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The frequency and specificity of Human Neutrophil Antigen Antibodies in a blood donor population

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Abstract

BACKGROUND—Transfusion related acute lung injury (TRALI) has been associated with both HLA and HNA antibodies. HNA antibody frequency, specificity, and demographic associations have not been well defined in the blood donor population.

METHODS—A subset of 1171 donors (388 non-transfused males, 390 HLA antibody negative females with three or more pregnancies, and 393 HLA antibody positive females with three or more pregnancies) from a larger leukocyte antibody prevalence study (LAPS) was tested for IgG and IgM HNA antibody using a granulocyte immunofluorescence flow cytometry assay. Additional testing on selected samples included monoclonal antibody immobilization of granulocyte antigen – flow cytometry and granulocyte genotyping.

RESULTS—Eight samples were HNA antibody positive (prevalence 0.7% [95% CI, 0.3 - 1.3%]). Three HNA antibodies (one IgG and two IgM) were found in non-transfused males (prevalence 0.8% [95% CI, 0.2 - 2.2%]); all were pan-reactive or non-specific. One HLA antibody negative previously pregnant female had an IgG HNA antibody with HNA-1a specificity (prevalence 0.3% [95% CI, 0.01-1.4%]). Four HLA antibody positive previously pregnant females demonstrated HNA antibodies, three IgG and one IgM (prevalence 1% [95% CI, 0.3 - 2.6%]). Two of these were HNA-1a specific, one HNA-4a specific, and one non-specific.

CONCLUSION—HNA antibodies occur with low frequency in the donor population and are present in both male and female donors. Despite the implementation of TRALI reduction strategies, HNA antibodies are still present in donor blood products. Though our data do not create a case for urgent implementation of donor HNA antibody testing, future new developments for high throughput HNA antibody screening, including for HNA-3a, may warrant reconsideration.

INTRODUCTION

Transfusion Related Acute Lung Injury (TRALI) is the development of non-cardiogenic pulmonary edema usually occurring within six hours of a blood transfusion. Its clinical importance is demonstrated by data from the FDA showing it to be the leading cause of transfusion related mortality.[1] The exact etiology and pathophysiologic mechanisms are still not fully characterized but it is believed that leukocyte antibodies present in donor plasma play a key causative role in most cases of TRALI.[2] In a classic study, Popovsky and Moore helped define the clinical aspects of TRALI and demonstrated that many cases were associated with the presence of Human Leukocyte Antigen (HLA) antibodies in donor blood units.[3] Since that report, there have been many studies implicating leukocyte antibodies,

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both HLA and Human Neutrophil Antigen (HNA) antibodies in the pathophysiology of TRALI.[4-7] For this reason many countries have adopted policies attempting to eliminate or reduce the likelihood that donor blood products will contain leukocyte antibodies. There have only been a small number of studies examining the type of leukocyte antibodies and their frequency in the donor population with most focusing on the presence of HLA antibodies rather than HNA antibodies.[8-12] HNA antibody detection is currently performed mainly in specialized laboratories, using tedious methodologies that are not conducive to large scale donor screening. The largest study to look at leukocyte antibodies in the donor population was recently published by Triulzi et al. as part of the Leukocyte Antibody Prevalence Study (LAPS) in which over 8,000 blood donors were evaluated for the presence of HLA antibodies. This study demonstrated that HLA class I and class II antibodies were found mainly in previously pregnant women and their frequency increased significantly with the number of pregnancies.[13] As a follow up to that study, we now report the prevalence of HNA antibody in a subset of these LAPS donors, its association with gender, pregnancy history, and HLA antibody status, and whether specific reactivities to HNA were identified. The results of this HNA antibody study may be useful to devise TRALI risk reduction strategies.

