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A preliminary comparison of the prevalence of transfusion reactions in recipients of platelet component from donors with and without HLA antibodies

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Abstract

Background—HLA antibodies have been implicated in transfusion related acute lung injury, but the probability that the transfusion of a blood component containing HLA antibodies will cause a reaction is not known. This study compared the prevalence of reactions associated with the transfusion of platelet components with and without HLA antibodies.

Study Design and Methods—This retrospective study tested 96 consecutive apheresis platelet donors for HLA class I and II antibodies. Matched-control donors without HLA antibodies were selected and records were reviewed to determine the proportion of components from each group that caused reactions. In addition, all apheresis platelet donors involved with 2 or more reactions were identified and tested for HLA Class I antibodies.

Results—Five of the 96 donors had antibodies to Class I or Class II antigens and of these, four had components transfused. The prevalence of reactions to components from these four donors with HLA antibodies and the 12 matched control donors without antibodies was similar (3 reactions to 167 transfusions or 1.8% vs 3 to 295 or 1.0% respectively, $p = 0.32$). A retrospective review of the transfusion records from all platelet donors found that components from 22 caused 2 or more reactions and 3 (13.6%) had antibodies to HLA Class I antigens compared to 4.2% of the consecutively selected donors ($p = 0.12$). None of the patients experienced transfusion related acute lung injury.

Conclusion—Reactions associated with transfusion of apheresis platelets containing HLA antibodies are unusual.

INTRODUCTION

Transfusion recipients experience a variety of reactions during or shortly after a transfusion.

¹ Some transfusions result in severe reactions such as acute lung injury that is characterized by shortness of breath, hypoxia and bilateral pulmonary infiltrates. In the 1980's the term "Transfusion Related Acute Lung Injury" or TRALI was first used by Popovsky and colleagues to describe these types of pulmonary transfusion reactions.^{2,3} TRALI has now surpassed ABO incompatibility as the leading cause of transfusion related mortality with the estimated mortality rate approximating 10%.⁴

The pathophysiology of TRALI has been linked to the transfusion of blood components containing HLA Class I, Class II, and neutrophil-specific antibodies which activate neutrophils and cause acute lung injury. Studies of prior recipients of blood components implicated in pulmonary transfusion reactions found that the transfusion of neutrophil antibodies results in reactions in up to 30% of transfusion recipients and TRALI in up to 22% of transfusion recipients.^{5,6} However, similar studies of blood components implicated in pulmonary transfusion reactions have found that the transfusion of HLA antibodies cause TRALI in less than 1% of subjects.^{7,8}

The aim of this retrospective case-control study was to compare the incidence of transfusion reactions between recipients of apheresis platelet components from donors with and without HLA antibodies. If HLA antibodies cause acute lung injury, we hypothesized that their transfusion should be associated with a higher incidence of milder reactions as well.

MATERIALS AND METHODS

Study Design

This study consisted of two parts. In the first part, 96 consecutive apheresis platelet donors were tested for HLA Class I and II antibodies in October of 2006. For each donor with HLA Class I or II antibodies, 3 control donors without HLA antibodies, but of a similar age and gender, were selected among the 96 donors. Three control donors were selected since it was expected that the prevalence of transfusion reactions would be low. For the donors with HLA antibodies and control donors, collection center and transfusion service records were reviewed to determine the total number of platelet components that were collected and transfused from each donor and the total number of platelet components from each donor that resulted in a transfusion reaction. Records from December 1999 to December 2006 were reviewed. Transfusion reaction records were reviewed for dyspnea, fever, rash, hives, change in blood pressure, change in pulse, change in blood gases, and chest x-ray changes. The National Heart Lung and Blood Institute (NHLBI) working group definition was used to determine if any of the transfusion reactions met the criteria for TRALI.⁵ The proportion of transfusions resulting in reactions was compared among donors with HLA antibodies and controls without HLA antibodies.

For the second part of the study all transfusion reaction records from December 1999 to December 2006 were reviewed and apheresis donors whose platelet components resulted in two or more reactions were identified. The donors implicated in 2 or more transfusion reactions were tested for antibodies to HLA Class I and neutrophil-specific antigens. The prevalence of leukocyte antibodies was compared among donors involved with 2 or more transfusion reactions and the 96 consecutive apheresis platelet donors.

All apheresis platelet components transfused were leukocyte reduced prior to storage. Recipients were not tested for the presence of the cognate HLA antigen.

