A Prospective Study of the Usefulness of the Measurement of Platelet-Associated IgG for the Diagnosis of Idiopathic Thrombocytopenic Purpura

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The measurement of platelet-associated IgG (PAIgG) is a potentially useful diagnostic test for idiopathic thrombocytopenia purpura (ITP). However, the restricted application of PAIgG measurements to thrombocytopenic populations primarily comprised of ITP patients will artificially enhance its diagnostic specificity. For this reason, we performed a prospective study in which the results of a sensitive technique for quantitating PAIgG were related to the cause of the thrombocytopenia. Over a 1-yr period, clinicians were invited to submit patient blood samples encompassing a wide spectrum of thrombocytopenic disorders as possible for PAIgG measurements. The physician was then contacted and requested to indicate the likeliest cause for the thrombocytopenia. The PAIgG was elevated in only 24 of 254 samples obtained from nonthrombocytopenic patients. In contrast, 134 (79%) of the 169 thrombocytopenic patients had elevated PAIgG results, and the increased levels were apparent in all diagnostic categories. The sensitivity of the PAIgG test for clinically diagnosed idiopathic thrombocytopenic purpura was 91% and the specificity was 27%. The positive predictive value for a raised PAIgG as a diagnostic test for ITP in a thrombocytopenic patient was only 46%, while the negative predictive value was 82%. This study indicates that the presence of increased PAIgG provides little additional information in the diagnosis of ITP. This study also suggests that immune mechanisms may mediate many more thrombocytopenic disorders than have been previously thought likely.

INITIAL RESULTS of the application of new diagnostic tests are often favorable, but more extensive clinical evaluation is required to obtain a true diagnostic perspective. For example, the diagnosis and classification of hemolytic anemias was greatly facilitated by the development of the direct antiglobulin test (DAT). The DAT allowed the delineation of hemolytic anemias into those of apparent immune and nonimmune etiology. For a number of years, a positive DAT was equated with hemolysis until clinicians began to perform red cell survivals in certain patients and it was recognized that a positive antiglobulin test did not always indicate hemolysis.

Idiopathic thrombocytopenic purpura (ITP) may be considered analogous to autoimmune hemolytic anemia, except the platelets are the target of the autoantibodies. There has been difficulty in developing a simple sensitive diagnostic test similar to the DAT for studying the binding of antibody to platelets; however, in recent years several techniques that measure platelet-bound immunoglobulin have been reported. These assays have shown that platelet-bound IgG is increased in approximately 90% of thrombocytopenic platelets with ITP when compared with the laboratory's normal ranges. These results suggest that the measurement of PAIgG was a particularly useful test for diagnosing ITP. As often happens with a new test, the patient population chosen to examine the sensitivity and specificity was primarily restricted to the condition that provided the rationale for the development of the test. This process can potentially lead to artificially favorable results, and when the test is applied to a wider spectrum of clinically and biologically related conditions, it may be shown to be less specific. Recently, one group of investigators measured platelet-bound immunoglobulins in other thrombocytopenic disorders and raised questions about the specificity of the test. For these reasons we performed a prospective study in which the results of a sensitive quantitative assay for platelet-associated IgG was related to the etiology of a variety of thrombocytopenic disorders.

MATERIALS AND METHODS

Study Design

For a 1-yr period, clinicians were invited to submit patient blood samples for analysis. The participating physicians were requested to include as wide a spectrum of thrombocytopenic disorders as possible. Two samples were solicited. One for whole blood platelet count, and the other for measuring of platelet-associated IgG (PAIgG). After the sample was analyzed, the physician managing the patient was contacted by letter and requested to indicate the likeliest cause for the thrombocytopenia. The result of the PAIgG assay was not used in designating the etiology of the thrombocytopenia.
Methods

