A Radiolabeled Antiglobulin Test for Crossmatching Platelet Transfusions

By Thomas S. Kickler, Hayden G. Braine, Paul M. Ness, Alice Koester, and Wilma Bias

Despite the use of HLA-matched platelets for alloimmunized recipients, transfusion failures occur. In order to reduce these failures, we investigated the use of a radiolabeled antiglobulin technique for platelet crossmatching. The principle of the test is that of an indirect Coombs test using labeled goat anti-human IgG. Incompatibility is determined by calculating a radioactivity antiglobulin test (RAGT) index. Using this technique, we performed 89 crossmatches on 19 leukemic or aplastic patients who were refractory to random donor platelets and receiving varying degrees of HLA-matched platelets. Effectiveness of the transfusion was assessed from the posttransfusion corrected platelet count increment (CCI) determined at 1 and 20 hr. When the RAGT index was 1.9 or less, the mean CCI at 1 hr was 17,570 ± 7003/cu mm, n = 55. When the RAGT index was 2.0 or greater, the mean CCI was 4237 ± 4100/cu mm, n = 34. At 20 hr when the RAGT index was 1.9 or less, the mean CCI was 8722 ± 3143/cu mm, n = 33, and when the index was 2.0 or greater, the mean CCI was 5712 ± 1286/cu mm, n = 23. Using this technique, one false negative resulted. Nine positive crossmatches with good increments at 1 hr were found; at 20 hr, however, the survival of these units was zero. These data suggest that this method is a useful adjunct in the selection of platelets in the refractory patient.

THROMBOCYTOPENIC PATIENTS receiving multiple transfusion can often become alloimmunized, resulting in refractoriness to random donor platelet transfusions. These refractory patients can then effectively be transfused with HLA-matched or selectively mismatched platelet.3,2 Despite close HLA matching, many of these selected platelet transfusions are failures. The immunologic causes of these failures may be unidentified HLA incompatibilities, non-HLA antibodies, or incompatibility in the HLA Bw4/Bw6 system of public specificities.3

In order to select the most compatible platelet transfusions for alloimmunized recipients, in addition to HLA matching, several laboratory techniques have been investigated. Earlier methods have included crossmatching by lymphocytotoxicity,4 platelet aggregation,5 complement fixation,6 and serotonin release,7 These procedures have generally proven nonreproducible, insensitive, or technically impractical.5-10 In the last few years, a number of sensitive procedures have been developed to measure platelet antibodies. These have included immunofluorescence assays,11,12 radiolabeled antiglobulin tests,13,14 enzyme immunoassays,15 and complement lysis inhibition assays.16 Many of these methods were developed initially to detect autoantibodies but also now have been shown sensitive in detecting platelet alloantibodies. Brand et al., using a qualitative immunofluorescence procedure along with lymphocytotoxicity crossmatching, were able to predict 93% of platelet transfusions correctly.11 The utility of their method is lessened by its subjectivity and the requirement of technology generally not available to blood banking facilities. More recently, Myers et al. have described a quantitative immunofluorescence procedure that yields an objective determination of incompatibility.12 In a preliminary evaluation of this method, their testing correctly predicted platelet survival in 15 of 17 transfusions. The predictive value of the complement lysis inhibition tests and enzyme immunoassay procedure in selecting platelet transfusions has not been studied.

In this study, we evaluated a radiolabeled antiglobulin test for platelet crossmatching as an adjunct to the use of HLA-matched donors. This method was developed by Cines and Schreiber for detecting platelet autoantibodies. We extended the technique to platelet crossmatching because of its simplicity and adaptability to routine clinical use.

MATERIALS AND METHODS

Patients Studied

Nineteen patients refractory to random platelet transfusion were studied (see Table 1). These patients had an extensive history of transfusions with red cells, random donor platelets, and (in many cases) granulocyte transfusions. All patients had severe bone marrow hypoplasia with baseline platelet counts of 10,000/cu mm or less.

The patients were considered refractory when on at least two occasions they had less than 5% of expected increment to 6 or more units of random platelets.