MATERIALS AND METHODS

LAPS enrollment was conducted between December 2006 and May 2007. It was a cross-sectional, multi-center study by the National Heart, Lung and Blood Institutes (NHLBI) Retrovirus Epidemiology Donor Study-II (REDS-II) program. All six REDS-II blood centers participated in the study. These included: American Red Cross New England region (Dedham, MA), American Red Cross Southern Region (Douglasville, GA), BloodCenter 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. Testing for HNA antibodies and HNA genotyping was performed by the Platelet and Neutrophil Immunology Laboratory, BloodCenter of Wisconsin.

Study Population

LAPS enrollment and study design have been previously described in detail.[13] Donors consenting to the study provided a blood sample for HLA class I and class II and HNA antibody testing and gave a detailed history of pregnancy and transfusion. A total of 8171 (6011 females, 2160 males) donors were enrolled; females and transfused males were intentionally over sampled. Due to budgetary and test logistic constraints, we were able to evaluate only a subset of LAPS donors for HNA antibody. Donors selected for this study had given consent for frozen repository sample (plasma, serum, and modified whole blood) storage of their enrollment sample. We established three approximately equal subgroups of donors, 388 non-transfused males (representing donors without known alloexposure), 390 HLA antibody negative females with three or more pregnancies (representing donors with alloexposure but without demonstrated immune response), and 393 HLA antibody positive females with three or more pregnancies (representing donors with alloexposure and with demonstrated immune response) and selected plasma samples for HNA antibody testing. HLA antibody status for class I and class II antibodies in the donors' plasma samples was previously determined using the One Lambda (Canoga Park, CA) Labscreen LSM12 Multi Antigen Bead kits[13]. HLA antibody positive donors had class I, class II or both class I and class II antibodies using NBG ratio > 10.8 for class I and > 6.9 for class II. These cutoffs were determined in the following manner. The manufacturer's package insert states that a

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cutoff for positive reaction be set at an NBG ratio of 2.2 (which was established through correlation in screening transplant candidates), or that laboratories establish their own cutoff value. Since the HLA antibody testing was performed on normal donors, the NBG cutoff was established by calculating the mean plus 3 standard deviations of the natural log-transformed distribution of NBG values in 1138 non-transfused male donors. HLA antibody negative donors had NBG ratio values < 2.2.

Neutrophil Antibody Testing

Neutrophils were obtained from donors who were previously typed for the neutrophil antigens HNA-1a, HNA-1b, HNA-1c, HNA-2, HNA-3a, HNA-4a/4b and HNA-5a/5b. Neutrophils were isolated from freshly drawn whole blood anticoagulated with ethylene diamine tetraacetic acid (EDTA) as previously described.[(5)] In brief, red blood cells were sedimented with 5% Dextran and leukocyte rich supernatant was centrifuged over ficoll-hypaque density followed by lysis of residual red cells with ammonium chloride solution.

Neutrophil-reactive antibody detection was performed using the Granulocyte Immunofluorescence test by flow cytometry (GIFT-FC) as previously described.[(14)] In brief, 4×10^5 donor neutrophils were incubated with 30 microliters plasma (diluted 1:2) for 30 minutes at ambient temperature. The cells were then washed three times in phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin (BSA) and 9 mmol/liter Na_2 EDTA and 0.2% PEB/BSA suspended in 0.2 ml of a 1:200 dilution of flouresceine isothiocyanate (FITC) – labeled goat anti-human IgG-Fc and phycoerythrin labeled anti-human IgM-Fc reagents (Jackson Immunoresearch Laboratories, West Grove, PA) and incubated in the dark for 20 minutes. The neutrophils were then washed once in PEB/BSA and fixed in 0.5% paraformaldehyde. Neutrophil bound fluorescence was detected by flow cytometry (FACS Calibur, Becton Dickinson, Mountain View, CA) and data were analyzed with computer software (CellQuest, Becton Dickinson).

Neutrophil testing was performed in three phases. Level 1 testing was a two cell antibody screen performed on all study samples. Level 2 testing was a repeat screen on those samples that were from known HLA class I positive female donors who had positive results in the Level 1 HNA antibody screen. Those samples underwent adsorption with platelets characterized for their HLA antigens in order to adsorb out any known HLA antibodies, leaving only neutrophil antibodies for those found positive. Level 3 was testing for neutrophil antibodies as described above, only using a larger panel of cells well-characterized for HNA antigens. Testing for levels 1, 2 and 3 are now described in greater detail.