Testing for HLA antibodies

Whole blood samples were collected in 10 mL red top tubes from apheresis platelet donors at the time of donation after obtaining informed consent. Serum was prepared and stored in frozen aliquots at -20°C in order to allow for batch testing. Testing for IgG antibodies to both HLA Class I and Class II antigens was performed using a solid phase mixed antigen ELISA assay (LAT Mixed, One Lambda, Inc, Canoga Park, CA). All samples reactive in the mixed antigen assay were also tested in another ELISA assay (LAT 1288, One Lambda) to determine the panel reactive antibody and specificity. For both ELISA assays, 10 μL of diluted (1:3) sample was added to 96-well Terasaki trays and the trays were incubated for one hour at $20-25^{\circ}\text{C}$.

After washing, 10 uL of alkaline phosphatase conjugated anti-human IgG was added to each well and incubated for 40 minutes at 20–25°C. After another wash the conjugate, 10 uL of colorimetric enzyme substrate, was added to each well and incubated for 10–15 minutes at 37°C. Stop reagent was added and the plates were read with an ELISA reader (ELX 800 microplate reader, One lambda).

Testing for neutrophil antibodies

Serum was tested for neutrophil antibodies by a reference laboratory using granulocyte agglutination and granulocyte immunofluorescence assays (American Red Cross, North Central Blood Services, St. Paul, Minnesota).

Statistical Analysis

The prevalence of HLA antibodies and transfusion was compared between groups using chi-square or Fisher exact methods.

RESULTS

Prevalence of reactions in recipients of platelet components with HLA antibodies

A total of 96 consecutive apheresis platelet donors were tested for antibodies to HLA Class I and II antigens. Their age ranged from 21 to 73 years with a mean of 53 years. Among the 96 donors were 55 men and 41 women. We found 5 donors with antibodies to either Class I or Class II HLA antigens. Among the 5 donors, 3 had antibodies to both HLA Class I and Class II antigens. All five donors with HLA antibodies were female (Table 1).

Among the five donors with antibodies to HLA Class I or Class II antigens, four donated components that were transfused. A total of 167 transfused components from these four donors with HLA Class I or Class II antibodies resulted in 3 or 1.8% transfusion reactions all of which were allergic reactions (Table 1). Among the 130 transfused components with HLA Class I antibodies, 3 or 2.3% resulted in transfusion reactions. Among the 164 transfused components with HLA Class II antibodies, 3 or 1.8% resulted in transfusion reactions and among the 127 transfused components with antibodies to both Class I and Class II antigens, 3 or 2.4% caused transfusion reactions.

The 12 matched control donors without HLA antibodies donated a total of 295 components that were transfused (Table 2). Among these 295 transfused platelet components, 3 or 1.0% resulted in transfusion reactions; all of which were allergic. There was no significant difference in the incidence of transfusion reactions among recipients of platelet components from donors with and without HLA antibodies ($p = 0.32$).

With the number of components transfused in the HLA antibody positive and HLA antibody negative groups (167 and 295 respectively), the approximate statistical power for detecting an increased rate of reactions in the HLA antibody positive group, using a standard 2-sided test with level of significance of 0.05, was: 40% if the incidence of reactions in HLA antibody positive group was 4 times higher (i.e. 4% vs. the observed 1.0% rate in the HLA antibody negative group); and 80% (a common study design target) if the incidence in the HLA antibody positive group were 6.25 times higher. If the incidence of reactions in the HLA antibody positive group were only 2% to 3%, the power would be below 20%. With twice as many components transfused in each group, a 3-fold increase would have been detected with approximately 50% power, and a 4-fold increase would have been detected with almost 80% power.

Prevalence of HLA antibodies in donors whose components frequently caused transfusion reactions

A retrospective review of transfusion reaction records from December 1999 to December 2006 identified 24 donors whose platelet transfusions were associated with 2 or more transfusion reactions. Platelet components from one donor resulted in 9 transfusion reactions and these components contained an antibody to HNA-2a. The nature of these transfusion reactions has been previously reported⁶ and this donor was excluded from the study. Another donor's platelet components resulted in two transfusion reactions. This particular donor was included in the first portion of the study and therefore was excluded from this analysis. Among the remaining 22 donors, 2 platelet components from each of 19 donors caused reactions and 3 platelet components from each of 3 donors caused transfusion reactions. None of the 22 donors had antibodies to neutrophil-specific antigens, but antibodies to HLA Class I antigens were found in 3 donors (13.6%) (Table 3). In the first portion of this study 96 donors were tested for HLA antibodies and components from 95 were transfused. The prevalence of antibodies to HLA Class I antigens in the 22 donors with 2 or more transfusion reactions was similar to the prevalence in the 95 consecutive people donating platelet components that were transfused (13.6% versus 4.2%, $p = 0.10$). Among the donors implicated in 2 or more transfusion reactions, there was no difference in the incidence of transfusion reactions to platelet components from the 3 donors with HLA antibodies and the 19 donors without HLA antibodies [7 of 126 (5.6%) vs 40 of 1,012 (3.9%) respectively, $p > 0.05$].