Whole blood was collected into ACD (6:1, v:v) and the platelets isolated using differential centrifugation. The amount of platelet-associated IgG was determined on three times washed platelets using an immunoradiometric assay.\(^1\) One-hundred microliters of varying dilutions of \(^{125}\)I-anti-IgG were incubated with 100 \(\mu\)l of \(^{125}\)I-anti-IgG diluted in 5% normal sheep serum and phosphate-buffered saline, 0.15 \(M\), pH 7.4. This antiserum was Fc monospecific and had activity against all subclasses of IgG. The subclass specificity was demonstrated using monoclonal IgG from patients with multiple myeloma. The anti-IgG was radiolabeled using the chloramine-T method and had a specific activity of 15 Ci/mg protein. Following incubation for 30 min at 37\(^{\circ}\)C, the unbound \(^{125}\)I-anti-IgG was measured by the addition of 100 \(\mu\)l IgG beads containing \(10^8\) beads. These beads were produced by covalent binding of pooled human IgG (Sigma Chemicals, St. Louis, Mo.) with Sephasorb beads (Pharmacia, Dorval, Quebec) using carbonyl-dimidazole. This concentration of IgG-beads could bind all available \(^{125}\)I-anti-IgG in 30 min at 37\(^{\circ}\)C. Control beads, which were handled identically but not labeled with IgG, bound less than 1%–2% of the \(^{125}\)I-anti-IgG.

After a further incubation for 30 min at 37\(^{\circ}\)C, the \(^{125}\)I-anti-IgG/IgG-beads were separated from the platelet fraction by centrifugation across a Ficoll-Hypaque gradient (6%:10%). Less than 2% of the platelets crossed this barrier. The concentration of platelets that inhibited 50% of the binding of the \(^{125}\)I-anti-IgG to the IgG-beads was related to the concentration of IgG standard, which produced an equal inhibition of binding.

The coefficient of variation of this assay was \(\pm 7\%\). As a control for the function of the assay, positive and negative laboratory-produced platelet controls were included on each test run.\(^2\) Positive controls were made by incubating washed group O platelets (500,000/\(\mu\)l) fixed with 2% formalin with an equal volume of 10 mg/ml human IgG, Cohn fraction II (Sigma Chemical Co., St. Louis, Mo.). Negative control platelets were made in the same way except the IgG was replaced by buffer.

In order to minimize sampling bias, an attempt was made to test all samples in a consecutive fashion. There were, however, samples that had to be excluded. The samples referred in from other cities were excluded because of the time interval between the collection of sample and measurement of PAIgG. Other exclusions included patients in whom a simultaneous platelet count was not performed, or the clinician did not indicate the cause of the thrombocytopenia. Some patients had multiple PAIgG assays performed, since certain physicians used this test to follow patient response to treatment, and in these cases only the initial sample was considered for analysis.

RESULTS

The normal level of platelet-associated IgG for our laboratory is less than 3 fg IgG/platelet, which is 2 SD above the mean result for 30 tests performed on different healthy nonthrombocytopenic individuals. The mean \(\pm\) SD and SE was 1.8, 0.6, 0.1 fg IgG/platelet and the mean age for the individuals was 31 \(\pm\) 6.2 yr. An illustrative standard curve using 2 different dilutions of anti-IgG is shown in Fig. 1. Over the 12-mo study period, a total of 859 samples were tested. Thirty-eight results were not analyzed because of technically inadequate tests. A further 107 results were excluded since they were out-to-town samples. In addition, there were 187 samples that were excluded because a simultaneous platelet count was not performed or the cause of the thrombocytopenia was not stated. Two-hundred and fifty-four samples were obtained from patients, of whom 22 had a previous history of idiopathic thrombocytopenic purpura or a connective tissue disorder. The remaining patients were thrombocytopenic. The results of the first PAIgG test performed on these patients is shown in Table 1. An elevated PAIgG had a strong association with decreased platelet count, irrespective of the etiology; 134 of 169 thrombocytopenic patients had elevated results compared with 24 of 252 nonthrombocytopenic patients, \(p < 0.005\) \((\chi^2)\). The results of the PAIgG test in patients with clinically diagnosed ITP, and in thrombocytopenic patients who were considered on clinical grounds not to have ITP, are shown as Table 2. The function of this table is to determine if the PAIgG test should be regarded as both sensitive and specific.
for ITP compared to other thrombocytopenic disorders. The sensitivity of the PAIgG test was 91% and the specificity was 27%. The positive predictive value for a positive PAIgG test for ITP in a thrombocytopenic patient was 46%. In contrast, the negative predictive value was 82%, indicating that a negative PAIgG test in a thrombocytopenic patient makes ITP unlikely.