Patients were excluded from the study on the basis of overwhelming sepsis, palpable splenomegaly, disseminated intravascular coagulation (documented by the presence of fibrin split products), previous history of autoimmune thrombocytopenia, and the administration of antilymphocyte globulin.
PLATELET CROSSMATCHING

Table 1. Patients Studied

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>HLA Phenotype</th>
<th>Lymphocytotoxic Antibody Levels*</th>
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AA, aplastic anemia; AML, acute myelogenous leukemia; BMT, bone marrow transplant; WCC, white cell concentrates.

*Percentage of cells on lymphocyte panel positive with a patient’s serum.

Effectiveness of Transfusion

Effectiveness of the transfusion was assessed from the posttransfusion platelet increment determined at 1 hr and also at 20 hr, in most cases.17 This increment was calculated as follows:

\[
\text{Posttransfusion platelet count} - \text{Pretransfusion count} \times \frac{\text{Body surface area}}{10^{12}}
\]

For a satisfactory transfusion outcome, our major criterion is a corrected platelet count increment at 1 hr of greater than 7500/cu mm. For 20-hr survival, a corrected count increment of greater than 4500/cu mm was considered adequate. All platelet counts were counted by phase-contrast microscopy.

Platelet Transfusions

Eighty-nine single donor platelet concentrates were obtained from a pool of HLA-typed donors at the Johns Hopkins Oncology Center, Chesapeake Regional Red Cross, and Southeast Wisconsin Blood Center. The recipients’ relatives were infrequent donors (only two transfusions given). No patients received repeat transfusions from the same donor. A and B matched platelets were used when available, otherwise C and D matched platelets were selected, avoiding HLA types against which the presence of an antibody was suspected. ABO-incompatible platelets were transfused on four occasions when ABO-compatible donors were not available. All platelets were collected using a Haemonetics Model 30. After collection, erythrocytes were removed from the platelet products by centrifugation at 22°C for 4 min at 1000 rpm (Sorvall RC-3). This also reduced mononuclear cell contamination to less than 10% unstimulated.

HLA Typing and Lymphocytotoxic Antibody Testing

HLA typing and screening of patients’ sera for HLA antibodies were done by standard microlymphocytotoxicity technique. The typing antisera were used against the following antigens: A1, A2, A3, A9, A10, A11, Aw19, Aw23, Aw25, Aw26, Aw28, Aw29, Aw30, Aw31, Aw32, Aw33, Aw34, Aw36, B5, B7, B8, B12, B13, B14, B15, Bw16, B17, Bw18, Bw21, Bw22, B27, Bw35, B37, Bw38, Bw39, B40, Bw41, Bw42, Bw44, Bw45, Bw47, Bw48, Bw49, Bw50, Bw51, Bw52, Bw53, Bw54, Bw55, Bw56. The degree of HLA match was categorized as follows. A—Identity of all HLA-A and B antigens. B—Three donor antigens match, the fourth antigen is unknown or cross-reactive; two donor antigens match, third and fourth donor antigens are unknown or cross-reactive. C—Three donor antigens match; one donor antigen is mismatched. D—Two or more donor antigens are mismatched.

Cross-reactivities between HLA antigens were defined according to Terasaki et al.18 Lymphocytotoxic crossmatch was performed by the standard microlymphocytotoxicity technique. Greater than 20% lymphocytotoxicity was considered an incompatible crossmatch. This procedure was performed only on the platelets collected at our institution.

Platelet Crossmatching Procedures

The assay procedure is an indirect radiolabeled antiglobulin test similar to the method of Cines and Schreiber.15 Donor platelets were obtained from the pheresis bag segments for use in the crossmatch test. An affinity-purified goat anti-human IgG was obtained from Kierkegaard-Perry (Gaithersburg, Md.) and was radiolabeled with 125I.19 On immunoelectrophoresis, the antibody reacted specifically with human IgG.

The test consists of a sensitization phase in which 108 platelets are incubated in 12 x 75 mm plastic tubes with 1 ml of patient plasma for 30 min at 37°C. After washing the sensitized platelets 4 times with Tyrode’s buffer, radiolabeled anti-human globulin is added, and the mixture is again incubated for 30 min at 37°C. After washing the platelets 4 times to remove the excess antoglobulin reagent, the platelet’s radioactivity is counted. All assays are run in duplicate and the results averaged.

