Red Blood Cell Alloimmunization in Multitransfused Patients in a Tertiary Care Center in Western India

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ABSTRACT

Objective: To investigate the seroprevalence and specificity of red blood cell (RBC) antibodies in multitransfused patients, in whom the risk of alloimmunization is especially high.

Methods: We conducted a retrospective study on blood specimens from 200 multitransfused patients. We evaluated all specimens for alloimmunization using various immunohematological tests via the column agglutination technique.

Results: The overall prevalence of RBC alloantibodies was 5.5%. Of the 11 specific types of alloantibodies identified, most (72.7%) belonged to the Rh blood group system, followed by the S, M, and Lewis blood group systems (9.1% each).

Conclusion: Most alloantibodies were of the Rh blood group specificity. To improve the quality of blood supplied, especially to patients with thalassemia, we recommend that Rh phenotyped, cross-match–compatible blood should be issued to prevent complications such as acute and delayed hemolytic reactions.

Keywords: alloimmunization, multitransfused, column agglutination technique, thalassemia, hemato-oncologic diseases, antibody identification

Abbreviations

RBCs, red blood cells; AA, aplastic anemia; SAGM, saline, adenine, glucose, and mannitol; AML, acute myeloid leukemia; MDs, myelodysplastic syndromes; CML, chronic myelogenous leukemia; NHL, non-Hodgkin lymphoma; ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; CKD, chronic kidney disease; SCD, sickle cell disease; EDTA, ethylenediaminetetraacetic acid; AABB, American Association of Blood Banks; CI, confidence interval; DHTFs, delayed hemolytic transfusion reactions; ATG, antithymocyte globulin

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Materials and Methods

We conducted a 3-year retrospective study with 200 multitransfused patients at the Department of Immunohematology and Blood Transfusion, Armed Forces
Medical College, Pune, India, from January 2010 through January 2013. Because ours is a tertiary care center, the patients are generally referred from surrounding area hospitals and had invariably received medications or transfusions prior to their referral. Records of all known multitransfused patients are maintained at our institution. Multitransfused patients are tested to determine the presence of any kind of auto- or alloantibodies that may have already developed. Depending on the results, we issue to the patient corresponding antigen-negative blood units. Routinely, we give all multitransfused patients ABO- and RhD-compatible, irradiated, and leucoreduced packed RBCs suspended in saline, adenine, glucose, and mannitol (SAGM) additive solution. Fresh blood (<7 days old) was given to all patients with thalassemia per the request of the treating physicians.

Inclusion criteria for the study consisted of having had multiple transfusions of more than 10 units of blood and/or blood products before referral and having received ABO- and Rh D-compatible blood and/or blood products in all of their previous transfusions (as indicated in their medical records). Exclusion criteria included previous out-of-group transfusions; having had any previous or present pregnancies or abortions; having any autoimmune diseases, such as systemic lupus erythematoses, lupus nephritis, or idiopathic thrombocytopenia; and currently having non-RBC transfusion requirements.

We divided the study participants into the following 4 groups: group 1 was made up of patients with thalassemia; group 2, patients with hemato-oncologic diseases, consisting of acute myeloid leukemia (AML), myelodysplastic syndromes (MDSs), aplastic anemia (AA), myeloproliferative syndromes such as chronic myelogenous leukemia (CML), lymphoproliferative diseases (Hodgkin lymphoma and non-Hodgkin lymphoma [NHL]), multiple myeloma, acute lymphocytic leukemia (ALL); and chronic lymphocytic leukemia (CLL); group 3, patients with chronic kidney disease (CKD), who have undergone regular hemodialysis for end-stage renal disease; group 4, patients having paroxysmal nocturnal hemoglobinuria, sickle cell disease (SCD), and/or hemolibria.

Clinical and transfusion records included the demographic characteristics, age, and sex of each patient, along with his or her transfusion history before the first visit to our blood bank. The transfusion history included the number of units of blood transfused, date of transfusion, indication for transfusion, age at first transfusion, transfusion of any other blood components, record of any out-of-group transfusion, age at splenectomy (if applicable), and drug history (especially for patients receiving chemotherapy). The study was approved by the institutional ethical committee.

