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Screening plateletpheresis donors for HLA antibodies on two high throughput platforms and correlation with recipient outcome

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Abstract

Background—To determine the prevalence and impact of transfusing plasma containing leukocyte antibodies, we compared two high-throughput HLA antibody screening assays, and prospectively examined the medical records of all platelet recipients to detect subtle manifestations of TRALI and other transfusion reactions.

Methods—Serum samples from 136 plateletpheresis donors were tested for HLA Class I and II antibodies using microbead (Labscreen PRA) and microchip (Dynachip) assays. Electronic medical records of all recipients were reviewed for vital signs and nursing documentation before and after transfusion.

Results—In the microchip assay with a cutoff value of 0.25, 2.9% of samples were positive for Class I, and 8.9% for Class II antibodies; with a cutoff value of 0.1, the results were 14.9% and 21.6% respectively. In the microbead assay (NBG ratio 1.5), 15% were positive for Class I, and 21% for Class II antibodies. The prevalence of HLA antibodies was 17% in donors without pregnancy or transfusion history, and 47% in donors with such history. The platelets were transfused in 265 episodes to 67 patients. There were no reported reactions; however, symptoms or vital sign changes were noted in 7 transfusion episodes. The incidence of reactions was 2.7% (2/75) for antibody-positive units and 2.6% (5/190) for antibody-negative units.

Conclusions—Microbead and microchip assays yielded similar results. The prevalence of HLA antibodies was greater in donors with a history of pregnancy or transfusion, but no increase in the incidence of transfusion reactions was noted in recipients of components from donors with HLA antibodies.

Keywords

HLA antibodies; neutrophil-specific antibodies; apheresis platelets; transfusion reactions; transfusion related acute lung injury

Introduction

Transfusion-related acute lung injury (TRALI) is a major cause of transfusion-related mortality and morbidity. It is associated with the presence of leukocyte antibodies in donor

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plasma. Transfusion guidelines in the United States now recommend that preventive measures be implemented by all blood donor centers to prevent the transfusion of plasma containing white blood cell antibodies to reduce the incidence of TRALI. HLA antibodies are more prevalent in multiparous women; in the United Kingdom and many U.S. blood centers, plasma for transfusion is no longer collected from multiparous women. The supply of apheresis platelets is more precarious and therefore TRALI preventive strategies applicable for apheresis platelets have centered around HLA antibody screening and discarding or not collecting components from women with antibodies. Earlier work by our group¹ piloted the use of a high-throughput HLA antibody screening assay in 96 consecutive apheresis platelet donors; the microbead assay detected antibodies in almost half of all donors; using ELISA as the gold standard, 5/96 (5%) of donors had HLA Class I or II antibodies. The prevalence of transfusion reactions to components from the donors with HLA antibodies was no different from that in 12 matched control donors without antibodies (1.8% versus 1.0%),² and there was no report of TRALI during this period.

The purpose of this study was to compare the use of two high-throughput assays for HLA antibody screening, and to further investigate the role of HLA antibodies in TRALI by prospectively examine the medical records of all platelet transfusion recipients to look for subtle symptoms and signs that may have been unrecognized as a transfusion reaction.

Materials and methods

Platelet donor selection

Whole blood samples were collected in 10-mL red top tubes from 137 volunteers donating apheresis platelets at the Platelet Center, Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, MD after appropriate informed consent. Any history of pregnancy or transfusion was ascertained from the platelet donor during the donor interview. All consecutive platelet donors from one week per month were selected for the study; each donor was tested once during the course of the study period. The protocol was approved by the Institutional Review Board of the National Heart Lung and Blood Institute (NHLBI), NIH, Bethesda, MD.

