Occurrence of Allogeneic HLA and Non-HLA Antibodies After Transfusion of Prestorage Filtered Platelets and Red Blood Cells: A Prospective Study

By Vera M.J. Novotny, René van Doorn, Marian D. Witvliet, Frans H.J. Claas, and Anneke Brand

The incidence and consequences of HLA and non-HLA immunization were evaluated in 229 patients with aplastic thrombocytopenia. All patients were transfused with prestorage filtered red blood cells and platelets. On admission, 29 patients presented with HLA antibodies due to prior immunization by pregnancy and/or blood transfusions. Of the 200 patients showing no detectable HLA antibodies on admission, 164 could be evaluated. HLA antibodies developed in 2.7% (3 of 112) of the patients with a negative risk history of prior immunization. The occurrence of HLA antibodies in patients with a history of previous pregnancies or prior non-leukocyte-depleted blood transfusions (risk history positive) was 31% (18 of 52). Of the total of 48 patients who were or became alloimmunized, 92% (44 of 48) had a positive risk history. Ten patients with broad multispecific HLA antibodies with a panel reactivity (PRA) of greater than 70% required transfusions with HLA-matched platelets. Patients with HLA antibodies with lower PRA could be supported by random donor platelets. Two patients developed platelet-specific antibodies, causing transfusion refractoriness that necessitated selecting platelets by the absence of a platelet-specific antigen. Using prestorage leukocyte depletion of red cells and platelets with less than 5 × 10^6 residual leukocytes, 95% of the patients, including patients with a previous risk history or with HLA antibodies with low PRA, can be supported with random donor transfusions for the entire duration of their thrombocytopenic periods.

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A problem associated with long-term platelet supportive care in patients with aplastic thrombocytopenia is the development of a state of refractoriness resulting in poor platelet transfusion increments.1,2 Nonimmunologic factors are frequently associated with reduced platelet survival after transfusion. Platelet survival may also be impaired by the presence of circulating antibodies directed against antigens expressed on the platelet membrane. HLA antibodies are the most common cause of immunologic platelet transfusion refractoriness.

HLA antibody formation is the result of a process that requires both allogeneic HLA-class I and HLA-class II bearing antigen-presenting cells.3,4 As HLA-class II antigens are not present on the platelet membrane, primary HLA alloimmunization can be prevented by the use of leukocyte-depleted transfusions. The use of leukocyte-depleted transfusions appeared not to be effective in preventing HLA antibody formation in patients with a history of previous pregnancies.5,6

Several retrospective and prospective studies have reported the incidence of HLA alloimmunization, ranging from 50% to 90% after transfusions of non-leukocyte-depleted blood and from 0% to 28% after leukocyte-depleted transfusions.7,8 The minimal amount of leukocytes capable of inducing primary HLA alloimmunization is not known, but studies indicate that leukocytes should be reduced to less than 10^7 per transfuse.9,10,11 Leukocyte depletion to this level can be obtained by filtration of red cells and by filtration or differential centrifugation of platelets. Platelets prepared by differential centrifugation (DC-PRPs) contain less than 10^7 leukocytes per donor unit. By subsequent pooling of multiple units, leukocyte contamination is variable, but is generally below 5 × 10^7. Filtration offers a better reproducibility and results in lower leukocyte levels as compared with DC-PRPs.12,13,14

Leukocyte depletion from non-stored platelet transfusions and red cells may be more effective in reducing HLA immunization17,18 and in preventing viral transmission.20-22 In addition, prestorage in contrast to pretransfusion filtration reduces the incidence of febrile transfusion reactions due to cytokines released by leukocytes.24

In a prospective study, the rate and incidence of alloimmunization after transfusion with prestorage filtered platelets and red blood cells were evaluated in relation to the patients’ histories of transfusions and/or pregnancies.

