Published in final edited form as:

Transfusion. 2009 September ; 49(9): 1825–1835. doi:10.1111/j.1537-2995.2009.02206.x.

The Effect of Previous Pregnancy and Transfusion on HLA Alloimmunization in Blood Donors: Implications for a Transfusion Related Acute Lung Injury (TRALI) Risk Reduction Strategy

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Abstract

Background—Antibodies to human leukocyte antigens (HLA) in donated blood have been implicated as a cause of transfusion related acute lung injury (TRALI). A potential measure to reduce the risk of TRALI includes screening platelet apheresis donors for HLA antibodies. The prevalence of HLA antibodies and their relationship to previous transfusion or pregnancy in blood donors was determined.

Study design and methods—8171 volunteer blood donors were prospectively recruited by 6 U.S. blood centers from December 2006 to May 2007. Donors provided a detailed history of pregnancy and transfusion, and a sample for HLA class I and II antibody testing by multi-antigen bead flow analysis.

Results—8171 donors were enrolled, 7920 (96.9%) had valid HLA antibody test results and 7841 (99%) of those had complete pregnancy and transfusion information. The prevalence of any HLA antibody was similar in non-transfused (n=1138) and transfused (n=895) men, 1.0 vs. 1.7% (p=0.16).

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Conflicts of Interest: The authors had no conflicts to disclose.

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HLA antibodies were detected in 17.3% of all female donors (n=5834) and in 24.4 % of those with a history of previous pregnancy (n=3992). The prevalence of HLA antibodies increased in women with greater numbers of pregnancy: 1.7%(zero), 11.2%(one), 22.5%(two), 27.5%(three) and 32.2% (four or more pregnancies), $p<0.0001$.

Conclusion—HLA class I and class II antibodies are detectable at low prevalence in male donors regardless of transfusion and in female donors without known immunizing events. The prevalence of HLA antibodies increases significantly with more pregnancies. These data will allow blood centers to estimate the impact of HLA antibody testing as a potential TRALI risk-reduction measure.

Keywords

HLA antibody; pregnancy; transfusion; transfusion related acute lung injury

Introduction

Human leukocyte antigen (HLA) antibodies mediate a number of important clinical effects including platelet transfusion refractoriness¹ and hyperacute, acute, and chronic organ rejection.² More recently HLA antibodies in donated blood have been implicated as the likely causative agent of most cases of transfusion related acute lung injury (TRALI).^{3,4} TRALI is a syndrome consisting of non-cardiogenic pulmonary edema with hypoxia occurring during or within 6 hours of transfusion.^{5,6} TRALI is now the leading reported cause of transfusion-related mortality in the U.S.⁷

Among blood donors, HLA antibodies are found most frequently in multiparous women.^{8–10} In 2003 the United Kingdom (UK) began a TRALI risk-reduction effort by supplying transfusable fresh frozen plasma (FFP) that was predominantly from male donors; this measure resulted in a substantial decline in reported TRALI cases in the UK.¹¹ A passive surveillance study of TRALI cases reported to the American Red Cross (ARC) from 2003–2005 indicated that 71% of the 38 reported probable TRALI-related fatalities and 75% (18/24) of the fatalities from transfused plasma were from leukocyte antibody positive female donors.¹²

The UK and ARC data prompted the AABB to publish an Association Bulletin (November 2006) outlining recommended measures to reduce the risk of TRALI and a timeline for implementation.¹³ In addition to using blood components only when indicated, the Bulletin recommended that blood collection facilities “implement interventions to minimize the preparation of high-plasma volume components from donors known to be leukocyte alloimmunized or at increased risk of alloimmunization”. High plasma volume components were defined as plasma components or apheresis platelets. The policy of supplying FFP or frozen plasma-24 hours (FP24) from predominantly male donors has now been widely implemented in the US.

In comparison, defining and implementing the appropriate measures to mitigate the risk of TRALI from apheresis platelets remain problematic. Specifically, diverting all female platelet apheresis donors to whole blood donation would likely seriously affect the apheresis platelet supply and impact the commitment of long time donors. Another potential strategy is to selectively divert a subset of apheresis donors who are most likely to have HLA antibodies; donors could be screened for a history of pregnancy or transfusion, and donors with a positive history could be deferred or tested for HLA antibodies. HLA alloimmunization in blood donors is known to be associated with pregnancy.^{8–10} Published studies have consistently demonstrated increasing HLA antibody prevalence with increasing parity although the number of donors in these studies is relatively small, and only the study by Powers et al¹⁰ used current, more sensitive testing methods. Because of the small number of donors in these previous studies, it was not possible with confidence to compare HLA antibody prevalence for each

parity level, to identify an independent effect of transfusion, or determine the effect of the time since the immunizing event.