Transfusion recipient tracing

Recipients from all study platelet donors were identified for review of their electronic medical record: for each platelet transfusion episode, vital signs and nursing documentation before and within 6 hours after the index transfusion were examined for fever, chills, rigors, shortness of breath, hypoxia, skin rash, hypotension, and hypertension. Fever is defined as temperature greater than 37°C and an increase of $\geq 1^\circ\text{C}$ compared to the pretransfusion temperature. Hypoxia is defined as a fall in hemoglobin oxygen saturation of $\geq 10\%$, an increase in respiratory rate of ≥ 10 breaths/minute, the initiation of oxygen therapy or increase in the amount of supplemental oxygen administered. Hypotension is defined as a fall in systolic blood pressure (SBP) to < 80 mmHg and a fall of ≥ 20 mmHg compared to pretransfusion SBP. Hypertension is defined as a SBP of ≥ 130 mmHg and an increase of ≥ 20 mmHg compared to pretransfusion SBP.

Donor testing for HLA antibodies

Serum was separated from the whole blood and stored frozen at -20°C for batch testing. Our existing high throughput method uses Luminex microbeads coated with HLA Class I and II antigens (Labscreen PRA Class I and II, One Lambda, Canoga Park, CA) added to undiluted serum. Determination of cutoffs for reactive samples was calculated by manufacturer software using normalized data; the normalized background (NBG) ratio makes use of reactions of the test sample and negative control serum with each bead, and

with a negative control bead. The cutoff for positive samples used for this study was the NBG ratio recommended by the manufacturer.

All samples were tested in parallel on a second high throughput microchip platform. Dynachip (Invitrogen, Carlsbad, CA) uses a glass microchip with purified HLA antigens fixed to the surface of the chip in a self-contained walk-away platform. Several dilutions of known patient samples were tested to determine the optimal dilution to be used for donor screening.

Donor testing for neutrophil-specific antibodies

Selected samples that were strongly positive for HLA antibodies by both methods were tested for human neutrophil antibodies (HNA) by a reference laboratory using granulocyte agglutination (GA), MAINA (monoclonal antibody immobilized neutrophil antigens) and granulocyte immunofluorescence (GIF-flow) assays (American Red Cross Neutrophil Serology Reference Laboratory, St. Paul, MN). The MAINA assay permits the detection of antibodies to specific neutrophil glycoproteins in the presence of HLA antibodies.

Donor screening for high-titer ABO antibodies

In response to several platelet-mediated hemolytic transfusion reactions, our transfusion service implemented universal donor screening for high-titer ABO antibodies using a single dilution cutoff (initially 1:150, then increased to 1:250 one year later), shortly after this study concluded. Group O donors in this study were cross-referenced against their most recent ABO high-titer screening result.

Statistical analysis

Comparison of HLA antibody prevalence between donor groups was performed using chi-square tests

Results

A total of 134 plateletpheresis donors comprising 85 males and 49 females underwent HLA antibody screening representing four weeks of testing over a four-month period; results from 3 other donor samples were invalidated due to unacceptable assay controls. Approximately two-thirds (88/134) of donors had no risk factors for immunization (pregnancy or transfusion). In pilot testing of the microchip assay, several dilutions of samples from patients known to be HLA alloimmunized were tested. A dilution of 1:12.5, with a cutoff value of 0.25, was found to yield similar results from the microchip assay and our laboratory's gold standard of ELISA assay. A dilution factor of 12.5 was used subsequently for donor testing with the microchip assay, and we analyzed results using two different cutoff values, 0.1 and 0.25. Using a cutoff value of 0.25, 4 of 134 (2.9%) samples were positive for Class I antibodies, and 12/134 (8.9%) samples were positive for Class II antibodies. Using a cutoff value of 0.1, 14.9% of samples (20/134) were positive for Class I antibodies, and 21.6% (29/134) samples were positive for Class II antibodies.

Next, results were considered in terms of testing the same sample in the microbead assay using a NBG ratio of 1.5, taking into account donor risk factors for immunization (Table 1). Fifteen percent (20/134) of all donors were positive for Class I antibodies, and 21% (28/134) were positive for Class II antibodies. These results are almost entirely concordant with those of the microchip assay using the lower cutoff of 0.1. Among the group of 88 donors without pregnancy or transfusion history, 17% (15/88) were HLA antibody positive (Class I or II or both); among the group of 38 donors with a history of pregnancy or transfusion, 47% (18/38) were HLA antibody positive. This difference is statistically significant ($p < 0.001$).