Pooled plasma from untransfused AB males is used as negative controls and crossmatched against three platelet donors tested in duplicate. The results of the test crossmatch are expressed as a ratio of test counts per minute (cpm) to the average cpm of the negative controls. This ratio is expressed as the radiolabeled antiglobulin test
index (RAGT index). A RAGT index greater than or equal to 2.0 is considered a positive test; a RAGT index of 1.9 or less is considered a negative crossmatch test. When 80 untransfused male blood donors were studied, the average RAGT index was 1.08 ± 0.3 (1 standard deviation). Hence, an RAGT index of 2 or greater is 3 standard deviations above normal. The coefficient of variation of the procedure done on 20 duplicate pairs was 10%.

When investigated, we found no difference in results if the platelets had been stored for 8 wk at 4°C in Tyrode’s buffer with 0.1% sodium azide.

RESULTS

One Hour Survival Prediction by the Crossmatch

The RAGT crossmatch was evaluated in 89 platelet transfusions administered to 19 patients. The result of the test were compared to the 1-hr corrected platelet count increments in Fig. 1. When the RAGT index equaled or exceeded 2.0, the mean corrected count increment was 4231 ± 4100/cu mm, n = 34. When the Index was 1.9 or less, the mean corrected count increment was 17,570 ± 7003/cu mm, n = 55 (p < .001). The association between the RAGT index and outcome of transfusion was significant at p < 0.005 using a χ² test.

Of the 26 transfusion failures, only one was not correctly predicted. There were 63 transfusions with satisfactory 1-hr increments. Fifty-four of these had negative crossmatches. Of the other 9 transfusions with good 1-hr increments but positive crossmatches, none had surviving platelets at 24 hr.

The relation of a positive RAGT crossmatch and degree of HLA match is noted in Fig. 2. In the 14 transfusion failures using “C” and “D”-matched platelets, 13 of them were predicted in vitro. With the “B”-matched platelets, all 11 failures were detected by the crossmatch.

Twenty-Hour Survival Prediction by the Crossmatch

Corrected platelet count increments at 20 hr were available for 56 transfusions. The other 33 transfusions were given as outpatients. Although 20-hr posttransfusion platelet counts were not done, those patients with normal 1-hr posttransfusion increments manifested normal hemostasis for the 4–7-day interval between clinic visits.

When the RAGT index equaled or exceeded 2.0, the mean corrected count increment at 20 hr was 571 ±
1286/cuu mm, \( n = 23 \). When the index was 1.9 or less, the mean corrected count increment was 8722 ± 3143, \( n = 33 \) (see Fig. 3).

There was one transfusion failure not predicted by the crossmatch test, the same transfusion failure not predicted by the 1-hr increment criterion. There were no false positive crossmatch results using the 20-hr increment. In contrast, when we compared the crossmatch results to the increments at 1 hr, of the 9 false positives (determined by 1-hr increment), 9 of these had no circulating platelets at 20 hr.

Only 4 ABO-incompatible transfusions were administered. All 4 had good 24-hr survival.

**Lymphocytotoxicity Crossmatch Results**

We found the lymphocytotoxicity crossmatch technique unsatisfactory for predicting the outcome of transfusion. Sixty-three units were crossmatched. Of the 15 transfusion failures, 4 positive crossmatches were observed for a predictive value of 25%. Of the 48 effective transfusions, 11 had positive crossmatches for a predictive value of 23%. The combined error rate for lymphocytotoxicity crossmatching was 35%.

**DISCUSSION**

Selection of platelet transfusions for the alloimmunized recipient has relied heavily on HLA matching. Despite this approach, transfusion failures still occur in the absence of other factors known to decrease platelet survival, suggesting an immune basis. These in vivo factors have generated many searches for an in vitro test to predict the transfusion outcome. Lymphocytotoxicity crossmatching has been proven to be of low predictive value for the outcome of platelet transfusion.\(^{10,20}\) False negative (negative crossmatches but poor transfusion outcome) have been shown to be as high as 40%.\(^{20}\) We also have confirmed the inadequacy of lymphocytotoxicity crossmatching. Other compatibility techniques have also been shown to have many false negative test results. Serotonin release demonstrated 60% false negative reactions, and platelet factor 3 release produced false negative test results in 76% of transfusions studied.\(^{10}\) Platelet aggregometry has produced conflicting data. Wu et al. reported that aggregometry was successful in selecting compatible platelets in 37 cases with only 3 false negatives and/or false positives. Filip et al., however, demonstrated an 83% false negative rate.\(^{10}\) In addition to the low predictive value of these methods, there is also the short-coming that fresh platelets are required to perform the assay.