The Leukocyte Antibody Prevalence Study (LAPS) was designed to measure the prevalence of HLA class I and class II antibodies in a large number of donors with or without a history of pregnancy or transfusion.¹⁴ Human neutrophil antigen antibody testing was also performed in a subset of donors and will be the subject of a subsequent publication.

Methods

LAPS was conducted between December 2006 and May 2007 as a prospective cross-sectional multi-center study by the National Heart, Lung, and Blood Institute's (NHLBI) Retrovirus Epidemiology Donor Study – II (REDS-II) program. All six REDS-II blood centers participated in the study; these included the American Red Cross New England Region (Dedham, MA), American Red Cross Southern Region (Douglasville, GA), Blood Center of Wisconsin (Milwaukee, WI); Blood Centers of the Pacific (San Francisco, CA), Hoxworth Blood Center/University of Cincinnati Academic Health Center (Cincinnati, OH), and the Institute for Transfusion Medicine (Pittsburgh, PA). The REDS-II coordinating center is Westat (Rockville, MD) and Blood Systems Research Institute (San Francisco, CA) serves as the REDS-II Central Laboratory.

Enrollment

The target enrollment goal for LAPS was 7,900 donors – 5,700 females, 1,100 non-transfused men and 1,100 transfused men. Females and non-transfused males were enrolled without targeted recruitment strategies with the assumption that their parity and demographics would approximate that of the overall donor population. However, targeted enrollment strategies were necessary to recruit previously transfused male donors in adequate numbers for the study. These strategies included direct mailings and phone contacts to ask previously transfused male donors if they could return to donate and potentially enroll in the study, or identifying these donors post-donation and asking them to enroll in the study at a later date. Details of the final LAPS enrollment by gender, transfusion status and parity are shown in Figure 1.

Donor screening

Donors enrolling in the study agreed to provide a sample of blood to be tested for leukocyte antibodies and to complete a short questionnaire. This self-administered questionnaire was used to collect details on history of pregnancy and transfusion (Appendix). Pregnancy history was elicited using 6 validated questions from the National Health And Nutrition Examination Survey (NHANES)¹⁵ addressing the number and outcome of all pregnancies, including live and still births, miscarriages, terminations, or tubal pregnancies, and the date of the last pregnancy. The pregnancy history obtained using the LAPS questionnaire correlated well (>95%) with pregnancy history for the same donors obtained as part of the routine donation process for all female donors at each of the participating REDS-II centers. Male and female donors were also asked if they ever had a transfusion, the number of transfusions, and the date of the last transfusion episode. Finally, donors were asked to consent to having a portion of their blood sample saved to a repository. A donor could only participate once as a LAPS subject.

HLA Class I and Class II antibody testing method

Testing for HLA class I and class II antibodies was performed on an EDTA plasma sample obtained from consented donors. Samples were frozen at -70°C and shipped in batches to the REDS-II central laboratory for testing. Screening tests for anti-HLA class I and II were performed with One Lambda (Canoga Park, CA) LabScreen LSM12 (LabScreen Mixed) multi-antigen bead kits according to manufacturer's instructions. The assay measures the binding of

antibody to fluorescein tagged microbeads. The assay uses 6 beads coated with purified class I antigens with up to 48 antigens per bead and 3 beads coated with purified class II antigens with up to 48 antigens per bead. The beads represent approximately 80 class I antigens and 25 class II antigens and were run simultaneously. Briefly, 5 μ l microbeads were incubated with 20 μ l plasma in a 96-well V-bottomed polystyrene plate (Whatman, Brentford, UK) for 30 minutes in the dark at 20–25°C with gentle agitation and then washed three times with wash buffer (One Lambda). Plates were centrifuged at $1300 \times g$ for 5 minutes between each wash step. 80 μ l of PE-conjugated anti-human IgG was added for a second 30 minute incubation at 20–25°C in the dark with gentle agitation, followed by two more washes. Negative control serum provided by One Lambda was included in each batch of specimens. Samples were acquired on a luminometer (Luminex, Austin, Tx), with the ability to discriminate up to 100 unique beads in one reaction.

Assay Interpretation

The version of the manufacturer's package insert available at the time of the study suggested that the cutoff for a positive test interpretation either be set at a normalized background ratio (NBG) of 2.2 (based on a correlation with the company's ELISA assay in screening organ transplant candidates) or that the cutoff be independently established by the individual testing laboratory. Since the assay was to be used to screen normal blood donors and not transplant recipients, the NBG cutoff used for this study was established by calculating the mean plus 3 SD of the natural log transformed distribution of NBG values in the 1138 non-transfused male blood donors tested as part of this study. The cutoff of 3SD was chosen based on plausible estimates of approximately 1% for the frequency of HLA antibodies in a non-alloexposed normal population and conventions for establishing normal ranges for laboratory screening assays. Based on this analysis, samples with an NBG >10.8 for any class I multi-antigen bead were considered positive for HLA Class I antibody and those with any class II multi-antigen bead NBG >6.9 were considered positive for HLA Class II antibody. For comparison with other recently completed studies, some data are also shown using an NBG value of 2.2 as the cutoff for designating a sample as positive for either Class I or Class II antibodies.