Four samples that were HLA antibody positive by both the microchip and microbead assays were tested for HNA antibodies. One sample was positive by GIF-flow and MAINA, with anti-HNA-1a specificity. The three other samples had weak reactivity by GIF-flow with no identifiable specificity and were not reactive in the MAINA assay. A fifth sample that was HLA antibody negative was tested for HNA antibodies because of a recipient symptom (hypoxia); no HNA antibodies were detected.

Transfusion recipient tracing

The 134 units of apheresis platelets were transfused in 265 episodes to 67 patients. There were no reported transfusion reactions involving any of the platelet components collected from the study donors. However, significant changes in the recipients' vital signs were noted in 7 transfusion episodes (Table 2). Two episodes involved platelet components that were from donors with HLA antibodies. The recipient of the first antibody-positive plateletpheresis unit had been diagnosed with pulmonary hemorrhage by bronchoalveolar lavage the day before, and experienced an episode of dyspnea two hours after platelet transfusion. The dyspnea resolved with bronchodilator therapy and was not evaluated by a chest radiograph. The recipient of the second antibody-positive unit was febrile pre-transfusion and his temperature continued to rise post transfusion; half of the plateletpheresis component had been transfused to another recipient, who did not have any symptoms or signs of a reaction. One recipient of a unit negative for both HLA and HNA antibodies developed hypoxia 15 minutes into the transfusion; chest x-ray and chest CT showed a new left lower lobe dense consolidation consistent with aspiration or pneumonia. The incidence of adverse events temporally associated with the transfusion was 2.7% (2/75) for HLA antibody-positive units and 2.6% (5/190) for antibody-negative units.

Platelets from the donor with anti-HNA-1a were transfused to one recipient, who did not have any symptoms or signs of transfusion reaction. Lookback review of 17 prior donations from this donor did not reveal any reports of transfusion reactions: there were 27 transfusion episodes involving 24 recipients.

Correlation between donors with high-titer ABO antibodies and donors with HLA antibodies

Sixty of the 134 donors were group O and had been screened for high-titer ABO antibodies, 28% (17/60) were classified as positive. Of the 17 donors with high-titer ABO antibodies, 47% (8/17) screened positive for HLA antibodies by either the microbead or microchip method. Of the 43 donors without high-titer ABO antibodies, 19% (8/43) tested positive for HLA antibodies. This difference is statistically significant ($p < 0.05$).

Discussion

TRALI is well recognized as a significant cause of transfusion-related morbidity and mortality. It accounted for half of all transfusion fatalities reported to the FDA 2005–2008 (www.fda.gov/cber/fatal08.htm). The initial description and case reports of TRALI,³ suggested that plasma-rich components such as fresh frozen plasma (FFP) and platelets were more likely to be associated with TRALI than red blood cells and this has been confirmed by retrospective and prospective case-controlled studies.^{4,5} Epidemiologic observations and animal models have pointed to the pathogenetic role of donor-derived anti-leukocyte antibodies as well as neutrophil-priming lipids that accumulate during blood storage in the development of TRALI.⁶ Leukocyte antibodies, which include HLA and granulocyte antibodies, are known to be commonly present in individuals exposed to foreign antigens during pregnancy or transfusion. Granulocyte antibodies activate the recipient's neutrophils in the pulmonary circulation, leading to pulmonary endothelial damage. A recent

prospective case-control study of TRALI in critically ill patients transfused in a tertiary care medical intensive care unit noted the development of TRALI in 8% of over 900 patients; compared to control subjects who were transfused but did not develop subsequent respiratory complications, the TRALI subjects were more likely to have received plasma or platelet components.⁷ The amount of plasma from female donors, and the amount of plasma from parous female donors were significant risk factors for TRALI, as was the number of transfused units positive for HLA Class II antibodies and granulocyte antibodies.⁷

A 10-year report from the hemovigilance system in the UK found that in 2003 the TRALI risk per component was 7 times higher for FFP and 8 times higher for platelets than for red blood cells.⁸ Based on these observations, the National Blood Service implemented a strategy in 2003 to preferentially use plasma from male donors for the production of FFP and for suspension of pooled platelets. In 2005–2006, the risk of probable TRALI attributable to FFP decreased 80%, from 15.5 to 3.2 per million units issued. In the United States, the AABB recommended TRALI risk reduction measures for plasma and apheresis platelets since 2007 and 2008, respectively. Many US blood centers have switched to male-only plasma for transfusion (except for group AB donors). The supply of apheresis platelets is more precarious and a TRALI risk reduction plan has proven more problematic.