We collected 5 mL of whole blood specimens in ethylenediaminetetraacetic acid (EDTA) from patients. Plasma and RBCs were separated within 30 minutes of specimen collection. The column agglutination technique (via the DiaMed ID Micro Typing Gel System, Bio-Rad Laboratories, Inc, Hercules, CA) was used for serologic evaluation. All tests were performed according to manufacturer instructions. We performed routine ABO and RhD blood grouping and subjected RBCs to direct antiglobulin testing.

Plasma specimens were processed for antibody screening and identification. We performed initial testing using LISS Coombs and NaCl/enzyme tests using a commercially available 3-cell panel (R\textsuperscript{a}, R\textsubscript{b}, and R\textsubscript{c}). If the results were negative, we presumed that the specimen contained no clinically significant alloantibodies. An autocontrol for each specimen is incorporated in the LISS Coombs microtube gel card, to detect the presence of any autoantibodies.

The specificities of antibodies were determined by comparing the reaction pattern with the antibody identification chart (antigram) provided by the manufacturer along with the 3- and/or 11-cell panel reagents. We considered patients to be alloimmunized if we could identify antibodies to 1 or more RBC antigens in their plasma.

Due to the low number of alloantibodies identified in this study, a multivariate analysis would not have led to a meaningful conclusion. Hence, we performed a univariate analysis by using \( \chi^2 \) or Fisher exact testing to determine any possible associations. A \( P \) value of less than .05 was considered significant.

**Results**

We divided the study populations into 4 groups based on their clinical diagnoses ([Table 1](#tab1)). Patients with hemato-oncologic diseases were further divided into subgroups:
MDS (n = 13), AA (n = 20), AML (n = 13), CML (n = 8), Hodgkin lymphoma (n = 2), NHL (n = 3), multiple myeloma (n = 7), ALL (n = 12), and CLL (n = 3). Table 1 and Table 2 illustrate the proportion of the male and female population among different clinical groups and among different age groups.

RBC alloantibodies were found in 11 out of the 200 multitransfused patients (5.5%). The alloantibody specificities identified were as follows: 8 (72.7%) belonging to Rh blood group system, and 1 each belonging to the S (9.1%), M (9.1%), and Lewis (9.1%) blood group systems. The 8 alloantibodies belonging to the Rh blood group system had the following subspecificities: anti-D, 1 (12.5%); anti-E, 4 (50.0%) and anti-c, 3 (37.5%). Details of the alloimmunized patients are provided in Table 3. No concurrent or multiple antibody specificity was observed. In all patients, the results of DAT and autocontrol testing were negative.

Females (8 of 11 [72.7%]) appeared to have a greater risk of RBC alloimmunization compared with males; the difference was statistically significant (Fisher exact 2-tail P value <.02). Of the total number of patients in the age group of 0 to 25 years, 6 (5.2%) were alloimmunized, followed by 4 (10.5%) in the age group of 26 to 50 years (Figure 1). The mean age of alloimmunization of the total study population was 28.7 years. We could not establish any relationship between age and the rate of alloimmunization ($x^2$ = 2.05; df = 2; P = .24).

The mean and median numbers of units transfused were 71.6 and 46.0 units, respectively (range, 10.0–498.0) in our study population. Among the total of 11 alloimmunized patients, 9 (81.8%) had received more than the median of 46 units, compared with 2 (1.6%) who had received less than the median of 46 units; the former characteristic was significantly associated with rate of alloimmunization (Fisher exact 2-tail P <.002).

In group 1, a total of 52 males (63.4%) and 30 females (36.6%) had thalassemia; among them, 5 females and 1 male were positive for alloantibodies. Six patients (7.3%) with thalassemia (of 82 total patients in this group) were positive for RBC alloimmunization. We did not find a significant correlation between the age of the patients in this group and the presence of alloimmunization. The mean number of units transfused in this group was 92.3 (range, 47.0–498.0). The mean age of onset of the first transfusion in this group was 22.8 months. Two (33.3%) alloimmunized patients were transfused blood after this mean age and 4 (66.7%) alloimmunized patients were transfused with their first blood unit before this mean age. However, the age at initial transfusion was not the same as the age at which alloimmunization was first detected. Hence, we could not establish an association between the age at initial transfusion and the rate of alloimmunization in patients with thalassemia because we could not determine with certainty the age at which alloantibodies were formed. Eleven (13.4%) patients with thalassemia out of the 82 total in this group had undergone splenectomy; we observed alloimmunization in 3 of these patients. The association between splenectomy and RBC alloimmunization in patients with thalassemia was significant (Fisher exact 2-tail P = .03).