For level 1 and 2 testing, two neutrophil cells isolated from two volunteer donors and characterized for all the HNA antigens described above were used to detect HNA IgG antibodies and two were used to detect HNA IgM antibodies. Assay cutoff values were determined by taking the mean plus three standard deviations (SD) of the natural log transformed distribution of fluorescence values in the 388 non-transfused male donors. This approach was chosen based on conventions for establishing normal ranges for laboratory screening assays and was also the approach used for establishing HLA antibody screening cutoffs.[13] The mean plus 3 SD values, calculated according to the above approach, were determined to be statistically significantly different for the two cells used to detect IgM antibody and, therefore, two different cutoff values were chosen for the two IgM cells (the corresponding non-log transformed cutoff values were 4.9 for one cell and 3.2 for the other cell). The two cutoff values were not statistically significantly different for the two cells used to detect IgG antibody and therefore, only one IgG cutoff value of 4.9 was chosen.

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For level 2 testing, since HNA-3a antigen is present on the surface of a number of cell types, including platelets, there was concern that any HNA-3a antibody would be removed by the platelet adsorption step resulting in the inability to detect it in Level 2 testing. For this reason all initially positive samples were genotyped for HNA-3a and those found negative were adsorbed with platelet donors lacking the HNA-3a antigen and again subjected to Level 2 testing to detect antibodies targeting this HNA specificity.[15]

Level 3 testing was performed on samples that were positive either by Level 1 testing or by Level 2 testing. For Level 3 testing, four to eight cells from volunteer donors carefully characterized for the HNA antigens described above were used to detect IgG and IgM. In order to be called positive, at least three of the panel cells were required to give positive results. Assay cut-off values were determined by taking the mean plus 3 SD of the natural log transformed distribution of fluorescence values obtained with testing 80 randomly selected, non-transfused male donors. The mean plus 3 SD values calculated according to the above approach were not statistically significantly different for any of the panel cells. Therefore, a single cutoff value was utilized for IgG (3.6) as well as for IgM (3.0).

Monoclonal Antibody Immobilization of Granulocyte Antigen – Flow Cytometry (MAIGA-FC)

Further characterization of HNA antibody was performed by MAIGA. HNA 1a, 1b, and 1c antibodies were identified using clone MBC 238.7 (CD16) (developed in house by BloodCenter of Wisconsin) and clone 3G8 (CD16) (a gift from J.C. Unkeless, M.D. Immunology Center, Mount Sinai School of Medicine, New York, NY). HNA-2 antibodies were detected using clone 7D8 (CD177) (a gift from David Stroncek, M.D., National Institutes of Health, Bethesda, MD). HNA 4 antibody was detected using clone Bear-1 (CD11b/18) (Abcam, Cambridge, MA). In this assay, neutrophils are incubated with test plasma, washed and then incubated with monoclonal antibodies specific for known membrane glycoproteins (GPs). After further washing and lysis in non-ionic detergent (1% Triton X-100), solubilized antibody GP complexes were captured in the wells of a microtiter plate coated with goat anti-mouse IgG antibody. After further washing, human IgG bound to the capture GPs was detected by enzyme-linked immunoassay (ELISA) with horseradish peroxidase – labeled goat antibody specific for human IgG-Fc.[14]

HNA genotyping was performed on isolated DNA by PCR amplification with sequence specific primers followed by electrophoresis of PCR products on ethidium-bromide stained gels and inspection for specific allelic bands as reported previously.[16]

Statistical Analysis

Fisher's exact tests were performed to assess differences in HNA antibody frequencies among the three groups (non-transfused males, HLA antibody negative females, and HLA antibody positive females) using SAS [9.1.3 (2004) SAS Institute Inc, Cary NC]. 95% confidence intervals (CI) for binomial proportions were calculated using the Clopper-Pearson method.

