The Effect of Lymphocytotoxic Antibody Reactivity on the Results of Single Antigen Mismatched Platelet Transfusions to Alloimmunized Patients

By Mohamad A. Hussein, Edward J. Lee, Robert Fletcher, and Charles A. Schiffer

Despite selection strategies that attempt to maximize the platelet donor pool, significant numbers of alloimmunized patients have few if any available donors. Although the number of potential donors increases when one antigen mismatched platelet transfusions (OAMPT) are considered, transfusions from such donors are often cited to fail to produce satisfactory platelet count increments. The presence of lymphocytotoxic antibody (LCTAB) correlates well with responsiveness to random donor platelet transfusions and serves as a good serologic screen for the diagnosis of alloimmunization. We therefore reviewed the results of OAMPT to alloimmunized patients and assessed the relationship between LCTAB levels in the recipient and posttransfusion platelet count increments. We noted an unexpectedly high percentage of good responses in our patient population: 73% of all OAMPT to recipients with LCTAB <60% reactive, resulted in successful increments. In recipients with LCTAB ≥60%, 58% of all transfusions were still successful. Despite a statistically significant inverse relationship between the level of LCTAB and the response of OAMPT to alloimmunized patients, 58% to 73% of recipients will have a satisfactory platelet recovery posttransfusion. These data support extending donor searches for alloimmunized patients to include any single mismatch particularly if a recipient’s LCTAB has lower reactivity.

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The Management of refractoriness to platelet transfusions secondary to alloimmunization represents a major clinical and research challenge in the field of platelet transfusion therapy. Despite increased availability of HLA type donors and a greater understanding of the factors that influence the results of such transfusions, a large percentage of allegedly histocompatible transfusions administered to alloimmunized patients are unsuccessful. In particular, it may be difficult to provide long-term platelet transfusion support for alloimmunized patients with unusual HLA types for whom fewer perfectly matched donors are available. Although the number of potential donors increases when donors whose platelets are mismatched for HLA antigens are considered, transfusions from such donors are often cited to fail to produce satisfactory platelet count increments. The presence of lymphocytotoxic antibody (LCTAB) correlates well with responsiveness to random donor platelet transfusions and serves as a good serologic screen for the diagnosis of alloimmunization. We therefore reviewed the results of a large series of one antigen mismatched platelet transfusions (OAMPT) to alloimmunized patients and assessed the relationship between lymphocytotoxic antibody levels in the recipient and the posttransfusion platelet count increments.

MATERIALS AND METHODS

We retrospectively analyzed the results of platelet transfusions mismatched for a single HLA A or B antigen administered to consecutive patients refractory to random donor pooled platelet concentrate transfusions. OAMPTs were used because that was the best available product at the time. All data were from patients treated between January 1976 and December 1989 at the University of Maryland Cancer Center. OAMPTs were categorized according to whether the donor’s mismatched antigen was serologically cross reactive with any of the recipient’s HLA antigens, and to whether LCTAB was present in the recipient.

Patients were considered clinically stable if they had a temperature lower than 101°F, no uncontrolled infection, severe hemorrhage, palpable splenomegaly, or disseminated intra-vascular coagulation. None of the patients evaluated received any therapy or product that might alter the transfusion results. Specifically, no intravenous immunoglobulins, high dose steroids, leucocyte-reduced platelet transfusions, or hematopoietic growth factors were administered. All transfusions were assessed by posttransfusion corrected count increments (CCI) obtained 10 to 60 minutes after transfusion, where CCI equals platelet count increment per microliter × body surface area (meter squared) divided by the number of platelets transfused × 1011.5.10 A CCI of ≥7,500 was considered to be an adequate response to transfusion. Refractoriness to platelet transfusion was defined as a posttransfusion CCI of <4,000 to random donor platelet concentrates on at least two consecutive occasions. All analyzed transfusions were administered after this defining point. Platelets were stored as single platelet concentrates at 22°C with horizontal to-and-fro agitation. The majority of transfused units were stored for no more than 3 days.

Lymphocytotoxic antibody testing was performed on patients’ serum at the time of hematologic diagnosis and was repeated at biweekly intervals during treatment, or more frequently if clinically warranted by changing response to random donor pooled platelet concentrates. LCTAB was determined as the percent of cytotoxicity of patients’ serum against a panel of lymphocytes of different antigenicity using standard methods. Antibody (LCTAB) cytotoxic to 20% of the panel lymphocytes obtained within 2 weeks of a particular transfusion was defined as positive.

