Prevention of Refractoriness and HLA-Alloimmunization Using Filtered Blood Products

By I. Sniecinski, M.R. O'Donnell, B. Nowicki, and L.R. Hill

Depletion of leukocytes from all blood products may decrease the incidence of alloimmunization to HLA antigens present on the white cells and thus delay the onset of refractoriness to random donor platelet support. In order to test this hypothesis, 54 patients with hematologic malignancy or marrow aplasia were entered on a prospective randomized trial using cotton-wool filtration as a method of leukocyte depletion of red cell and platelet concentrates. Forty patients were considered evaluable; 20 patients received filtered products and 20 patients in the control group received standard unfiltered products. The filter was 99% efficient in removal of leukocytes (average number of WBC/platelet product, 6 x 10^6). Platelet loss by this technique was 8%. Alloimmunization was assessed by detection of de novo formed lymphocytotoxic and platelet specific antibodies by microcytotoxicity test, Staph A protein radioimmunoassay, and solid phase red cell adherence test. In the group receiving filtered products, three of 20 (15%) patients developed lymphocytotoxic antibodies while ten of 20 (50%) patients in the control group developed cytotoxic antibodies (P = .01 by actuarial methods). Platelet antibodies were detected in seven of ten alloimmunized patients in the control group and three of three patients in the study group. Clinical evidence of refractoriness was seen in three of 20 patients in the filtered group and ten of 20 in the control group (P = .01 by actuarial methods). The cost of filtration was a fraction of the cost of a plateletpheresis product. Filtration appears to be an effective and economical method for reducing alloimmunization and clinical refractoriness to random donor platelets in patient receiving long-term transfusion support.

Alloimmunization to HLA antigens occurs in 40% to 70% of patients with hematologic disease who require transfusion support over extended periods of time. This leads to a clinical problem of refractoriness to random donor platelet support in more than half of those patients with laboratory evidence of alloimmunization. The management of such patients represents a difficult challenge. There has been much interest in modifying transfusion practices to minimize the risk of alloimmunization. One method is to use either sequential single donor blood products or HLA compatible products; this is both cumbersome and expensive. Another approach is to deplete leukocytes that are the presumed source of HLA antigen exposure in blood products. The results of prior trials using leukocyte depletion to diminish alloimmunization have been conflicting. We performed a prospective randomized trial in patients with hematologic disorders comparing standard blood transfusion support with a program in which all blood products were depleted of WBCs using a simple inexpensive cotton-wool filter. We report here the efficiency of the technique and the incidence of alloimmunization and clinical refractoriness to platelet transfusions.

Materials and Methods

Patient Population

Fifty-six patients with hematologic malignancy or aplastic anemia who were expected to require repeated red cell and platelet transfusions were entered into the study when they were referred to the City of Hope National Medical Center. Two patients were found to have HLA antibodies in the serum at presentation and were excluded from the study. Fifty-four patients negative for HLA antibodies were randomized between two transfusion support programs; the control group (26 patients) received standard red cell concentrates and pools (4 to 10 units) of random donor platelets while in the study group (28 patients) all blood products were filtered. A computer generated randomization scheme was employed using a pseudo-random number generator and stratifying for age and previous transfusions or pregnancies. Fourteen patients had received one to four platelet or red cell transfusions in the week before admission; four patients had late leukemic relapses (three acute lymphoblastic leukemias [ALL] and one acute myelogenous leukemia [AML]) and had received red cell and platelet transfusions 2 to 3 years previously. Only patients who had received five or more platelet transfusions were considered evaluable for alloimmunization and clinical refractoriness. Eleven patients (six in the control group and five in the study group) were considered non-evaluable because they received less than five platelet transfusions; three patients with aplastic anemia originally randomized to filtered group received single related donor plateletpheresis products at the discretion of the attending physician. The evaluable patients in the control group received between five and 37 transfusions of pooled platelet concentrates and between four and 32 red cell concentrates. The patients in this group were followed-up from 13 to 678 days. Those in the study group received between five and 62 pooled platelet concentrates and between three and 46 red cell concentrates and were followed-up for 72 to 1,068 days. The characteristics of the evaluable patient groups are shown in Table 1. There were no statistical differences between the two groups as measured by the Fisher exact test and Wilcoxon signed-rank test.
pool of six random donor platelet concentrates. After priming the eight minutes, concentrates at 4°C for less than ten days before transfusion.6 Pooled platelet standard technique (centrifugation four minutes, 2,500 rpm) and stored HLAtyped comprehensive antibodies by the standard micnolymphocytotoxicity Antibody Screening model IG-500.7'9 One filter was used to process a unit of RBC, or a let concentrates were prepared using the Imugard cotton-wool filter, to 48 hours before transfusion.6

