma patients with elevated levels of IgG had elevated PAIgG levels. Only 1 showed a low platelet count of 80,000 cu mm. Two patients with IgA myeloma and 1 patient with kappa light-chain myeloma had normal PAIgG. Four of 7 patients (57%) with thrombocytopenia, noted during the course of sepsis, had elevated PAIgG levels. Four patients with idiopathic autoimmune acquired hemolytic anemias showed normal PAIgG levels.

**DISCUSSION**

Currently available techniques for quantitating PAIgG, including Fab anti-Fab, antiglobulin consumption, radioactive labeled Coomb’s test, and $^{125}$I-labeled staph protein A binding, are complex and require rigid standardization. It has been suggested that a few regional centers be utilized for the investigation of patients suspected of having platelet antibodies. Quantitation of PAIgG by radial immunodiffusion obviates this restriction because of its relative simplicity. Immunoplates are commercially available and have a shelf life of 1 yr. The minimal requirement of 160 ng of IgG is easily met by obtaining $0.25 \times 10^8$ platelets from patients with ITP even with very low platelet counts. Ten to 30 milliliters of whole blood is sufficient for most situations. Platelets can be analyzed immediately or stored frozen at $-20^\circ$C.

The results of the present method and their clinical correlation are similar to the reports utilizing various other techniques. In the present study, 85% and 100% of patients with acute and recurrent ITP, respectively, had elevated PAIgG. Similarly, elevations of PAIgG were reported in 76%–100% of cases of ITP using the antiglobulin consumption test, and $^{125}$I using staph protein A binding, in 90% using the radiolabeled Coomb’s test, and in 94% using the Fab anti-Fab method. Elevated PAIgG levels were also found in all of our patients with IgG myeloma regardless of the platelet count, confirming the observation of McGrath et al. and emphasizing the necessity for measuring serum IgG levels in patients with hypergammaglobulinemia and elevated PAIgG levels. This study also found elevated PAIgG levels in patients with sepsis and thrombocytopenia (57%) and in patients with SLE and thrombocytopenia (40%), as has been reported by others.

The measurement of PAIgG by radial immunodiffusion is adaptable to most clinical laboratories. Hopefully, this technique will provide wider experience with PAIgG measurement and allow further insight into its clinical significance.

**ACKNOWLEDGMENT**

The authors wish to thank the Calbiochem-Behring Corporation for the generous supply of LC-Partigen immunoplates.

**REFERENCES**

Quantitation of platelet-associated IgG by radial immunodiffusion

BS Morse, D Giuliani and M Nussbaum