Human Subjects

The LAPS protocol and donor consent was approved by each of the six participating centers and by the Westat Institutional Review Board.

RESULTS

Table 1 provides the results of Level 1 and Level 2 testing indicating that 15 of 1171 (1.3%) donor samples were positive by the screening assays. Among the 393 women with known

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pre-existing HLA antibody 37 were initially positive in the HNA antibody screen. To detect HNA specific antibodies these plasma samples required preadsorption with platelets to remove HLA antibodies, followed by rescreening for HNA antibodies. Six of these samples screened positive for HNA antibody following the adsorption. No HNA-3a antibodies were detected in these 37 samples. The percentage of women with 3 or more pregnancies who screened positive for HNA antibody was similar (1.5%) whether HLA antibody was present or not, whereas the proportion of males screening positive for HNA was about half of that or 0.8% (Table 1). However, there was no statistical difference for the proportions testing positive among the three groups (p=0.59).

Table 2 depicts Level 3 testing done to confirm that HNA antibodies were present. Eight of the 15 samples positive in the two-cell HNA antibody screen were confirmed to have HNA antibody present. For the seven non-confirmed samples a review of their original results (data not shown) demonstrated that the original screening positive fluorescence values were near the IgG or IgM cutoff values. This led to concern that these samples could be negative and, indeed, with the larger panel, these were demonstrated to be HNA antibody negative. The 3 non-transfused males all remained HNA antibody positive at Level 3 testing with one donor demonstrating IgG antibody and two IgM antibodies (overall prevalence of 0.8% [95% CI, 0.2 – 2.2%] in the non-transfused male group of samples). Only one of the 390 HLA antibody negative females demonstrated an HNA antibody, which was IgG (0.3% [95% CI, 0.01 – 1.4%]). Four of the six originally positive females with greater than three pregnancies and having HLA antibodies demonstrated HNA antibody specificity (overall prevalence of 1.0% [95% CI, 0.3 – 2.6%]) in the HLA antibody positive female group), three with IgG and one with IgM reactivity. Overall, the prevalence of HNA antibodies did not significantly differ among the three groups (p=0.46). Further subhypotheses showed no statistical difference between HLA antibody positive and HLA antibody negative female donors (p=0.37), nor between allo-exposed (pooling both groups of females) and non-alloexposed (non-transfused male) donors (p=0.53). The overall prevalence of HNA antibody was 0.7% among these 1171 donors evaluated (95% CI, 0.3 – 1.3%). While these 1171 donors are not representative of a donor population (e.g. no low parity female donors are included), this overall prevalence can be tentatively interpreted as the expected HNA antibody prevalence in a donor population (since we found no difference in prevalence among any of the analyzed subgroups).

Table 3 depicts the detailed testing for the eight Level 3 positive donor samples. Two of the eight samples were pan-reactive, that is, reacting with all of the panel cells but failing to demonstrate HNA antigen specificity. The nature of these antibodies is unclear but they may represent autoantibody in view of the fact these antibodies were found in two supposedly non-alloexposed males. Three donor samples were found to have antibody specificity to HNA-1a, one donor sample had specificity for the HNA-4a neutrophil antigen and two samples gave nonspecific reactivity (one from a non-transfused male, one from a woman with 3 or more pregnancies). Of the 3 HNA-1a positive donor samples two showed IgG reactivity and one showed IgM reactivity. The two IgG HNA-1a specific antibodies were tested by the MAIGA (detects only IgG antibodies) and these were both positive using an anti-CD16 antibody, again, confirming the assignment of HNA-1a specificity. All three of the HNA-1a positive antibody samples were genotyped for HNA-1a, -1b and -1c and were determined to be HNA-1a negative, indicating that the antibody was an alloantibody with the assigned specificity. Similarly, the donor sample found to have anti-HNA-4a was genotyped and found to lack the HNA-4a antigen, confirming that this was an alloantibody with HNA-4a specificity.