for platelet antibodies in the solid-phase red cell adherence test.14 The mean of at result was defined as greaten than three SD above the against a panel of platelets collected from 20 donors. A positive positive results represented cytotoxicity against 10% of the cell panel.

Platelet antibody testing. Antibodies to platelets were detemined by radiolabeled Staphylococcal Protein A (125I-SPA) assay according to the method of Yam et al.15 Serum samples were tested against a panel of platelets collected from 20 donors. A positive result was defined as greater than three SD above the mean of at least three negative controls. In addition, serum samples were tested for platelet antibodies in the solid-phase red cell adherence test.14

Statistical analysis. Statistical comparisons were performed using the Fisher exact test for contingency tables, the log-rank test and Cox model for actuarial analysis.15

Blood Component Preparation

Standard blood components. Red cell concentrates were prepared from single-unit, whole blood volunteer donations using standard technique (centrifugation four minutes, 2,500 rpm) and stored at 4°C for less than ten days before transfusion.16 Pooled platelet concentrates consisted of 6 units of platelets prepared from multiple random donors (first centrifugation, four minutes, 2,500 rpm, second centrifugation, eight minutes, 3,800 rpm) and stored at 22°C for 24 to 48 hours before transfusion.16

Filtered blood components. Leukocyte-poor red cell and platelet concentrates were prepared using the Imugard cotton-wool filter, model IG-500.17'18 One filter was used to process a unit of RBC, or a pool of six random donor platelet concentrates. After priming the filter with 70 mL of sterile saline solution, the red cells or platelet concentrates were passed through the filter. The red cells were filtered under a pressure of 50 mm Hg using pressure cuff. The platelet concentrate filtration was accomplished by gravity flow. At the end of filtration, 100 mL of saline was passed through the filter to remove trapped platelets. Filtration was completed in 20 to 30 minutes. Table 2 shows the characteristics of filtered red cell and platelet components with regard to total volume, platelet, red cell, and leukocyte counts.

Red cell transfusions were administered for active bleeding or for hematocrits <30%. Platelet transfusions were given for control of thrombocytopenic bleeding or prophylactically when platelet counts were <20,000/μL. Platelet counts were obtained at one hour following each platelet transfusion. The platelet count increment one hour after transfusion was calculated according to the following formula:

\[
\text{% Increment} = \frac{\text{Post - Pre Platelet Count} \times BV \times 100}{\text{Number of Platelets Transfused} \times 0.67}
\]

The number of platelets transfused was estimated by multiplying the number of units by \(5.5 \times 10^{10}\).16 Blood volume (BV) was calculated by multiplying the patients weight in kilograms by 69 mL/kg for males and by 65 mL/kg for females.16 The factor 0.67 was used to account for splenic pooling. Percent increment in nonrefractory patients ranged from 35% to 65%. Platelet counts were also obtained 24 hours after transfusion in 90% of all platelet transfusion episodes. They were used only as an approximation of platelet survival to gauge the frequency of transfusions.