Statistics

The study sample size was selected to have a 90% power to detect differences in a strictly monotonic increasing HLA antibody prevalence in female donors with regard to previous pregnancies (categorized as zero, 1, 2, 3, and 4 or more). The prevalence of HLA antibodies in these groups (plus groups of non-transfused males and transfused males) was compared using Chi square tests. Statistical significance was assigned to p values <0.05 . Adjusted and unadjusted logistic regression models were constructed to evaluate the effect of parity and transfusion parameters on prevalence of HLA antibody (SAS 9.1.3 (2004) SAS Institute Inc, Cary NC). Box plots were developed for NBG ratios (class I and class II) among samples deemed HLA antibody positive.

Human subjects

The LAPS study protocol and donor consent forms were approved by each participating center IRB and the Westat IRB. The survey instrument (i.e. questionnaire) was approved by the U.S Office of Management and Budget.

Results

Over the six month enrollment period, 8171 adult whole blood and apheresis donors consented to participate in the study (Figure 1). The estimated participation rate of approached donors exceeded 90%. Of the 8171 enrollees, 7920 (96.9%) had valid HLA antibody test results. This resulted in a final sample for analysis consisting of 895 transfused males, 1138 non-transfused

males, 1816 never pregnant females and 3992 ever pregnant females. There were 53 males with unknown transfusion status and 26 females with unreported pregnancy history. The demographics (age, parity and race/ethnicity) of LAPS donors with HLA testing results and the overall REDSII donor population are shown in Table 1. LAPS donors were more likely to be female due to intentional oversampling, and more likely to be age 45 or older. The distribution of race/ethnicity was similar. Among LAPS donors there was a lower prevalence of never pregnant donors and a higher prevalence of donors with 3 or more pregnancies.

Transfused male donors were intentionally over sampled to estimate the prevalence of HLA antibody in this population. The prevalence of Class I and/or Class II HLA antibody was low in both transfused and non-transfused males, albeit slightly but not statistically significantly higher in transfused than in non-transfused males (Figure 2, prevalence of any HLA antibody 1.7% vs. 1.0%, $p=0.16$). Using the manufacturer's suggested NBG cutoff of 2.2 the rates were 6.8% and 7.1% in transfused vs. non transfused males, also not statistically different. ($p=0.79$).

Overall, HLA antibodies were identified in 1006/5834 (17.2%) of female LAPS donors. Among female donors who reported a previous pregnancy, 973/3992 (24.4%) were positive for one or both classes of HLA antibodies. A history of previous pregnancy was a sensitive (96.7%), 973/1006 method to detect HLA alloimmunization in women. In addition, a history of no previous pregnancies had a negative predictive value of 98.3%. As shown in Figure 3, there was a significant increase in the prevalence of having any HLA antibody in women with greater numbers of pregnancies: 1.7% (zero pregnancy), 11.2% (one), 22.3% (two), 27.5% (three) and 32.2% (four or more pregnancies), overall trend $p<0.0001$. The groups were also statistically significantly different when compared individually to one another i.e., 0 vs. 1, 1 vs. 2, 2 vs. 3, and 3 vs. ≥ 4 . Among previously pregnant women, the prevalence data suggest that the first two pregnancies are the strongest alloimmunizing pregnancy events. A similar number of women had class I antibody (603 or 10.4%) as had class II antibody (687 or 11.7%). Using the manufacturer's suggested NBG cutoff of 2.2 there was a similar increase in the prevalence of HLA antibodies in women with increasing numbers of pregnancies; - 8.3% (zero), 20.2% (one) 33.6% (two), 40.3% (three), and 45.4% (four or more), ($p<0.0001$).

A small proportion (1.7%) of women at low risk of alloimmunization (never pregnant or transfused, $n=1732$) had HLA antibodies. This prevalence was similar to that of transfused (1.7%) or non-transfused (1.0%) males, $p=0.29$.

A box-plot showing the distribution of all NBG values above the 3SD cut-off for HLA class I antibodies (10.8) observed in non-transfused males, transfused males, and females by parity is shown in Figure 4. The box-plot shows a similar distribution of NBG values in men (transfused or non-transfused) and never pregnant women supporting the similarity of the three groups. As shown in figure 4, the distributions of observed NBG values showed the greatest difference between zero and 1, and between 1 and 2 pregnancies, paralleling the prevalence data. Similar results were obtained for HLA class II antibodies. (data not shown).