In the second group, 2 of the 8 patients (25.0%) with CML were RBC alloimmunized; their mean age was 48.5 years. The mean number of transfused units in this subgroup was 21.3 (range, 10.0–38.0). CML was not significantly associated with alloimmunization ($x^2$ = 2.424; df = 1; P = .12). Of the 13 patients with MDS, 8 were male and 5 were female. Of the 11 alloimmunized patients, 3 (27.3%) had MDS; all 3 were female, with a mean age of 45.7 years. The mean number of transfused units in this subgroup was 106 (range, 56–166). MDS appeared to confer a significant risk for RBC alloimmunization ($x^2$ = 4.55; df = 1; P <.03).

Of the 31 patients in group 3, 21 (67.7%) were male and 10 (32.3%) were female (mean age, 43.9 years). The mean number of transfused units in this subgroup was 20.7 (range, 10.0–32.0). None in this group was alloimmunized. In group 4, there were 4 males and 2 females for a total of 6 patients. The mean age in this group was 26.0 years, and the mean number of transfused units was 24.5 (range, 10.0–45.0). None of the patients in this group developed RBC alloantibodies.

Discussion

Alloimmunization to RBC antigens is usually stimulated by the transfusion of blood products and is one of the complications of RBC transfusion. Other than RBC alloimmunization, immunological complications of repeated RBC transfusions include difficulties obtaining compatible blood, development of autoantibodies, acute or delayed hemolytic transfusion reactions, and hemolytic disease of the newborn. Blood banks with limited technical facilities and scarce donor resources need to take these complications into consideration when planning the long-term support of transfusion-dependent patients.
Table 1. Sex Distribution of Study Participants According to Clinical Conditions

<table>
<thead>
<tr>
<th>Group</th>
<th>Disease</th>
<th>No. (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thalassemia</td>
<td>52 (63.4)</td>
<td>30 (36.6)</td>
<td>82 (41.0)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Hemato-oncologic</td>
<td>52 (64.2)</td>
<td>29 (35.8)</td>
<td>81 (40.5)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CKD</td>
<td>21 (67.7)</td>
<td>10 (32.3)</td>
<td>31 (15.5)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Others</td>
<td>4 (66.7)</td>
<td>2 (33.3)</td>
<td>6 (3.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>129 (64.5)</td>
<td>71 (35.5)</td>
<td>200 (100)</td>
<td></td>
</tr>
</tbody>
</table>

*CKD, chronic kidney disease.

Table 2. Age and Sex Distribution of the Study Participants

<table>
<thead>
<tr>
<th>Age Group (y)</th>
<th>Males No. (%)</th>
<th>Females No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–25</td>
<td>77 (66.4)</td>
<td>39 (33.6)</td>
<td>116 (58.0)</td>
</tr>
<tr>
<td>26–50</td>
<td>22 (57.9)</td>
<td>16 (42.1)</td>
<td>38 (19.0)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>30 (65.2)</td>
<td>16 (34.8)</td>
<td>46 (23.0)</td>
</tr>
<tr>
<td>Total</td>
<td>129 (64.5)</td>
<td>71 (35.5)</td>
<td>200 (100)</td>
</tr>
</tbody>
</table>

*Mean age of the entire study population, 27.12 years (range, 2 to 90 years).