MATERIALS AND METHODS

Patients. From 1989 to 1992, all patients with transfusion-dependent aplastic thrombocytopenia admitted to the University Hospital Leiden (Leiden, The Netherlands) were included in the study. A total of 229 patients conforming to the following criteria were entered: (1) thrombocytopenia due to bone marrow depression, (2) registration of transfusion history (with information about product transfused), (3) registration of previous pregnancies, and (4) registration of presence or absence of HLA and/or non-HLA antibodies on admission. Patients were considered eligible for evaluation of immunization by prestorage filtered blood components at the end of the study when (1) the total number of platelet transfusions was at least three during the thrombocytopenic period; (2) the follow-up period was ≥ 2 weeks, unless antibody formation occurred before 2 weeks; and (3) serum for antibody screening was available 2 weeks after the last transfusion. A total of 164 patients were eligible for evaluation of immunization.

Diagnosis and therapeutic protocols. The 229 patients entered in the study had the following diagnoses: severe aplastic anemia (AA; n = 34), acute nonlymphocytic leukemia (ANLL; n = 81), acute lymphocytic leukemia (ALL; n = 27), non-Hodgkin’s lymphoma (NHL; n = 37), Hodgkin’s disease (n = 11), myelodysplastic syndrome (MDS; n = 13); and other hematologic diseases (n = 26).

Patients with AA (n = 34) were treated with rabbit or horse antithymocyte globulin in combination with high-dose methylprednisolone and/or cyclosporin or were treated with corticosteroids and androgens. Eligible patients <45 years of age with an HLA identical
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sibling received an allogeneic bone marrow transplantation or if no siblings were available, an autologous bone marrow transplantation. A total of 58 patients received an allogeneic bone marrow transplantation and had the following diagnoses: AML (n = 23), ALL (n = 11), AA (n = 6), NHL (n = 2), Hodgkin’s disease (n = 1), and other hematopoietic diseases (n = 15). Autologous bone marrow transplantation was performed in 42 patients with the following diagnoses: ANNL (n = 17), ALL (n = 7), NHL (n = 13), Hodgkin’s disease (n = 4), and other hematopoietic diseases (n = 1). All patients were treated according to the protocols of the European Organization for Research and Treatment of Cancer (EORTC).

Prestorage filtered red blood cells. Citrate-phosphate-dextrose (CPD)-anticoagulated erythrocytes, from which the buffy coat was removed within 4 to 16 hours after withdrawal, were filtered within 36 hours after donation through a cellulose acetate filter (Celselect B1005-888 NPBI; Emmer Compasceum, Emmen, The Netherlands), leaving a maximum of 5 x 10⁹ leukocytes in 1 U of red blood cells. Filtered red blood cells were subsequently stored for up to 35 days in 100 mL SAG-M (877 mg NaCl, 900 mg glucose O·H₂O, 16.9 mg adenosine, 525 mg mannitol) preservation solution.

Prestorage filtered platelets. Filtered and subsequently stored platelet suspensions were prepared from buffy coats as previously described. After filtration, platelet-rich plasma (PRP) contained less than 10⁹ leukocytes and approximately 3.25 x 10¹¹ platelets. Filtered PRP was stored at room temperature on a horizontal reciprocator for a maximal period of 96 hours. The pH was checked before transfusion. Platelets were transfused if pH was within the range of 6.0 and 7.2. In 95% of the cases, the pH was ≥ 6.4. Before transfusion, PRPs were centrifuged, and platelets were resuspended in 20 mL of the residual plasma.

Indications for platelet transfusion. Platelets were transfused on a prophylactic basis when platelet count dropped below 10 x 10⁹/L or above this value in case of a therapeutic intervention or bleeding. Adult patients received a standard transfusion of 6 donor units per transfusate, and children received a dose of 1 donor unit per 10 kg bodyweight.