A patient was considered to be refractory to random donor platelets if two consecutive platelet transfusions produced one hour posttransfusion increments that were <20% of the predicted value in the absence of clinical factors known to affect platelet recovery such as active bleeding, disseminated intravascular coagulation, fever (T > 38.5°C), septicemia, or splenomegaly. The clinical end points for the study were (1) development of refractoriness to random donor platelets necessitating subsequent HLA-matched single donor platelet transfusions; (2) alloergic marrow transplantation since all transplant recipients at the City of Hope receive single donor HLA matched platelet support; (3) administration of granulocytes for uncontrolled sepsis; and (4) death. Eight patients are still being followed-up. One patient from the study group was removed at 9 weeks without evidence of alloimmunization for bone marrow transplant. Three patients from the control group developed alloantibodies before bone marrow transplantation. One patient in each group required granulocytes for uncontrolled sepsis at 300 days and 388 days following entry to the study without evidence of alloimmunization.

RESULTS

There were 684 platelet transfusion events in this study; one-hour counts were available in 584 of the events (85%) and 24-hour counts were available in 603 events (88%). As

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>Filtered</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>39 (19-66)</td>
<td>46 (9-71)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>11/9</td>
<td>11/9</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>ALL</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Aplastic anemia</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Prior transfusions</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Prior pregnancy</td>
<td>7</td>
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Table 2. Preparation of Blood Components

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Red Cell Concentrate</th>
<th>Platelet Concentrate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
<td>Filtered</td>
</tr>
<tr>
<td>Mean volume</td>
<td>276 mL</td>
<td>310 mL</td>
</tr>
<tr>
<td>Mean red cell count</td>
<td>2.40 x 10^{12}</td>
<td>2.26 x 10^{12}</td>
</tr>
<tr>
<td>Mean platelet count</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mean leukocyte count</td>
<td>3.9 x 10^8</td>
<td>5.0 x 10^7</td>
</tr>
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</table>

*Pool of six random donors.
previously stated, only the 584 transfusion episodes with available one-hour counts were evaluated for evidence of refractoriness.

Among the 584 evaluable platelet transfusion events, there were 66 in which the increment was <20% of the expected increment. In 25 episodes, the poor increment coincided with fever (T > 38°) with bacteremia documented in 17 of these febrile episodes; three episodes coincided with increasing splenomegaly in patients with chronic myelogenous leukemia (CML) blast crisis and one patient had disseminated intravascular coagulation. In the remaining 37 events, there was no clinical indicator of increased consumption or sequestration. In seven instances, the poor increment was a random happening with subsequent transfusions giving adequate increments. In the other 30 instances, two or more consecutive platelet transfusions gave poor increments and were considered to be evidence of platelet alloimmunization.

The incidence of clinical refractoriness and alloimmunization to random donor platelets is shown in Table 3. Ten of 20 patients (50%) receiving unfiltered transfusions became clinically refractory whereas only three of 20 patients (15%) receiving filtered products had poor responses to random pools of platelets. Five of the patients in the unfiltered group became clinically refractory within the first 21 days, while the others developed refractoriness 66 to 567 days after initial transfusion. None of the patients receiving leukopoor products developed refractoriness within the first 21 days; the times of refractoriness were 106 days, 429 days, and 512 days after entry into the study. Using actuarial methods, the times to refractoriness differed in the two groups (P < .01) even after correction for number of transfusions (Fig 1). The number of transfusions until refractoriness also differed significantly (P < .01).

Ten patients in the unfiltered group developed HLA specific alloantibodies; nine of these patients were also clinically refractory. One patient developed a persistent low titer antibody after 66 days but continued to have good response to platelet transfusion for an additional 5 months. One patient who became clinically refractory at 173 days and died ten days later did not have detectable antibodies. Three of the patients receiving filtered blood products developed alloimmunization at 16 days, 387 days, and 466 days.