Impact of time since last transfusion on the prevalence of HLA antibodies

The effect of time since the last transfusion on HLA class I or class II antibody prevalence was examined in transfused male donors. The median time since the last transfusion was 267 months (22.3 years). There was no significant difference in the prevalence of HLA antibodies between male donors transfused within the last 10 years ($n=241$, 1.2%), those transfused >10–20 years ago ($n=144$, 2.1%), those transfused >20–30 years ago ($n=159$, 0.6%), or those transfused >30 years ago ($n=303$, 2.3%), $p=0.48$.

Impact of time since last pregnancy and type of pregnancy on the prevalence of HLA antibody

The effects of time since last pregnancy and type of pregnancy (delivery vs. miscarriage/early termination) on HLA antibody prevalence was also evaluated (Table 2). Time since last pregnancy was categorized in 10 year intervals. We observed a decline in the prevalence of HLA antibodies, from 31.3% to 18.3% ($p<0.0001$), when more time had elapsed since the last pregnancy (Table 2), unadjusted for number or type of pregnancies. The effect of a pregnancy resulting in a delivery (live and still births) or a lost pregnancy (miscarriage or termination) on HLA antibody prevalence is shown in Table 2. HLA antibody prevalence increased with each incremental delivery, from 2.0% with no deliveries to 35.0% with ≥ 4 deliveries ($p<0.0001$), unadjusted for time since last pregnancy or number of lost pregnancies. HLA antibody prevalence also increased with each lost pregnancy, from 14.4% with no lost pregnancies to 27.0% with > 2 lost pregnancies, ($p<0.0001$), unadjusted for time since last pregnancy or number of deliveries. To evaluate whether this association represented an independent effect of lost pregnancies on HLA antibody prevalence or whether this effect could be attributed to the higher number of deliveries among women who had lost pregnancies, we conducted adjusted analyses. When looking at one or more lost pregnancies and adjusting for number of deliveries and time since last pregnancy, the effect of a lost pregnancy was no longer significant, $p=0.10$, while number of deliveries and time since last pregnancy effects persisted (both $p<0.0001$). Among women with only one pregnancy, the prevalence of any HLA antibody in women whose pregnancy was a delivery was 15.4% (71/462) vs. 1.2% (2/164) in women whose single pregnancy was lost ($p<0.0001$). Thus, early pregnancy loss did not appear to independently affect HLA antibody prevalence.

Estimating the potential impact of HLA antibody testing on apheresis donations

Of 42,325 apheresis donations given by female donors at the REDS-II centers in 2007, 33% were given by never pregnant females, 12% by those with one pregnancy, 23% by those with two pregnancies, and 32% by those with three or more pregnancies.¹² Using the observed prevalence of HLA antibodies from Figure 2, HLA antibody prevalence would be expected to be approximately 16% (95% CI 14.7, 16.9) among all female apheresis donations and 24% among donations from women with a previous pregnancy history. Data from the REDS-II donation database showed that in 2007 approximately 38% of all apheresis donations were given by women.¹⁶ The net impact of screening and diverting only previously pregnant female apheresis donors based on HLA antibody testing would be an estimated loss of approximately 6% ($0.38 \times 16\%$) (95% CI 5.6, 6.4) of all apheresis platelet donations using the NBG cutoffs of 10.8 and 6.9.

Discussion

Surveillance data from 2003–2005 reported by the American Red Cross¹² and a recent prospective clinical surveillance study for TRALI¹⁷ suggest that plasma and apheresis platelets have the highest per component risk of TRALI. In the US, a strategy that has gained favor to reduce the risk of TRALI from transfusable plasma components (i.e. FFP, FP24, cryoprecipitate-poor plasma) is the preferential use of plasma from male donors and diversion of plasma from female donors to fractionation only. Adoption of this approach for apheresis platelet donors, however, would likely result in unacceptable loss of platelets as our data indicate that in the six REDS centers, 38% of apheresis donations are given by women.¹⁶ Reducing the amount of plasma in apheresis platelets by using platelet additive solutions has also been proposed to reduce the risk of TRALI, but this option is currently limited by the absence of an FDA-approved platelet additive solution in the US.

Both animal models and clinical studies implicate leukocyte antibodies as a major cause of TRALI.^{3, 4, 18, 19} Most cases of TRALI do appear to be linked to donor units that contain

leukocyte antibodies,¹⁹ and since these TRALI cases appear to be of greater severity than the non-leukocyte antibody associated cases,⁴ risk reduction strategies have focused on interventions to reduce exposure to plasma-rich components which are known to be at higher risk of containing leukocyte antibodies.

