CONCISE REPORT

The Use of Radiolabeled and Fluorescein-Labeled Antiglobulins in Assays to Predict Platelet Transfusion Outcome

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We used both radiolabeled and fluorescein-labeled antiglobulins in assays to detect antibodies against platelets in multiply transfused patients to determine the value of these tests in predicting the outcome of platelet transfusion in such patients. In 15 allosensitized patients, we studied 68 single-donor platelet transfusions, 43 (63%) of which had a poor outcome, defined as a corrected count increment (CCI), < 10,000. The results obtained with either test were significantly correlated with the CCI following transfusion (p < 0.001), but the assay using the radiolabeled antiglobulin had slightly better sensitivity, specificity, and predictive value. When the assays were used in combination, there was again significant correlation with the CCI of the transfusion, p < 0.001. When both assays predicted failure of the transfusions, 31/31 (100%) such transfusions resulted in a CCI of < 10,000, and when both assays predicted success of the transfusions, 14/15 (93%) such transfusions resulted in a CCI of > 10,000. Both assays are useful in predicting the outcome of the platelet transfusions; when the assay results were concordant, almost total predictive accuracy was obtained.

The efficacy of each platelet transfusion was determined by the corrected count increment (CCI):

$$CCI = \frac{(Posttransfusion count - Pretransfusion count) \times BSA (sq \ m)}{No. \ of \ platelets \ transfused \ (\times 10^9)}$$

The posttransfusion count was obtained 1–2 hr after the transfusion was finished. All platelet counts were measured using an ELT-8 (Ortho Instruments, Westwood, MA). If the CCI was > 10,000, the transfusion was considered to be a success, while a CCI of < 10,000 indicated failure of the transfusion.

HLA Typing and Lymphocytotoxic Antibody Screening

HLA typing was done by standard microlymphocytotoxicity technique. Lymphocytotoxic antibody screening was performed on a 30-donor cell panel that included most of the HLA antigens.

The HLA type of each platelet apheresis unit was compared with the HLA type of the recipient. The following HLA grading system was used:

- A: 4 antigen match
- B: 3 antigen match (1 antigen mismatched, cross-reactive, or unknown)
- C: 2 antigen match (2 antigens mismatched, cross-reactive, or unknown)
- D: 0 or 1 antigen match

Random: apheresis unit, with no HLA match requested

Reagents

The fluorescein-labeled antiglobulin was a polyclonal goat anti-human IgG, conjugated with fluorescein isothiocyanate in a high

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1245
Platelet Crossmatch

Platelets were obtained from the donor apheresis bags prior to transfusion. They were washed 3 times with GVB-EDTA and resuspended to a concentration of $500 \times 10^6$/ml. Patient sera were either used immediately or stored for no more than 24 hr at 4°C. Approximately 10^6 washed platelets (0.2 ml) were incubated with 0.5 ml patient’s pretransfusion serum for 60 min at room temperature (RT). This mixture was then washed 3 times with GVB-EDTA and resuspended to 0.3 ml.

At this point, the suspension of sensitized donor platelets was divided for the two assays. In the assay using the radiolabeled monoclonal antiglobulin (RMA test), 0.1 ml sensitized platelets were incubated with 0.1 ml 125I-monoclonal anti-IgG for 45 min at 37°C. Three 0.05-ml aliquots were spun through phthalate esters to remove unbound antiglobulin and counted with a gamma counter. In the assay using the fluorescein-labeled antiglobulin (FLA test), 0.1 ml sensitized platelets was incubated with 0.1 ml fluorescein-labeled anti-IgG for 30 min at RT in the dark. Following incubation, the platelets were washed 3 times with GVB-EDTA to remove unbound antiglobulin and then viewed with an epifluorescence microscope.

In each RMA test, the donor platelets were also incubated with GVB-EDTA as a negative control. GVB-EDTA incubation was used as the control, because, in the RMA test, autologous serum controls give results very similar to GVB-EDTA controls (data not shown). The amount of antiglobulin bound on platelets incubated with patient’s serum divided by the amount bound on platelets incubated with GVB-EDTA determined the RMA index. An index of 1.5 or greater was considered to be a positive test result, indicating the presence of antibody. In the FLA test, the platelets were given a score of 0–4, based on the degree of fluorescence observed. A value of 0 or 1 was considered background, while a value of 2, 3, or 4 was positive fluorescence, indicating the presence of antibody. A negative control (donor platelets incubated with GVB-EDTA) and a positive control (donor platelets incubated with a strong anti-P1A1 antibody) were viewed with each FLA test. All samples were viewed without knowledge of the patient being tested for the outcome of the transfusion.