Evaluation of transfusion response. Transfusion results were expressed as corrected count increment (CCI), calculated as follows:

\[ [(\text{Posttransfusion Platelet Increment} \times 10⁹/L)] \times \text{Body Surface Area (m²)} \]
\[ \text{Platelets Transfused} \times 10¹¹ \]

Transfusion failure and platelet transfusion refractoriness. A transfusion was judged as a failure when the 1-hour posttransfusion CCI was less than 7.5 and/or the 20-hour posttransfusion CCI was less than 2.5. Refractoriness was defined as two repeated transfusion failures on fresh (less than 24 hours old) ABO-matched transfusions.

Calculation of the weeks exposed to platelet transfusions. The period in which patients received platelet transfusions was used to determine the weeks of exposure. To correct for long intervals between therapeutic courses without transfusion therapy, only weeks in which patients received platelet transfusions were used to calculate the exposure period.

Nonimmunologic factors. During transfusion episodes, patients were screened for the presence of nonimmunologic factors known to cause impaired platelet survival. The following factors were included: fever, infection, bleeding, venoocclusive disease, graft-versus-host disease, diffuse intravascular coagulation, antibiotic or anti-myotic drugs, and splenomegaly.

Antibody screening. Patient sera were screened on admission, every 2 months, in case of clinical refractoriness, and 2 weeks after the last platelet transfusion.

Lymphocytotoxicity assay. Lymphocytotoxic antibody screening of patient sera was performed by the standard National Institutes of Health (NIH) complement-dependent microlymphocytotoxicity test (LCT) against a panel of 21 to 50 selected donors carrying the majority of the defined HLA-A and HLA-B specificities. A positive reaction was defined as greater than 30% dead cells per well and expressed as percentage panel reactive antibodies (PRA): the number of positive donors divided by the number of donors tested. The screening was considered positive when the PRA was ≥ 20%.

Platelet immunofluorescence assay. Platelet IgG and IgM antibodies were determined with an immunofluorescence test (PIFT) against a panel of 10 randomly selected donors. The number of fluorescent platelets per 200 platelets was estimated. A reaction was defined as being positive when the percentage of platelets showing fluorescence was ≥ 20%. The PIFT screening was defined positive when ≥ two panel donors showed ≥ 20% fluorescence.

Monoclonal antibody immobilization platelet assay (MAIPA). Antibody specificity was defined using the MAIPA with CaHu-IgG. In selected cases, sera were tested for the presence of antibodies reacting with platelet-specific glycoproteins Ia/IIa, IIb/IIIa, P₃, IX, or HLA (w6/32). Positive MAIPA reactions were further tested against donor platelets typed for the following human platelet antigens: HPA1a1b, HPA2a2b, HPA3a3b, and HPA5a5b.

Statistical analysis. Analysis was performed with the Mantel-Haenszel χ² test.

RESULTS

Patients. Of the 229 patients entered in the study, 144 were males, 85 were females, and 34 were children aged less than 16 years (Table 1). HLA antibodies were detectable in 29 patients on admission, with platelet-reactive antibodies detected in one patient. Thirty-five patients were excluded from the analysis because they did not meet the criteria for evaluation. In 20 of these excluded patients, no serum was available 2 weeks after the last transfusion. All of these patients had sufficient increments after platelet transfusions. The other 15 patients were excluded because they received either less than three platelet transfusions or the transfusion period was less than 2 weeks.

The remaining 164 patients (males, 107; females, 57) were analyzed for the development of antibodies. These 164 patients received a median number of 17 platelet transfusions (range, 3 to 220). The median number of red cell transfusions was 18 (range, 0 to 158), and the median number of plasma transfusions was four (range, 0 to 198). The median exposure period was 7 weeks (range, 1 to 78).

HLA antibodies. In 29 patients HLA antibodies were detectable on admission (Table 2). This was the case in 14 patients with previous pregnancies. Of these 14 patients, four had also received prestorage filtered red blood cells and platelets that had been leukocyte-depleted with a differential centrifugation technique (DC-PRP). HLA antibodies were also present on admission in six patients with a history of nonfiltered red blood cell transfusions for reasons other than their actual disease and in eight patients who received DC-PRPs and prestorage filtered red cells before admission to our hospital.