Table 3. RBC Alloantibody Specificities in 11 Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Diagnosis</th>
<th>Units Transfused, No.</th>
<th>Antibody Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>55</td>
<td>MDSs</td>
<td>166</td>
<td>Anti-M</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>48</td>
<td>MDSs</td>
<td>56</td>
<td>Anti-c</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>34</td>
<td>MDSs</td>
<td>69</td>
<td>Anti-E</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>49</td>
<td>CML</td>
<td>11</td>
<td>Anti-c</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>48</td>
<td>CML</td>
<td>38</td>
<td>Anti-S</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>21</td>
<td>Thalassemia</td>
<td>324</td>
<td>Anti-E</td>
</tr>
<tr>
<td>7</td>
<td>Female</td>
<td>10</td>
<td>Thalassemia</td>
<td>174</td>
<td>Anti-E</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>19</td>
<td>Thalassemia</td>
<td>342</td>
<td>Anti-c</td>
</tr>
<tr>
<td>9</td>
<td>Female</td>
<td>12</td>
<td>Thalassemia</td>
<td>498</td>
<td>Anti-E</td>
</tr>
<tr>
<td>10</td>
<td>Female</td>
<td>13</td>
<td>Thalassemia</td>
<td>271</td>
<td>Anti-Le*</td>
</tr>
<tr>
<td>11</td>
<td>Female</td>
<td>7</td>
<td>Thalassemia</td>
<td>214</td>
<td>Anti-D</td>
</tr>
</tbody>
</table>

*MDSs, myelodysplastic syndromes; CML, chronic myelogenous leukemia.

*Mean age of alloimmunized patients, 28.7 years. Mean number of units transfused in those alloimmunized patients, by disease: MDS, 97; CML, 24.5; thalassemia, 303.8.

The overall frequency of alloantibodies in our study was 5.5%, which is virtually identical to the reported rates of 5.3% by Karimi et al. and 5.2% by Sirchia et al. However, this rate is low among the wide range of frequencies reported in the literature. The highest reported frequency, in the United Kingdom, is 76%, followed by reports from the United States, at 42.9%, 34.8%, and 34%. Reports from Taiwan (37%), Kuwait (30%), and Greece (21.1%) were all higher than our frequency of 5.5%. Most of the aforementioned studies specifically focused on patients with thalassemia and SCD only.

We reported anti-E being most common (36.4%), followed by anti-c (27.2%). Other antibody specificities were anti-D (9.1%), anti-M (9.1%), anti-S (9.1%), and anti-Le (9.1%). Most of these antibodies are clinically significant and have been implicated in delayed hemolytic transfusion reactions (DHTRs). The high incidence of anti-E and anti-c reflects a heterogeneous distribution of these antigens in our population. Very few studies from India have reported antibody specificity due to alloimmunization. Various other researchers have reported a higher percentage of anti-E and anti-c in ethnic Asian populations. The
incidence of the R\textsubscript{1}R\textsubscript{1} Rh phenotype in India has been reported as 50% in the north and 70% in the ethnic Mongol population in east India. The alloantibodies of anti-Rh specificities that we detected in this study suggest an exposure to R\textsubscript{1}R\textsubscript{1} phenotype blood units, the prevalence of which is approximately 1% in the ethnic Indian population.

Anti-D alloantibody specificity reported in our study, in a case of thalassemia had multicentric transfusion support, per medical documents available with the patient. The reason for alloimmunization may be the presence of a partial-D variant in the patient, which could have led to anti-D formation during any of the previous exposures to Rh D-positive blood units. Anti-S, a clinically significant alloantibody that causes DHTR and hemolytic disease in newborns was observed in a case of CML. The prevalence of the S antigen is approximately 55% in the population of India. The other alloantibodies that we detected, namely, Anti-Le\textsuperscript{a} and Anti-M, were not clinically significant.

Female sex was associated with an increased risk of RBC alloimmunization. This finding is consistent with results of studies. This is biologically plausible because women are exposed to alloantigens during pregnancy and childbirth. However we eliminated this possibility because we excluded females who were or had been pregnant. We do not know whether our findings suggest an unknown pathophysiologic mechanism or whether random variation in the small number of subjects produced the association. Only 1 study reported no associations between sex and rate of alloimmunization.

The number of blood units transfused was associated with alloimmunization: 9 patients (12.3%) who had received more than the median 46 units had significantly higher rate of alloimmunization. Exposure to such a large number of blood donor units is likely to expose the blood recipient to common foreign RBC antigens, such as non-D Rh, Fy, Jk, and S.