The ideal platelet crossmatching procedure would utilize a stored bank of donor platelet samples to select the appropriate donor for subsequent plateletpheresis. Hence, an in vitro test that does not depend on cell viability but intact cells would simplify the logistic demands of supporting alloimmunized patients. Many of the new techniques that measure platelet-associated immunoglobulin have the potential for meeting this requirement.

We found that the radiolabeled antiglobulin test can be performed on stored platelets and, in addition, is rapid, simple, and reproducible. It uses readily available reagents and technology commonly used in blood centers. The standardization and cut-off points of incompatibility are entirely objective. The determination of incompatibility based on negative controls makes the final results a semiquantitative value. More experience will be necessary to show whether each laboratory will find a different index as being predictive for a transfusion failure.

We found that the test predicted incompatible transfusions for HLA-A, B, C, and D matched platelets. In this study, too few HLA-A matches were transfused to evaluate the test's predictive value for this group of HLA matches. The ability to predict transfusion outcome when using mismatched platelets is important. This capability may reduce the size of matched donor pools needed to provide HLA-matched platelets.

Besides being able to detect HLA antibodies in a sensitive fashion, platelet-specific antibodies can also be detected. The importance of platelet-specific antibodies in refractoriness to platelet transfusions is uncertain. Most identified platelet antigens are of high frequency and less than 5% of patients could be expected to be refractory on this basis. In the current series, only four “A”-matched transfusions were administered. One patient who received HLA-identical platelets, however, did not achieve a satisfactory increment. This transfusion failure was predicted by our RAGT crossmatch. It is possible that this transfusion failure may have been due to platelet-specific antibody, although the other possibilities of nonimmune platelet destruction cannot be ruled out.

The major criterion for the effectiveness of transfusion was a platelet count performed 1 hr after transfusions completion. Secondarily, we also evaluated the crossmatch procedure to predict 20-hr survival. In the absence of splenomegaly or septic shock, the 1-hr increment has been reported to distinguish antibody-mediated platelet destruction from poor response caused by fever, disseminated intravascular coagulation bleeding, or milder infections.\(^{17}\) We interpret 20-hr increments more cautiously because of the concern that other clinical conditions besides alloimmunization can result in accelerated platelet destruction.\(^{17}\)

We observed only one false negative result, i.e.,
negative crossmatch but poor platelet increment. False negatives might be explained by consumption of the platelets on a nonimmune basis despite our efforts to exclude those conditions that can lead to increased peripheral consumption. Alternatively, the level of antibody present may be below the sensitivity of the test.

Nine positive crossmatches with adequate 1-hr increments were observed. Despite a satisfactory 1-hr increment, by the next day all nine of these transfusions had no survival. In one outpatient, two false antibody present may be below the sensitivity of the test. We cannot rule out the possibility of nonimmune destruction.

In the 56 transfusions that had platelet counts measured at 20 hr, there was one false negative crossmatch that was also a false negative according to the 1-hr criterion. Outpatient transfusions did not have follow-up platelet counts performed at 20 hr. Clinically, these patients did not have any signs or symptoms of thrombocytopenia when the crossmatch was compatible.

In summary, we have found the radiolabeled antiglobulin test a useful adjunct to the selection of platelet transfusions in refractory patients. If a crossmatch result is negative, the predictive value is 98%. This level is sensitivity promises to reduce transfusion failures—the ultimate goal of the platelet transfusion program. A positive RAGT crossmatch may predict poor long-term platelet survival despite having a satisfactory initial increment to the platelet transfusion.

REFERENCES


17. Daly PA, Schiffer CA, Aisner J, Wiernik PH: One hour post-transfusion increments are valuable in predicting the need for HLA-matched preparations. JAMA 243:435, 1980


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