At our center, we previously found 5 out of 96 consecutive apheresis platelet donors have HLA antibodies when tested via an ELISA assay.² These antibody-positive donors contributed 167 transfused components over 7 years with 1.8% incidence of reported transfusion reactions, all allergic (no TRALI); this incidence is not significantly different from that of patients who received antibody-negative platelet transfusions from matched control donors. We then tested the 96 donors by a high through-put mixed-antigen microbead flow analyzer method, confirming with a microbead PRA assay: 44% of donors had Class I or Class II antibodies; as mentioned above, there was no report of TRALI from these 96 donations.¹

Our current study incorporates donor pregnancy and transfusion history in relation to HLA antibody screening on two high-throughput platforms. The microchip assay is an automated assay combining ELISA with microarray technology. When using a less sensitive cutoff of 0.25 with this assay, the results were comparable to our traditional ELISA, our gold standard for patient testing. At a more sensitive cutoff of 0.1, the results were comparable to the LabScreen PRA assay: both methods identified 15% of donors to be Class I positive; LabScreen identified 21% of donors to be Class II positive, while Dynachip identified 22% of donors to be Class II positive, with 88% concordance between the two methods. Consistent with other studies, donors with a history of pregnancy or transfusion were much more likely to be antibody-positive than donors without such history.

The prevalence of alloimmunization is higher in our study than in two larger studies. Powers reported a prevalence of 25% in 1009 female donors, half of whom had a history of pregnancy, using an NBG cutoff of 2.4 (LabScreen mixed antigen assay).⁹ Importantly, pregnancy history had a negative predictive value of 94%. The Leukocyte Antibody Prevalence Study (LAPS) screened 3992 parous females, 1816 nulliparous females, 1138 non-transfused males, and 895 transfused males: HLA antibody prevalence was 24%, 1.7%, 1.0%, and 1.7% respectively.¹⁰ Importantly, the LAPS study calculated the NBG cutoff to be the mean plus 3 standard deviations of the log-transformed distribution of NBG values in the cohort of non-transfused males. In our current series, we confirmed HLA antibody specificity in some non-transfused males, consistent with the observation that HLA antibodies may be naturally occurring.¹¹ One could speculate that these naturally occurring HLA antibodies are unlikely to be implicated in TRALI, given the epidemiologic association between TRALI cases and female plasma.

Our cumulative experience with screening for HLA antibodies and prospective followup of transfusion outcomes suggests that HLA antibodies infrequently cause TRALI, even though our population of platelet transfusion recipients comprises many patients with hematologic malignancies, a group considered high risk for pulmonary transfusion reactions.¹² Specifically, the incidence of adverse events temporally associated with the platelet transfusions was the same for antibody-positive units compared to antibody-negative units. Arguably, our recipient population comprising complex hematology/oncology patients almost always had underlying conditions that increase the risk of fever or hypoxia at any given time. In the absence of a full investigation which would include a hemolysis workup, for instance, we have chosen to consider all these events as possibly related to transfusion. The primary goal of our study was to determine if there is a significant difference in transfusion reactions - reported and subclinical -among recipients of platelets with and without HLA antibodies. Since we did not find a significant difference in reactions between the two groups, we did not pursue antibody specificities and the corresponding presence/absence of cognate antigens on the recipients.