Based on the 2006 AABB bulletin, a potential approach to TRALI risk reduction from apheresis platelets would be to screen such donors for a history of transfusion or pregnancy, perform leukocyte (i.e. HLA) antibody testing on those donors who give a positive history, and divert those donors found to have leukocyte antibodies away from the donation of high plasma volume components.

Although there have been studies showing an increasing prevalence of HLA antibody with increasing parity^{8–10} these studies tested relatively small numbers of donors and two^{8,9} did not use the more sensitive methods for HLA antibody testing that are now available. Current HLA antibody testing methods include enzyme linked immunosorbent assays (ELISA) or flow cytometry and Luminex-based assays.²⁰ These methods are more sensitive than lymphocytotoxicity (LCT) assays and can be performed in a high throughput manner compatible with donor screening.^{10,20} A recent single center study by Powers et al¹⁰ used the microbead flow assay used in this study, but at a different cutoff derived from renal transplant candidates. The current study was designed to study a sufficient number of donors to establish the prevalence of HLA class I and class II antibodies in blood donors with a history of transfusion or pregnancy. The multi-antigen microbead luminex method was selected to maximize sensitivity and throughput.

We found that a history of previous transfusion had little impact on HLA class I or class II antibody prevalence based on reactivity rates and NBG values among positives in a large sample of transfused and non-transfused males. Based on an NBG cutoff of the mean plus 3SD of a natural log transformed distribution of values in non-transfused male donors, we found that the prevalence of HLA antibody in both non-transfused and previously transfused male donors was low and not statistically different. The time since last transfusion among donors was long, with a median interval of 22 years. To our knowledge, our study is the first to document this long interval between blood donation and a previous transfusion history. Although we showed that HLA antibody detection rates were stable over time in transfused males within the time frames observed, it is important to note that we did not evaluate recently transfused persons. Previous studies in which HLA antibody was evaluated within several months of transfusion showed a prevalence of 10–15%.²¹ One possible explanation for these markedly different findings may be loss of detectable HLA antibodies in the initial months to years after sensitization, similar to what has been observed in females after pregnancy.²² Our data cannot exclude the possibility that a recent history of transfusion could be associated with an increased, albeit transient prevalence of HLA antibodies.

Our data showing that class I or class II HLA antibody prevalence is low and is similar in transfused and non-transfused males donors indicates that transfusion history would not be helpful in discriminating donors at greater risk of HLA alloimmunization. Therefore, from a policy perspective, we believe that that testing of male apheresis donors for HLA antibody is not indicated and that asking apheresis donors about transfusion history as part of a triage algorithm for HLA antibody screening is also not indicated. Women who did not report a previous history of pregnancy had an HLA antibody prevalence and a distribution of NBG values that was similar to that found in men. From a policy perspective, our data and those of Powers et al suggest that the strategy of inquiring about pregnancy history, and electing not to perform HLA antibody testing on those who report no history of pregnancy is reasonable.

The cause of the low level of HLA antibodies in non-transfused males (1.0%) and never pregnant, non-transfused women (1.7%) is unknown. HLA antibodies in these individuals could be naturally occurring autoantibodies,²³ alloantibodies related to potential immunizing events such as vaccination,²⁴ cross-reacting antibodies to bacterial antigens,²⁴ or false positive results.

We enrolled a sufficient number of women to examine the influence of parity parameters on HLA antibody detection. We found that the prevalence of HLA class I and class II antibodies increased with each pregnancy at least through to the upper limit of our analysis (i.e. 4 or more pregnancies) confirming previous studies of smaller numbers of donors.^{8–10} In addition, each pregnancy appeared to be a strong HLA immunizing event; this was particularly true for the first 2 pregnancies which resulted in disproportionately more alloimmunization compared to the third or fourth or more pregnancy. We hypothesized that a pregnancy resulting in a delivery (live or still birth) would have a greater immunizing effect than a pregnancy loss (miscarriage or termination). The data showed that each delivery of a live or still birth, at least up to 4, was indeed a strong independent alloimmunizing event. When analyzed in women who also had deliveries, pregnancy loss was non-significant after adjusting for number of deliveries. Furthermore, when looking at women with only a single pregnancy, a lost pregnancy (as opposed to a delivery) did not appear to have any statistically significant effect on HLA alloimmunization. The dominant effect of deliveries may be the result of the greater duration of maternal exposure to fetal antigens in pregnancies that progress to delivery as compared to the shorter duration of alloexposure that may occur in pregnancy loss. We also examined the impact of time since last pregnancy on HLA antibody detection, and found that HLA antibody prevalence diminished over time although the effect was modest. In a recent study conducted in a small number of donors, Powers et al did not observe a decline in HLA antibody prevalence over time in a cohort of donors using a low NBG cutoff. Nevertheless, our data and that of others demonstrate that long-term HLA antibody persistence can be seen.^{10,25} The reasons for this long term persistence at a somewhat lower rate are not known, but may relate to the requirement for persistent microchimeric fetal cells in the mother to sustain alloantibody levels and hence detectability.