Of the 164 patients (12%) lacking alloantibodies on admission, 19 (12%) developed HLA antibodies during the study period. HLA antibody formation was demonstrated in 3 of 112 patients (2.7%) with a negative history for transfusions or pregnancies. One of these three patients, a 60-year-
Table 1. Histories of the 229 Patients Entered in the Study

<table>
<thead>
<tr>
<th>History</th>
<th>No. of Patients Excluded</th>
<th>No. of Patients Eligible</th>
<th>HLA Antibodies (platelet antibodies)</th>
<th>Assessable</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3 TR and/or FU &lt;2 wks Serum11</td>
<td>14</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-leukocyte-depleted TR†</td>
<td>6</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonfiltered platelets†</td>
<td>8</td>
<td>12</td>
<td></td>
<td></td>
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<tr>
<td>No history of presensitization§</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>164</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: TR, platelet transfusions; FU, follow-up period.
* Transfusions because of actual disease (9/42 patients).
† Standard red blood cell transfusions because of other than actual disease.
‡ Platelets prepared in a differential centrifugation technique and prestorage filtered red blood cells.
§ No history of pregnancies or nonfiltered transfusions.
|| No serum available 2 weeks after the last transfusion.
¶ Assessable for antibody formation on prestorage filtered red cell and platelet transfusions.

old man, developed multispecific HLA antibodies after a period of 6 weeks and required HLA-matched transfusions. The second patient developed transient HLA antibodies disappearing after 4 weeks, during which time the patient could be sufficiently supported by random platelet transfusions. The third patient was a child who developed HLA antibodies with a PRA of 50% within 1 week after six platelet transfusions (15 donor units). He had a history of receiving prestorage filtered red blood cells but poststorage filtered platelets. In this case, the platelet dosage was increased, and no HLA-matched platelet transfusions were necessary. Nine of the 25 patients with prior pregnancies developed HLA antibodies. One of these nine patients also had received DC-PRPs and prestorage filtered red cells previously. Four of the other 16 patients who did not develop HLA antibodies had received DC-PRPs and prestorage filtered red cells before admission. In 6 of 15 patients with a history of non-leukocyte-depleted red blood cell transfusions, HLA antibodies were formed. Of 12 patients with a transfusion history of DC-PRPs and prestorage filtered red cells, one formed HLA antibodies. Altogether, HLA antibodies were detected in 48 patients; 29 had HLA antibodies on admission, and 19 developed HLA antibodies during the study period. In 44 of these 48 patients, a risk history of prior pregnancies and/or exposure to nonfiltered blood products was present.

Non-HLA antibodies. One patient showed platelet-reactive antibodies against all of the 10 panel cells in the PIFT on admission, while the LCT was negative. In this case, platelet antibody specificity using a MAIPA or PIFT against HPA-typed donors could not be defined. Platelet-reactive antibodies developed in 8 of the 164 patients. In two of these eight patients; antibodies were directed against platelet-specific antigens. In one patient typed as HPA-1a (Zw⁺)-negative, anti–HPA-1a was produced. In this case, HPA-1a (Zw⁺)-negative platelets had to be transfused. One patient of Japanese origin formed antibodies reacting with all of the platelet panel cells, including HPA-1a-negative donors, while coprecipitating with glycoprotein IIIa in the MAIPA. It was suspected that the antibodies were reactive with the HPA-4a (Yuk⁺) antigen. Unfortunately, family members were not available for confirmation.