Using actuarial methods, the time to alloimmunization differed significantly between the two groups (P < .01) (Fig 2). The rate of alloimmunization did not correlate with prior exposure to HLA antigen by transfusion or pregnancy. Six of 12 patients in the unfiltered group with prior exposure developed antibodies as compared with four of eight patients.
<table>
<thead>
<tr>
<th>Method of WBC Removal</th>
<th>Leukocyte (%)</th>
<th>Percent Removed</th>
<th>Refractory Patients No. and (%)</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC, Epoxy filter</td>
<td>66/0.0</td>
<td>97%</td>
<td>26/28 (83%)</td>
<td>Control</td>
</tr>
<tr>
<td>PLTS, centrifugation</td>
<td>10/0.0</td>
<td>100%</td>
<td>10/18 (63%)</td>
<td>Control</td>
</tr>
<tr>
<td>RBC, frozen or washed</td>
<td>50/0.0</td>
<td>98.7%</td>
<td>5/25 (20%)</td>
<td>Control</td>
</tr>
<tr>
<td>PLTS, centrifugation</td>
<td>12/0.0</td>
<td>81%</td>
<td>15/31 (48%)</td>
<td>Control</td>
</tr>
<tr>
<td>RBC, Imaged G-500</td>
<td>8.0/0.0</td>
<td>99.6%</td>
<td>7/31 (23%)</td>
<td>Control</td>
</tr>
<tr>
<td>PLTS, centrifugation</td>
<td>2.4/48.0/0.0</td>
<td>70-95%</td>
<td>3/19 (15%)</td>
<td>Control</td>
</tr>
</tbody>
</table>

Table 4. Transfusion of Leukocyte Blood Components: Comparison of Efficacy and Rates of Allotransimmunization
without prior exposure. In the filtered group the proportion was two of ten patients with prior HLA exposure developed antibodies as compared with one of ten without exposure. All patients who developed clinical refractoriness received either family member plateleteraphesis product or unrelated HLA matched platelet product with variable response. No patient was rechallenged with pooled unrelated platelets. All patients who developed positive HLA antibodies continued to show evidence of significant antibody titers for 2 to 18 months following their last platelet transfusion. Using the method of Yam et al, we did not detect any platelet antibodies. However, when using the solid phase red cell adherence test, we have detected the platelet antibodies in seven of ten alloimmunized patients in the control group and three of three patients in the study group.

DISCUSSION

Current chemotherapeutic approaches to the treatment of acute leukemia stress repeated courses of intensive myelo-suppressive therapy. The chemotherapy is frequently coupled with allogeneic or autologous marrow transplantation. Patients thus require more protracted periods of blood and platelet support with consequent increased risks of alloimmunization and clinical refractoriness to platelet transfusion. Management of the alloimmunized patient is difficult and expensive, requiring single HLA matched platelet products. Results of the current study are compared with three previous studies using various methods of platelet products. The rates of alloimmunization in both the control groups and the study groups are very similar in all four studies. In the first three studies the patients were followed-up for 3 to 6 months following induction chemotherapy, while the immunized patients in our study have been followed-up for up to 2½ years following induction, consolidation, and frequently, reinduction chemotherapy. While most patients develop alloantibodies within the first 8 weeks of exposure we have seen a quarter of our patients developing antibodies after 6 months of exposure.

The clinical end points for refractoriness to random donor products is much more subjective and thus one expects more variability in the groups. The incidence of refractoriness in the control group in the Eernisse and Brand study is much higher than generally expected. In the other two studies and in our own study roughly 20% to 25% of patients became clinically refractory during their first course of chemotherapy. However, we saw an additional 25% of patients who developed signs of refractoriness during intensive consolidation therapy or reinduction therapy up to 18 months later. With many centers using repeated courses of myeloablative chemotherapy or autologous transplantation in the treatment of hematologic malignancies the period of support and thus risk for alloimmunization is increased. Filtration of all blood products is a simple efficient and relatively inexpensive technique for significantly reducing exposure to HLA antigen when compared with the use of centrifugation or frozen RBCs. It can obviate the need for HLA matched apheresis products or cryopreservation of autologous platelets and is more practical than using random single donor plateleteraphesis products to restrict HLA antigen exposure since the procedure can be performed in a community hospital setting.

ACKNOWLEDGMENT

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Prevention of refractoriness and HLA-alloimmunization using filtered blood products

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