A standard linear regression method was used to fit the relationship between CCI values and the corresponding degree of positivity of the LCTAB percentage. The slope parameter’s P value was determined from a t-distribution and tested for statistical significance. The only variables used in the regression equation are CCI and percent LCTAB, where the former is the dependent variable and the latter is the independent variable. All statistical analyses were performed using Statistical Analysis Software (SAS Institute, Cary, NC).

RESULTS

Three hundred sixty-nine single donor transfusions that were mismatched for a single HLA antigen were adminis-

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tered to 83 stable patients with acute leukemia. Transfusions were divided into five groups according to the level of the lymphocytotoxic antibody at the time of transfusion. The results of all transfusions are analyzed in Table 1. Although there was a statistically significant inverse relationship between the percentage of reactivity of the LCTAB and post-transfusion increments by regression analysis, the differences were most apparent in patients with more reactive antibodies. Seventy-three percent of all OAMPTs to all patients with LCTAB <60% resulted in a successful platelet increment with a mean CCI of 14,680. The CCI dropped significantly (P value <.001) to a mean CCI of 9,800 in transfusions to patients with LCTAB ≥60%. Furthermore, in the least biased group of transfusions (Table 2) the percentage of successful transfusions dropped from 67% in patients with LCTAB <60% to 53% in recipients with LCTAB ≥60% (P value .039).

Because we were somewhat surprised to see minimal effects of less reactive antibodies, we then attempted to correct for the effects of a number of known donor selection factors that could have modified these results. Repeated transfusions from donors whose platelets produced good increments and who donated repeatedly for the same patient (n = 80) were then eliminated, so that only the first transfusion for each donor/recipient pair was considered. Because transfusions of platelets from donors with antigen mismatched for HLA B12 and its splits (HLA B 44 and 45) produce satisfactory results in approximately 70% of the incidents, all mismatches for this antigen and its splits were deleted (n = 34). To minimize the influence of lymphocytotoxic antibody known to be reactive against the mismatched antigen such transfusions were eliminated (n = 20). Finally, transfusions with platelets from donors mismatched for antigens that are splits or strongly cross-reactive with recipient antigens were eliminated (n = 24) to provide a group of 211 transfusions, including donor-recipient pairs that have been used only once, were not mismatched for HLA-B12 or its splits, were not mismatched for any antigen splits or strong cross-reactive antigen groups, and were given to patients without lymphocytotoxic antibody against the mismatched antigen (Table 2).

The mean CCI and the percent of successful transfusions were slightly lower in the more selected group of transfusions presented in Table 2 (13,970 and 7,780 in recipients with LCTAB <60% and ≥60%, respectively) and notably in patients with LCTAB >80%. Although the pattern of results was similar. If only transfusions with CCI >7,500 are considered, the significant difference in the mean CCI between the two levels of LCTAB is maintained (Table 3). That is, even when transfusions were “successful” the average increment was lower in patients with higher LCTAB levels.

When available, lymphocytotoxic antibody specificity against mismatched antigens was evaluated. Antibody specificity was available for 202 of the transfusions. Antibody against the mismatched antigen was detected in only 20 transfusions to 14 recipients. Ten of the twenty transfusions to nine patients resulted in a successful increment (CCI ≥7,500) with six of the ten transfusions producing CCI ≥10,000.

**DISCUSSION**

There has been a striking increase in the use of platelet transfusions in recent years, primarily as a consequence of an increase in the number of patients with leukemia and other cancers who are treated with more intensive and prolonged chemotherapeutic approaches. Despite improvements in the transfusion support of these thrombocytopenic patients, refractoriness to platelet transfusions remain a serious problem, thereby potentially limiting curative forms of therapy. Although there are selection strategies that attempt to max-

<table>
<thead>
<tr>
<th>LCTAB (%)</th>
<th>0-19</th>
<th>20-39</th>
<th>40-59</th>
<th>60-79</th>
<th>80-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of transfusions</td>
<td>47</td>
<td>47</td>
<td>37</td>
<td>75</td>
<td>163</td>
</tr>
<tr>
<td>Mean CCI × 10⁶</td>
<td>13.67</td>
<td>13.53</td>
<td>17.41</td>
<td>10.80</td>
<td>9.43</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.18</td>
<td>1.21</td>
<td>1.76</td>
<td>0.85</td>
<td>0.67</td>
</tr>
<tr>
<td>95% Confidence interval for mean CCI</td>
<td>11.36-15.98</td>
<td>11.16-15.90</td>
<td>13.96-20.86</td>
<td>9.13-12.47</td>
<td>8.12-10.74</td>
</tr>
<tr>
<td>% CCI ≥7,500</td>
<td>74.4</td>
<td>70.2</td>
<td>75.6</td>
<td>69.3</td>
<td>53.9</td>
</tr>
<tr>
<td>Regression coefficient (95% confidence interval)</td>
<td>-1.34 (-0.74, -1.95)</td>
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<tr>
<td>P value of regression coefficient (t-test)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
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Transfusions from donors used once, not mismatched for HLA B12 or its splits, no antibody against mismatched antigen, and no mismatches for split or cross-reactive antigen.