The other six patients developed platelet-reactive antibodies with until now undefined specificity. In two cases these

Table 2. Comparison of HLA and Non-HLA Immunization Rate by History

<table>
<thead>
<tr>
<th>Patient History</th>
<th>No. of Patients With Antibodies</th>
<th>Total No. at Risk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HLA Antibodies</td>
<td>Non-HLA Antibodies</td>
</tr>
<tr>
<td></td>
<td>At Entry</td>
<td>During Study</td>
</tr>
<tr>
<td>Pregnancies with/without TR*</td>
<td>42</td>
<td>3</td>
</tr>
<tr>
<td>Non-leukocyte-depleted TR†</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>Nonfiltered platelets‡</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>No history of presensitization§</td>
<td>142</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>229</td>
<td>35</td>
</tr>
</tbody>
</table>

Abbreviations: TR, transfusions; NA, not assessable.
* Transfusions because of actual disease.
† Transfusions because of another than actual disease.
‡ Platelets prepared in a differential centrifugation technique and prestorage filtered red blood cells.
§ No history of pregnancies or nonfiltered transfusions.
|| One patient with a history of prestorage filtered red blood cells but poststorage filtered platelets.
antibodies were directed against IIb/IIIa and IIIa, respectively.

Refractoriness: Immunologic and nonimmunologic factors. Table 3 lists the presence of nonimmunologic and immunologic factors, in relation to the occurrence of platelet transfusion refractoriness. Patients included are the 164 evaluated for the occurrence of immunization and the 30 patients with detectable HLA or non-HLA (n = 1) antibodies on admission. These 194 patients received a median number of 17 platelet transfusions (range, 1 to 220). Transfusion refractoriness developed in 79 of these 194 patients. In 57 of 79 (72%), clinically compromising factors were present; in 31 of 79 (39%), HLA antibodies were present; and in 4 of 79 (5%), platelet-reactive antibodies were present. In two patients platelet-specific antibodies were demonstrated, and in the other two, specificity could not be defined. In 7 of 79 (9%) patients, neither immunologic nor nonimmunologic explanations could be found for the transfusion failures. In 115 nonrefractory patients, nonimmunologic clinical factors were present in 59 (51%) and HLA antibodies in 17 (15%), and in five (4%), non-specific platelet reactivity was demonstrated.

Clinical factors contributing to reduced platelet survival and non-HLA antibodies were equally divided among patients with and without refractoriness. The presence of HLA antibodies was more predictive for refractoriness. In 31 of the 48 patients (65%) with detectable HLA antibodies, refractoriness occurred, compared with 17 patients with HLA antibodies and sufficient transfusion increments (P < .001). Of the 31 refractory patients with HLA antibodies, 10 patients had multispecific HLA antibodies against more than 70% of the panel cells and required HLA-matched platelet transfusions. The other 21 patients with platelet refractoriness showed a PRA of 40% to 60%. They could be supported by frequently administered, higher dosages of random donor platelets. The 17 patients who showed sufficient increments in the presence of HLA antibodies had a PRA of 20% to 40% in the LCT.

Transfusion reactions. Of the 4,238 platelet transfusions administered, seven transfusion reactions were reported in six patients. Fever and chills were observed on two occasions, and chills with or without fever and hot flashes three times; these reactions were associated with absence of plate-let increment. Dizziness and abdominal pain occurred twice in the same patient. In the three patients without platelet increments, HLA antibodies were found. In the other three patients, all routine investigations of transfusion reactions including blood cultures were negative. The patient who showed two successive transfusion reactions associated with abdominal pains subsequently received 11 platelet transfusions during 4 weeks without reactions.

DISCUSSION

Several studies in patients with platelet transfusion-dependent thrombocytopenia showed a reduced incidence of HLA immunization after leukocyte-depleted blood products. The reported range in immunization is still wide, probably resulting from patient selection and different leukocyte depletion protocols used. The induction of HLA and non-HLA antibodies could be analyzed in 164 patients. Transfusion of prestorage filtered platelets and red blood cells was associated with 2.7% (3 of 112) HLA alloimmunization in risk history-negative patients and with 31% (16 of 52) in risk-positive patients. The combined results of all patients, including those with detectable HLA antibodies on admission, show that pregnancy and non–leukocyte-depleted transfusions are highly associated with the occurrence of HLA antibodies. Of all 48 HLA-immunized patients, 44 (92%) had a positive risk history.