RESULTS

Figure 1 shows the correlation between the RMA test results and the CCI of the platelet transfusions. Figure 2 shows the correlation between the FLA test results and the CCI of the platelet transfusions. The results of both tests were significantly related to the CCI ($\chi^2 = 29.5, p < 0.001; \chi^2 = 18.8, p < 0.001$, respectively). Table 1 compares the two assays and shows that the RMA test was correlated slightly better with the CCI than the FLA test. Sensitivity was defined as the number of unsuccessful transfusions (CCI <10,000) correctly predicted by the assay (positive test result). Specificity was defined as the number of successful transfusions (CCI >10,000) correctly predicted by the assay (negative test result).

When the results of the two assays were used in combination, there were three possibilities: (1) both tests were positive, suggesting presence of antibody; (2) both tests were negative, suggesting absence of antibody; and (3) one test was positive, and one test was negative. Figure 3 shows the significant relationship
between concordant assay results and the CCI following the platelet transfusions ($\chi^2 = 39.4, p < 0.001$). When both tests were positive, suggesting presence of antibody, 31 of 31 such transfusions had a CCI <10,000, with a mean CCI of 2,341 ± 3,268. When both tests were negative, suggesting absence of antibody, 14 of 15 such transfusions had a CCI >10,000, with a mean CCI of 17,191 ± 7,275. When the test results were concordant, a situation occurring in 46/68 (68%) of the transfusions, the outcome was correctly predicted in 45/46 (98%) of these transfusions. When the test results were discordant, i.e., one positive and one negative test result, 22 such transfusions had a mean CCI of 12,248 ± 8,488.

The grade of the HLA match was not related to the CCI of the transfusion (Table 2). Most of the transfusions failed, even with grade A or B matches, due to strong allosensitization to HLA and platelet-specific antigens. The transfusions using random apheresis units resulted in higher CCIs because they were usually given to patients who were not so broadly allosensitized.

**DISCUSSION**

Allosensitization is a major obstacle to effective platelet transfusions in the multiply transfused patient.

There is a great need for a simple and accurate platelet crossmatch. The most recent methods use anti-IgG labeled with either radioactivity or fluorescein as the detector molecule.

Helmerhorst and coworkers compared the sensitivity of three of the newer techniques for the detection of platelet antibodies. They used a quantitative antiglobulin consumption assay, a platelet radioactive antiglobulin test (PRAT), and a platelet suspension immunofluorescence test (PSIFT) to detect autoantibodies and alloantibodies in titration. They found the PSIFT and PRAT to be quite sensitive, but they did not use the tests in a clinical setting. Myers et al. have used quantitative immunofluorescence as a platelet crossmatch technique, correctly predicting 15/17 transfusions in 8 allosensitized patients.

A recent study by Kickler et al. used a radiolabeled antiglobulin test as a potential platelet crossmatch technique. They reported excellent results, with 96% sensitivity and 86% specificity at 1 hr posttransfusion, using a CCI of 7,500 as the cutoff for a successful transfusion.

We have used a RMA test and a FLA test as a potential platelet crossmatch technique. In a clinical setting of strongly allosensitized patients (43/68 or 63% of the transfusions had poor outcomes), we have compared the assays for accuracy in predicting the outcome of platelet transfusions. We found that each assay provided acceptable accuracy, and when the assay results were used in combination, the outcome of transfusion was usually correctly predicted. When the test results were concordant, 98% predictive accuracy was obtained.

In our study, false positives occurred when the transfusion resulted in a CCI of >10,000, but one of the assays gave a positive result, suggesting the presence of antibody. These false positives may arise in patients whose serum contains IgG, which binds to the platelet surface but does not cause immune clearance. Of the 11 false positive assay results in our study, 5 were on a single patient. False negatives occurred when the transfusion CCI was <10,000, but one or both assays gave negative results, suggesting the absence of antibody. Occasional high background values on the negative controls could cause false negatives, as could
nonimmune clearance of the platelets in the recipient. We did not include transfusions in patients with known causes for nonimmune clearance, such as sepsis or hemorrhage, but mild or subclinical bleeding and infection could consume platelets and give false negative results. Of the 13 false negative assay results in our study, 10 occurred in 2 patients, both of whom were severely granulocytopenic and had intermittent fever.

The entire crossmatch technique, performing both assays simultaneously, required only 3–4 hr. The relative ease of the procedure, the low cost of reagents, and the accurate results are attractive features of this crossmatch technique using two labeled antiglobulins. The routine use of these assays could improve the effectiveness of platelet transfusions in allosensitized patients.

REFERENCES


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R Ware, EG Reisner and WF Rosse