 Patients in group 1 (thalassemia) were the majority of alloimmunized patients, comprising 7.3% of subjects. Higher rates of alloimmunization, namely 30%, was reported from ethnic Arab patients with thalassemia and 22% in patients with thalassemia who are of Asian descent and live in the United States. Conversely, lower alloimmunization rates have been reported from ethnic Arab patients with thalassemia (range, 19.0–63.0). A study conducted in 1983 reported 11% alloimmunization in AA. Immunosuppressive drugs such as cyclosporin or antithymocyte globulin (ATG) were not available during the previous studies, which may account for the higher rate of alloimmunization.

Moreover, 6.1% of multitransfused patients in our study with hematological malignancies were alloimmunized. Fluit et al reported alloimmunization in 11.8% of multitransfused recipients and Schonewille et al reported 9% seroprevalence in hematopoietic stem cell patients. Patients with myelodysplastic and myeloproliferative disorders may exhibit a high antibody response after transfusion, as we observed in our study population. However, none of the other hematopoietic stem cell patients (group 2) tested positive for alloantibodies. One study concluded that RBC alloantibodies can be lost or go undetected at some point in the progression or regression of disease in the patient. Earlier studies suggested that chronic lymphocytic leukemia, a lymphoproliferative disorder, protects against alloimmunization, as we also observed in our study. This is biologically plausible because the malignant clone may displace the functional T and B cells.

Although patients with AA are prone to develop alloantibodies, none of our AA patients did. The mean number of transfused units in this subgroup was 46.7 (range, 19.0–63.0). A study conducted in 1983 reported 11% alloimmunization in AA. Immunosuppressive drugs such as cyclosporin or antithymocyte globulin (ATG) were not available during the previous studies, which may account for the higher rate of alloimmunization.

Similarly, none of our patients with CKD developed alloantibodies, which contrasts with the previously
The use of cyclosporine and availability of recombinant human erythropoietin may explain the difference.

Aloimmunization rates vary with disease status: patients with SCD historically have rates of alloimmunization that approach 40%. None of the patients in group 4 who had SCD cases had RBC alloantibodies, but the number of SCD patients in our study was very small.

Prevention of RBC alloantibody formation in multitransfused patients extends life expectancy and reduces the amount transfused blood required. Several authors advocate that transfusions given to patients who are likely to become transfusion dependent over a longer period of time should be matched for antigens other than ABO and D in an attempt to prevent alloimmunization. Prophylactic matching for C, E, c, e, and K antigens theoretically can reduce clinically significant antibody formation by 70%; adding Fy\(^a\), Jk\(^a\), and S antigens will increase the preventive strategies of alloimmunization to almost 90%. However, no randomized controlled studies have yet been performed to evaluate this strategy. Further, regarding patients who receive transfusions in more than 1 center, receiving unmatched transfusions in 1 institution can negate the efficacy of antigen matching at another institution. Cost considerations and the question of whether extended antigen matching of transfused blood would truly benefit the patient make this an important issue for transfusion services.

Extended antigen matching (c, E, and K), intended to prevent the formation of most RBC antibodies in patients who chronically receive transfusions, has been advocated for selected patient populations. Some reports have shown a significant drop in the alloimmunization rate and delayed hemolytic transfusion reactions when extended antigen matched units are used in vulnerable patients. The patient population most likely to benefit from this strategy is still unclear. However, as in our study results, extended antigen matching for patients with thalassemia may be beneficial.

Because the frequency of RBC alloimmunization in our multitransfused patients is low, and because most alloantibodies are to Rh blood group antigens, we recommend that use of extended matched blood products in ethnic Indian multitransfused patients should be restricted to those who develop 1 or more RBC alloantibody. Otherwise, ABO- and Rh-phenotyped cross-matched compatible blood can be issued to multitransfused patients. Routine use of extended phenotyping of donor units is not warranted because the cost effectiveness of this approach has not been established.

**Figure 1**
Age-wise distribution of red blood cell alloimmunization and number of patients.

**Figure 2**
Age-wise distribution of red blood cell alloimmunization and mean number of units transfused.
References