The results show that prestorage leukocyte depletion to a level of less than 5 x 10^6 virtually prevents primary HLA alloantibody formation, but also show that after a prior contact with allogeneic leukocytes, a memory immune response is apparently boosted by HLA-class I–bearing blood components. This is in agreement with a prospective randomized study in patients with previous pregnancies, comparing standard and filtered single-donor platelet transfusions.

In the present study, prestorage filtered platelets were used. Although no studies are available comparing HLA alloimmunization after transfusion with prestorage versus poststorage filtered platelets in non-presensitized patients, our results differ from those of Williamson et al who found a higher rate of HLA immunization, i.e., 22.4% when stored platelet transfusions were administered by bedside filtration. However, the studies cannot be compared because Williamson et al found no differentiation between patients with or without a history of blood transfusions or pregnancies.

Our study confirms the results of van Marwijk Kooy et al, who showed in a randomized study that 42% of risk history-negative patients developed HLA antibodies after receiving platelets that had been leukocyte-depleted by centrifugation compared with 7% after filtered platelets.

Platelet antibodies were present on admission in one patient and developed in eight further patients. The exact specificity of the antibodies could be defined only in one case as allo-HPA-1a (Zw'), and in one patient there was a strong suspicion for allo-HPA-4 (Yk'). In the first case, HPA-1...
(Zw')-negative platelets were transfused, resulting in good platelet increments. In the second case, no compatible donors were available. In these two cases (one male and one female) antibodies were formed after four and three platelet transfusions, respectively; in the absence of a history of prior sensitization.

Non-HLA platelet-reactive antibodies are not associated with transfusion refractoriness, unless these are directed against platelet-specific antigens. In five of the seven patients with non-specific platelet antibodies, transfusion increments were sufficient. This was also observed by Godeau et al., and it cannot be excluded that such antibodies are drug-dependent or autoantibodies.

Clinical factors such as infection associated with fever and administration of multiple antibiotic or antimyotic drugs were not predictive for transfusion failures, as similar clinical conditions were present in 59 of the 115 nonrefractory patients. This was previously recognized by Friedberg et al.29 and Doughty et al.30

In 7 of the 79 refractory patients (9%), no clinical or immunologic explanation could be found to explain platelet refractoriness despite extensive analysis. Four of these seven patients had acute myelogenous leukemia (AML). Diagnoses for the other three patients were, respectively, MDS, NHL, and chronic lymphocytic leukemia.

Strong HLA antibodies with a broad PRA (greater than 40%), as was demonstrated in 31 patients, is significantly associated with platelet transfusion refractoriness. This was in contrast to 17 nonrefractory patients who showed a PRA below 40%. Although HLA antibodies were significantly associated with transfusion failures, their presence explains refractoriness in less than 40% of the 79 cases. HLA immunization is no longer the major cause of platelet transfusion refractoriness. This decreasing percentage of patients (less than 5%) dependent on HLA-matched donors calls into question the cost and effort of maintaining regionally HLA-type blood donor facilities exclusively for this purpose and argues for centralization and combined use of HLA-matched donors by blood banks and bone marrow donor registries.

As transfusion history is essential with respect to the occurrence of HLA-antibodies, all patients presenting with a disease requiring long-term platelet transfusion support should be transfused from the moment of diagnosis onwards with leukocyte-depleted red cells and platelets prepared in a technique that ensures leukocyte depletion to a level of less than 5 × 10⁶ per transfusate.

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Occurrence of allogeneic HLA and non-HLA antibodies after transfusion of prestorage filtered platelets and red blood cells: a prospective study [see comments]

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