The frequency of HLA antibodies observed in previously pregnant LAPS donors is somewhat higher than that reported by Densmore et al.,⁸ most likely because the multi-antigen bead Luminex HLA screening method used in LAPS is more sensitive than the LCA methods used in that study. The Luminex assay was also used in two recent smaller studies of HLA alloimmunization in blood donors, both of which reported higher rates than what we observed at the 3SD cutoff but similar to our results at the NBG cutoff of 2.2.^{10,26} Powers et al tested 1027 women and 26 males with a history of transfusion. They identified HLA antibodies in 25.4% of all female donors (vs. 17.3% at 3SD and 27.4% at NBG 2.2 in our study), 5.9% in never pregnant nor transfused women (vs. 1.7% at 3SD and 8.3% at NBG 2.2 in our study), and 12.0% of non-transfused males (vs. 1.0% at 3SD and 6.8% at NBG 2.2 in our study). A smaller study by Fadeyi et al.²⁶ also reported a higher prevalence of HLA antibodies in 96 apheresis donors; finding HLA antibodies in 56% of females and 35% of males. Fadeyi et al.²⁶ also tested each sample for HLA antibodies by ELISA and reported that 5/44 (9%) of women and 0/55 (0%) men were positive. These ELISA results are almost identical to the results of a larger study of 1043 donors reporting HLA antibodies in 9.8% of females and none in males.²⁷ The most likely explanation for the high prevalence using microbead flow assays observed in the two smaller studies^{10,26} is that they used the manufacturer's suggested NBG cutoff of 2.2. We found similar results in our larger study when we used the same cutoffs. The 2.2 NBG cutoff is derived from transplant recipients in contrast to the 3SD cutoff derived from normal donors. High sensitivity is paramount in screening transplant candidates because of the possibility of an anamnestic response in the recipient that could lead to organ rejection. However, in the setting of screening apheresis donors for TRALI prevention, the issue is

detection of passively transfused antibody, not an anamnestic response and, thus, sensitivity requirements are likely different. Validation of the multiantigen bead flow cytometry assay cutoff in normal donors has not been previously established. For this reason we used non-transfused males to establish a “background” rate of HLA antibodies in a population with no known alloimmunizing events. Clinical surveillance or large-scale retrospective studies of TRALI from units with HLA antibodies will be required to establish the clinical significance of different levels of NBG reactivity and consequently the appropriate HLA antibody assay cutoff. If a strategy of HLA antibody testing were to be applied to previously pregnant female platelet apheresis donors, our data indicate that approximately 6% of platelet apheresis donations would be lost. A similar strategy could also be applied to female plasma apheresis donors. Under this scenario, implementation of HLA antibody testing would require that a donor screening question regarding new pregnancy events since the last donation be added. Women found to have HLA antibodies can be asked to become whole blood donors and their plasma can be used for fractionation

There may be some variance from our donor loss estimates at individual blood centers if the distribution of pregnancy history or the proportion of female apheresis donations differs from that at the REDS-II centers; in addition, the relative frequency with which apheresis collections from female and male donors are made into multiple platelet apheresis (or so called split) products will also influence the overall impact of an HLA antibody screening program.

The efficacy of a TRALI risk reduction strategy based on HLA antibody screening of apheresis donors has been questioned. Fadeyi et al.²⁸ reviewed transfusion service records for passive reports of transfusion reactions from 167 components provided by four apheresis donors with HLA antibodies. The only reactions reported were allergic and the rate, 1.8%, was not significantly different than that observed in controls (4.2%). The study was limited by its small size and passive reporting of reactions. In contrast, a small look back study by Powers et al, which traced HLA antibody positive units transfused to 26 recipients, identified two possible cases of TRALI.¹⁰ In another retrospective look back study of 187 components from 62 female donors with HLA antibodies, Maslanka reported 1 case of TRALI from a red cell transfusion. 27 Although these and other data indicate that the large majority of components collected from donors with HLA antibodies do not result in TRALI, this does not negate the adoption of an HLA antibody screening strategy that identifies those components that carry increased risk, even if such a strategy is relatively non-specific, provided that platelet availability is not substantially compromised. The clinical relevance of TRALI is seen in recent prospective TRALI surveillance studies which have reported an incidence of TRALI from 1:500 patients²⁹ to as high as 8% in ICU patients,¹⁷ with the latter reporting a higher risk from plasma and apheresis platelets.