**DISCUSSION**

This prospective study confirms that the measurement of platelet-associated IgG (PAIgG) is highly sensitive (91%) for the diagnosis of patients with idiopathic thrombocytopenic purpura. However, this study also indicates that a positive PAIgG test is a relatively nonspecific finding, and there are increased amounts of platelet-bound IgG in a wide spectrum of thrombocytopenic disorders.

A newly developed technique was used to directly quantitate platelet-associated IgG. However, the results we obtained cannot be attributed to potential artifacts of measurement. This method, like most techniques that quantitate platelet-bound immunoglobulin, depends on immunologic recognition of platelet-bound IgG. A standard curve with known concentrations of IgG was performed each time a test sample was assayed. The amount of radiolabeled anti-IgG that bound to the test platelets was related to an equal amount of binding of the anti-IgG to the IgG standard. Thus, the principle is similar to the standard antiglobulin consumption assay, a technique that has been validated by several different laboratories.

Although this study demonstrates that the measurement of platelet-associated IgG has a low specificity for the diagnosis of ITP, it does not provide information concerning the biologic or clinical implications of the finding of elevated platelet-bound immunoglobulin. It is possible that immune mechanisms mediate many more thrombocytopenic disorders than previously has been thought likely. Indeed, some and perhaps many of the clinical disorders listed in Table 1 could be mediated by immune mechanisms. For example, there is indirect evidence that pre-eclampsia is a disorder of altered immune tolerance, and it is possible that the elevated PAIgG reflects platelet-bound immune complexes that can occur in this disorder. Similarly, immune complexes have been reported in serum from patients with thrombotic thrombocytopenic purpura and leukemia, and there is evidence of increased platelet turnover in both of these disorders. However, in the absence of the performance of platelet survivals in these patients, the sensitivity and specificity of PAIgG for these thrombocytopenic states cannot be evaluated. Other investigators have reported that PAIgG is usually not elevated in “nonimmune thrombocytopenic” disorders. The reasons for the apparent discrepancy of results between studies is uncertain, but could in part reflect our inclusion of many thrombocytopenic disorders that are likely to be mediated by immune mechanisms, for example, the thrombocytopenia complicating bacterial septicemia.

The sensitivity and specificity of a test is proportional to the prevalence of disorder in the series exam-
ned. Ideally, this study should have evaluated every thrombocytopenic patient present in the hospital. This would have included a significantly higher proportion of patients with hematologic malignancies and chemotherapy-induced thrombocytopenia. Examination of Table 1 indicates that the inclusion of more of these patients would have led to a further lowering of specificity. However, in clinical practice, the diagnostic performance of the test is influenced by appropriate patient selection and hence the group studied in our series is an attempt to give a perspective.

This study indicates that the measurement of PAIgG provides limited additional information in the diagnosis of ITP. A positive result does not confirm the diagnosis, although a negative result makes the diagnosis much less likely. This study, however, does not indicate that PAIgG measurements should not be performed, and its suggests that platelet-bound IgG could mediate many more thrombocytopenic disorder than was originally thought likely. It is possible that the measurement of platelet-bound immunoglobulin taken in conjunction with the results of platelet survivals and other in vivo and in vitro studies will lead to a better understanding of the pathophysiology of various thrombocytopenic disorders.

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