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</tr>
<tr>
<td>Mean CCI × 10⁶</td>
<td>13.74</td>
<td>12.49</td>
<td>16.20</td>
<td>11.00</td>
<td>7.74</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.56</td>
<td>1.67</td>
<td>1.92</td>
<td>1.21</td>
<td>0.85</td>
</tr>
<tr>
<td>% CCI ≥7,500</td>
<td>66.6</td>
<td>64.2</td>
<td>71.4</td>
<td>71.0</td>
<td>45.2</td>
</tr>
<tr>
<td>Regression coefficient (95% confidence interval)</td>
<td>-1.56 (-0.79, -2.33)</td>
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imize the number of potential donors by using donors mismatched for cross-reactive antigens as described by Duquesnoy et al,1 and matching for HLA B12 and its splits by Schiffer et al,13 there is a significant number of patients who have few if any available donors. The use of donors mismatched for one antigen could potentially expand the donor pool.1

Our study reviewed a large number of OAMPTs to LCTAB positive and negative patients. This group of patients was refractory to random donor pooled platelet concentrate. In contrast to older1 and more current reports14 we were surprised to find that 73% of all antigen mismatched transfusions resulted in successful increments if the lymphocytotoxic antibody was <60% reactive. In recipients with LCTAB ≥60%, 58% of all transfusions were still successful. The mean CCI for successful transfusions to recipients with LCTAB levels <60% was comparable with those noted following random donor platelet concentrate transfusions administered to stable patients and comparable with the success of transfusions mismatched for HLA B12 and its splits.13,14

Duquesnoy et al1 analyzed the response of 29 patients to transfusions mismatched for one or more HLA antigens and demonstrated that 15 patients responded well to such transfusions on two or more occasions and that 14 patients consistently failed to respond. Unfortunately, the data of cross-reactive and non-cross-reactive matched platelets were pooled together. More recently, McFarland et al15 evaluated factors influencing the transfusion response to HLA matched platelets in refractory patients and noted that in patients refractory to platelet transfusion with a LCTAB >20%, only 35% of OAMPT to all patients resulted in a successful recovery.

The unexpectedly high percentage of good responses in our patient population is not related to donor/recipient bias since the results were unchanged when only the first transfusion from a particular donor was considered. Moreover, the high frequency of successful transfusions is not related to using a particular antigen as a mismatch. Also, HLA B12 and its splits did not appear to account for these good results because eliminating such transfusions did not affect the outcome. Some specificity of LCTAB could be demonstrated in >50% of the transfusions and when possible, we attempted to select donors with mismatched antigens against which the recipient did not demonstrate a specific antibody (88% of all transfusions with LCTAB specificity available). Nonetheless, 50% of the transfusions administered to patients with specific antibody against the mismatched antigen were successful.

Platelet transfusions from donors selectively mismatched for cross-reactive and certain non-cross-reactive HLA antigens were found to be more effective in HLA A2 negative than in HLA A2 positive alloimmunized thrombocytopenic patients.16 Comparable with previous reports, 54% of our patients were HLA A2 positive. The presence or absence of the HLA A2 antigen in the recipients did not influence the relationship between the LCTAB and the percentage of successful transfusions or the mean 10 minute or 1 hour CCI (data not shown).

Our results further demonstrate that alloimmunization to platelet transfusions is a complicated process with LCTAB levels reflecting only one aspect of this immune reaction.17 LCTAB antibody levels can fluctuate over time in leukemia patients and often can disappear despite continued transfusion.18 Such fluctuations could account for occasional discrepant transfusion outcomes since antibody levels were not always determined the same day as the transfusion. Nonetheless, such variations in antibody are unlikely to be a major factor, since most antibody specimens were obtained within 2 weeks of the transfusion that was analyzed. Despite a statistically significant inverse relationship between the level of LCTAB and the response of OAMPT to alloimmunized patients, 58% to 73% of recipients will have a satisfactory platelet recovery posttransfusion.

Even though LCTAB levels did not discriminate between successful versus unsuccessful responses to OAMPTs these data support extending donor searches for alloimmunized patients to include any single antigen mismatch, particularly if the recipients’ LCTAB has lower reactivity. This strategy will increase the donor pool and hopefully decrease the search time for an appropriate donor.

### REFERENCES


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