A limitation of this study is the lack of a gold standard for HLA antibody testing that would allow the selection of a definitive cutoff for positivity in the Luminex screening assay. While this creates some uncertainty about the absolute prevalence of HLA antibodies in our donor population, this has little effect on the comparison of prevalence estimates between various donor groups. We saw the same relationships whether we selected a 3SD cutoff or the manufacturer's suggested NBG cutoff of 2.2. We used a single method (Luminex) for HLA antibody testing based on optimal sensitivity and high throughput. However, repository samples were saved so that comparison with other methods can be performed. It is possible that REDS-II and LAPS donors are not representative of the entire U.S. blood donor population. Demographic data from Table 1 indicate that LAPS donors are similar but not identical to the larger population of REDS-II donors. Since the 6 REDS-II blood centers collect about 7% of the U.S. blood supply representing more than 1,000,000 units annually, we believe the background donor population from which LAPS enrollees were selected is likely to be similar to the US donor population as a whole, although it may differ from certain regional donor

populations. There were differences in the age and parity distribution of LAPS donors compared to the overall REDSII donor population, however the differences were modest and unlikely to affect the ability to generalize our results. Lastly, we are currently unable to definitively comment about the clinical relevance of the HLA antibodies detected in LAPS donors regarding TRALI risk. REDS-II will soon begin a lookback study of recipients of a large number of plasma rich components from LAPS donors with and without HLA antibodies which should provide valuable insight into the clinical relevance of these antibodies and may provide the information needed to determine the appropriate cutoff for the multiantigen bead Luminex assay.

In conclusion, we showed that the prevalence of HLA antibodies in blood donors is clearly related to the number of previous pregnancies. Never pregnant women have a low prevalence of HLA alloimmunization which does not appear to differ from that of non-transfused or transfused men. Transfusion appears to have no or only minimal impact on HLA antibody prevalence in a donor population. These data can be used to develop and assess the impact of implementing an HLA testing strategy on apheresis donors and apheresis platelet supply. In the REDS-II donor population, the estimated impact would be a loss of approximately 6% of apheresis donations. Blood centers will need to weigh the projected donor loss expected with an HLA testing strategy targeted to some or all apheresis donors versus other measures to decide on the best TRALI risk reduction strategy.

Acknowledgments

The authors thank would like to thank Mila Lebedeva and Deborah Bunch who performed the HLA testing and the research and donor staff at all six participating blood centers. Without their help, this study would not have been possible.

This study was supported by contracts N01HB47168; N01HB47169; N01HB47170; N01HB47171; N01HB47172; N01HB47174; N01HB47175; N01HB57181 from the National Heart, Lung, and Blood Institute.

Appendix

LAP Study Questionnaire

Apply bar code here

Blood Center's Donation Number:

Study-related Unique Identification Number:

Today's Date Date of Birth

M M Y Y Y Y M M D D Y Y Y Y

Gender: ☐ Male ☐ Female Ethnicity: Are you of Hispanic/Spanish origin?
☐ No ☐ Yes

If yes: ☐ Cuban ☐ Puerto Rican ☐ Mexican, Mexican-American or Chicano ☐ Other

Race (list all that apply):
☐ White ☐ American Indian ☐ Black ☐ Asian Indian ☐ Chinese
☐ Filipino ☐ Hawaiian Native ☐ Japanese ☐ Vietnamese ☐ Eskimo
☐ Other

Question 1: Have you ever received someone else's blood? ☐ Yes ☐ No ☐ Don't Know

Question 2: If you answered yes to Question 1, please indicate below in what month and year was your:

Last transfusion episode ☐
 Don't Know (best estimate) M M Y Y Y Y

Prior transfusions ☐
 Don't Know (if any; before the last transfusion episode) M M Y Y Y Y M M Y Y Y Y

For Female Donors:

Question 3: Have you ever been pregnant? Please include live births, miscarriages, terminated pregnancies, still births, and tubal pregnancies.
☐ Yes ☐ No ☐ Don't Know

Question 4: How many times have you been pregnant in your life? Again, be sure to include all pregnancies including live births, miscarriages, terminated pregnancies, still births, and tubal pregnancies.
 Enter Number of Pregnancies ☐
 Don't Know

Question 5: How many of your pregnancies resulted in a live birth? Please count the number of total pregnancies, not number of live-born children. For example, if you had twins or other multiple births, count as a single pregnancy.

img1.

Enter Number of Pregnancies Resulting in Live Birth ☐

Don't Know

Question 6: How many of your pregnancies resulted in still birth? Again, please count the total pregnancies.