We selected 5 samples for HNA testing; 4 with strong HLA antibodies in both the microbead and microchip assays and one from a donor whose transfusion was associated with dyspnea. Only one of the five samples was definitely positive for HNA-1a antibody, in association with HLA Class I and II antibodies, in a parous female donor. The three other samples that were tested for HNA antibodies based on high PRAs were weakly positive in the GIF assay and no antibody specificity was detected. All three donors were parous females. The platelet components from these four donors and 17 lookback components from the donor who had anti-HNA-1a were not associated with any adverse transfusion outcomes. However, there is strong evidence from other lookback studies and hemovigilance data that HNA-3a and HNA-2a antibodies consistently cause pulmonary transfusion reactions.¹³⁻¹⁸ In the German hemovigilance experience, HNA-3a antibodies were implicated in 6/10 fatal TRALI cases (HLA Class II antibodies in the remainder).¹⁷ Therefore, donors who are demonstrated to have neutrophil-specific antibodies should be deferred from plasma or apheresis platelet donation¹⁹.

Antibodies specific for HNA-1a and HNA-1b more often cause alloimmune neonatal neutropenia than those specific for HNA-2a or HNA-3a,²⁰ yet the latter antibodies are more likely to cause TRALI. This may be due to the fact that plasma contains soluble HNA-1a and -1b antigens,²¹ but not HNA-2a or HNA-3a. Passively transfused antibodies specific for HNA-1a or HNA-1b may react with soluble antigen rather than with neutrophil-associated antigen.

It is often speculated that TRALI is under-recognized and/or under-reported, since transfusions often occur in patients with complex underlying medical conditions, but we found no evidence of this. We did find evidence that certain symptoms and signs temporally associated with transfusion are under-reported as potential transfusion reactions. There were no reported transfusion reactions in association with the platelets collected from the study donors, but review of pre- and post-transfusion vital signs uncovered four instances of fever, one case of urticaria, and two cases of respiratory symptoms temporally associated with the platelet transfusion within the 6-hour window. While we do not believe any of these 7 cases represents TRALI, fever, allergic and respiratory symptoms in relation to platelet transfusion do merit appropriate evaluation. Electronic medical records lend themselves to built-in mechanisms to prospectively identify potential adverse outcomes from transfusion^{22,23}.

We hypothesized that HLA antibodies may be associated with high-titer ABO antibodies, both produced in response to cross-reactive epitopes found in microorganisms or cellular material through such immune stimuli as vaccination. Although our ABO high-titer

screening method is a single dilution cutoff and not stepwise serial titrations, and the dataset is small, the apparent association we detected merits exploration in future studies. There is also at least one report of HLA alloimmunization being associated with RBC alloimmunization in sickle cell patients.²⁴

In summary, high through-put methods for HLA antibody screening appear to be highly sensitive; specificity is enhanced and cost-effectiveness improved if antibody screening is performed only in parous female donors. Individual facilities will need to determine their own cutoff with each testing method to minimize the impact on apheresis platelet inventory and donor retention, although most HLA laboratories will be familiar with PRA testing in organ transplant recipients. A cutoff based on NBG values of a reference group of nontransfused male blood donors is a reasonable option, while recognizing the possibility of naturally occurring HLA antibodies.

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Table 1

HLA antibody screening of 134 donors by high throughput microbead assay

	Number (%) of Donors with Reactive Assays		
	All Donors (n=134)	Donors with pregnancy or transfusion (n=38)	Donors without pregnancy or transfusion (n=88)
Class I Antibody only	7(5)	5 (13)	2 (2)
Class II Antibody only	15(11)	7 (18)	7 (8)
Class I and II antibodies	13(10)	6 (16)	6 (7)
Any HLA antibody	35(26)	18 (47)	15 (17)

Table 2

Summary of *adverse events* (potential transfusion reactions) in 7 recipients of plateletpheresis transfusions and the results of donor testing for HLA and HNA antibodies

Recipient symptoms	Timing after platelet transfusion	Donor HLA antibody screen	Donor HNA antibody screen
Tachypnea, cough (known diagnosis of pulmonary hemorrhage)	2 hours	Positive (<i>Class II</i>)	Not tested
Fever	Received RBC before platelets	Negative	Not tested
Pruritus, urticaria	1 hour	Negative	Not tested
Fever	3.5 hours	Negative	Not tested
Fever	1.5 hour	Negative	Not tested
Hypoxia	15 minutes	Negative	Negative
Fever (patient was febrile pre-transfusion)	3.5 hours	Positive* (Class I & II)	Not tested

* Second recipient of this unit did not have a reaction