Reactions to the transfusion of plasma containing blood components are more common in recipients of components from female donors.⁷⁻¹⁰ Interestingly, only 6 of the 24 donors whose components caused 2 or more transfusion reactions were female (25%) compared to 43% of the consecutive donors.

DISCUSSION

This retrospective study did not detect a case of TRALI among 167 patients receiving platelet components with HLA Class I or II antibodies. There was a more than 2-fold increase in transfusion reactions in patients given platelets with HLA antibodies compared to those given components without HLA antibodies. However, the study was not large enough to detect a significant difference. Based on the prevalence of HLA antibodies in platelet donors and the frequency of transfusion reactions, in order to detect a difference in the prevalence of transfusion reactions associated with the transfusion of platelets, a retrospective study would have to include 5 to 10 times more donors. Since some transfusion reactions are not reported to the transfusion service, a prospective study may require the analysis of fewer donors.

Among the 167 transfused apheresis platelet components from 4 randomly identified donors with HLA antibodies none caused TRALI. This is similar to two other studies. One study found only one case of TRALI in 211 recipients of blood components from donors with HLA antibodies.¹¹ Another study of prior recipients of blood components from a TRALI-implicated donor with broadly reactive antibodies to HLA Class I and II antigens identified TRALI in 1 in 103 patients.¹² It is estimated that 1 in 1,000 to 1 in 10,000 transfusions cause TRALI. Our study and the two others suggest that the transfusion of HLA antibodies with a platelet component is associated with only a slight increase in risk of transfusion reactions. However, larger, prospective, controlled studies are needed to more precisely define any increased risk associated with the transfusion of HLA antibodies.

We also compared the prevalence of HLA antibodies in platelet donors associated with 2 or more transfusion reactions with the prevalence in randomly selected apheresis donors. Donors implicated in 2 or more transfusion reactions were greater than 3-fold more likely to have HLA antibodies, but this difference did not reach statistical significance. The majority of donors

whose platelet components were implicated in multiple transfusion reactions contained no HLA or neutrophil specific antibodies, suggesting that other factors are responsible for most transfusion reactions.

In conclusion, we identified a trend toward a greater prevalence of allergic reactions in recipients of platelet components with HLA antibodies. However, additional, larger prospective, controlled studies are needed to better characterize the effects of the inadvertent transfusion of HLA antibodies with plasma-containing blood components. Recipient HLA typing should be included. Since few recipients of platelet components with HLA antibodies had allergic reactions or any reactions for that matter, any decision for universal exclusion of donors with HLA antibodies is premature. A decision to exclude donors with HLA antibodies should be made on an individual basis.

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Table 1
Donors with HLA antibodies, the number of components from each donor that were transfused, and the number associated with a transfusion reaction among 96 consecutive platelet apheresis donors

Donor	Gender	Age	Race	Class I Antibody Results		Class II Antibody Results		Components Transfused		Components Associated with Reactions	
				Screen	PRA	Screen	PRA	Number		Number	Percent
852	F	51	Caucasian	Neg	1	Pos	59	37		0	0
869	F	36	African American	Pos	98	Neg	0	3		0	0
871	F	52	Caucasian	Pos	82	Pos	26	82		1	1.2
892	F	66	Caucasian	Pos	75	Pos	80	45		2	4.4
891	F	57	Asian	Pos	87	Pos	57	0		0	--

PRA = Panel reactive cells

Pos = positive

Neg = negative

Control donors without HLA antibodies, the number of components from each donor that were transfused, and the number associated with transfusion reactions

Donor	Gender	Age	Race	Components Transfused		Components Associated with Reactions		Type of Reaction
				Number		Number	Percent	
841	F	50	Caucasian	12		0	0	-
865	F	55	Caucasian	6		0	0	-
867	F	47	Caucasian	24		0	0	-
919	F	36	Caucasian	16		1	6.2	Allergic
915	F	40	Caucasian	8		0	0	-
858	F	42	Caucasian	14		1	7.4	Allergic
859	F	51	Caucasian	39		1	2.6	Allergic
868	F	52	Caucasian	10		0	0	-
914	F	54	Caucasian	49		0	0	-
803	F	67	Caucasian	16		0	0	-
916	F	62	Caucasian	77		0	0	-
860	F	67	Caucasian	24		0	0	-

Donors whose platelet components were associated with two or more transfusion reaction and who had HLA Class I antibodies: age, gender, number of platelet transfusions, and proportion of platelet transfusions associated with reactions

Table 3

Donor	Gender	Age	HLA antibody PRA (%)	Components Transfused		Components Associated with Reactions	
				Number	Percent	Number	Percent
1	M	47	28	17		2	11.8
2	M	57	40	27		3	11.1
3	F	52	90	82		2	2.4

PRA = panel reactive cells