Enter Number of Pregnancies Resulting in Still Birth ☐

Don't Know

Question 7: How many of your pregnancies resulted in miscarriages or terminated pregnancies?

Enter Number of Pregnancies Resulting in Miscarriage/terminated pregnancy ☐ Don't Know

Question 8: The last time you were pregnant, in what month and year did the pregnancy end?

Don't Know ☐

M M Y Y Y Y

img2.

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Figure 1.
LAPS enrollment figures and distribution of donors by gender, transfusion, and pregnancy history. Targeted enrollment was used to enroll sufficient numbers of transfused male donors.

Figure 2. HLA antibody prevalence for class I, class II, class I and II and any HLA antibody at a 3SD cutoff in transfused and non-transfused males. The difference in HLA antibody prevalence was not statistically significant ($p=0.16$).

*Overall difference comparing all groups

Figure 3.

HLA antibody prevalence for class I, class II, class I and II and to any HLA antibody at a 3SD cutoff in never pregnant women and women with one or more pregnancies is shown. There is a statistically significant increase in the prevalence of class I, class II or any HLA antibody with an increasing number of pregnancies, from one to four or more (overall trend $p<.0001$). The first two pregnancies were associated with the greatest increase in prevalence of HLA antibodies.

Figure 4.

Box plot of the distribution of the observed HLA class I NBG values above the cutoff of 10.8 in each donor group. The distribution of NBG values observed in transfused males, non-transfused males and never pregnant females is similar. Higher NBG values are seen in women with 2 or more pregnancies compared to women with one pregnancy.

Table 1

Demographic characteristics of subjects enrolled in the LAPS study with HLA antibody screening results (n=7920) and those in the overall REDSII database

		LAPS Subjects [*]		REDS-II donors for the same time period ⁺ as the LAPS enrollment [^]	
		n	%	n	%
Gender	Male	2086	26.3	232485	50.3
	Female	5834	73.7	229299	49.7
Age	<=24	812	10.3	120910	26.2
	25-44	2246	28.4	133192	28.8
	45-64	4036	50.9	176450	38.2
	>=65	824	10.4	31240	6.8
Mean Age (SD)		47.1 years +/-14.6		40.3 years +/-16.6	
Race/Ethnicity	White	7145	90.4	399867	87.1
	Black	219	2.8	28834	6.3
	Hispanic	234	2.9	14582	3.2
	Asian	189	2.4	8868	1.9
	Other	117	1.5	6955	1.5
Parity	Zero	1816	31.3	86914	43.3
	1	634	10.9	21042	10.5
	2	1307	22.5	40296	20.1
	3	1058	18.2	28109	14.0
	4	993	17.1	24184	12.1

^{*} some subjects in LAPS were missing demographic information (age (n=2), race/ethnicity (n=16), or parity information (n=26))

⁺ REDS-II Donors between October 2006-May 2007

[^] some donors are missing demographic information (gender (n=11), age (n=3), race/ethnicity (n=2689), or parity information (n=28,754))

Table 2

Effect of various parity/pregnancy parameters on HLA Antibody prevalence

Time since last pregnancy in years*			
	Positive/n (%)	unadjusted OR (95% CI)	Adjusted OR (95% CI)
<10	288/921 (31.3)	2.04 (1.64, 2.53)	2.64 (2.09, 3.32)
10–20	271/1118 (26.0)	1.57 (1.27, 1.95)	1.77 (1.42, 2.21)
20–30	213/951 (22.4)	1.29 (1.03, 1.62)	1.47 (1.17, 1.86)
>30	166/909 (18.3)	1.0	1.0

Deliveries (Live and Still Births combined)*			
	Positive/n (%)	unadjusted OR (95% CI)	Adjusted OR (95% CI)
0	41/2097 (2.0)	1.0	1.0
1	117/687 (17.0)	10.3 (7.1, 14.9)	6.1 (3.1, 12.0)
2	402/1660 (24.2)	16.0 (11.5, 22.3)	10.3 (5.4, 19.8)
3	283/906 (31.2)	22.8 (16.2, 32.0)	15.3 (7.9, 29.6)
≥4	162/463 (35.0)	27.0 (18.8, 38.8)	19.8 (10.1, 38.7)

Lost pregnancies (Pregnancies medically or spontaneously terminated)*			
	Positive/n (%)	unadjusted OR (95% CI)	Adjusted OR (95% CI)
0	614/4250 (14.4)	1.0	1.0
1	239/1007 (23.7)	1.84 (1.56, 2.18)	1.07 (0.89, 1.28)
≥2	151/560 (27.0)	2.19 (1.78, 2.69)	1.24 (0.99, 1.55)

* data missing time since last pregnancy for 119 donors, deliveries for 21 donors, and lost pregnancies for 17 donors.