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DISCUSSION

The frequency with which HNA antibodies are implicated in TRALI is not well established. This is because many cases of TRALI probably go unreported and others have a limited serological evaluation, with only HLA antibody evaluation and not HNA antibody assessment. Despite this, there are a number of studies documenting the association of donor HNA antibodies with TRALI. Reil et al. reported on 36 TRALI cases of which 12 were associated with HNA antibodies. Of these, 10 were HNA-3a specific antibodies, one was HNA-1a and one was HNA-2a. Of the 10 fatalities among the 36 cases, six were associated with HNA-3a antibody.[12] Chapman et al. reported on 195 cases of TRALI from 1996 through 2006 in the United Kingdom. Of 96 cases investigated for leukocyte antibodies, 73 identified at least one leukocyte antibody positive donor, including 13 with granulocyte antibodies (5 with HNA-1a, one with HNA 3a and 7 non-specific) that were concordant with recipient neutrophil antigens.[17] Eder et al. from the American Red Cross reported 38 fatalities from TRALI in a surveillance analysis conducted from 2003 to 2005. Of these, 24 had a serological evaluation of donor specimens and two cases were associated with HNA antibodies, both HNA-3a.[18] Other cases of TRALI associated with HNA antibodies, most with HNA-3a specificities, have also been reported. One particularly informative case was reported by Kopko et al. describing a lookback of 50 patients who received blood components from a donor who possessed an HNA-3a antibody that was associated with a TRALI fatality. Of 36 patient charts available for review, 15 patients experienced TRALI-like reactions, 7 mild / moderate and 8 severe.[19] Surprisingly, only two of the 15 cases were reported to the blood center, suggesting that many cases of TRALI caused by HNA-3a antibodies may go unrecognized. These publications clearly establish HNA antibodies as a serious and life-threatening cause of TRALI.

Our data demonstrated that the frequency of HNA antibodies in the subset of the donor population in this study was low, 0.7% (95% CI, 0.3 – 1.3%). Five of the eight HNA antibody positive samples were from females with a previous history of pregnancy, with four of the five also being positive for HLA antibodies. With most blood centers adopting TRALI reduction strategies of using male only or never pregnant female plasma products, the risk of HNA antibody exposure by plasma transfusion should be reduced from historical levels. For platelet apheresis collections, many centers have adopted HLA antibody screening of female donors with a history of pregnancy.[(20)] The low frequency of HNA antibodies in HLA antibody negative multiparous donors in our study implies that routine HNA antibody testing would potentially lead to a relatively small increment in safety.

Three of the HNA antibody positive donors were non-transfused males. Two of these donors had pan-reactive antibodies (reacting with 4 of 4 panel cells and 6 of 6 panel cells respectively), while the third gave non-specific reactivity (5 of 8 reactive panel cells). These results are in contrast to Reil et al. who did not find any neutophil specific antibodies in 229 tested male blood donors.[12] The significance of a pan-reactive or non-specific HNA antibody is not established, although data from the United Kingdom SHOT system identified 7 TRALI cases associated with donors who had non-specific neutrophil antibodies and Reil et al. found 9 such antibodies in screening of 5,561 donors.[(12,17)] These pan-reactive or non-specific antibodies may be autoantibodies or elevated levels of immune complexes in donor plasma. Autoantibodies to neutrophils have been associated with autoimmune neutropenia in both adults and children, have included IgG and IgM antibodies, and have been found in males and females.[(21,22)] Thus, our finding of neutrophil pan-reactive antibodies, including in male blood donors, may not be surprising. Additional research to evaluate the role of pan-reactive or non-specific HNA antibody in the development of TRALI is needed. REDS-II is currently conducting patient record review for TRALI events after transfusion of blood

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components obtained from donors with HNA antibodies identified in this study to further evaluate the significance of these antibodies.

Three of the eight HNA antibody positive donors had IgM antibodies only. Two (one non-specific, one pan-reactive) were from male donors and one was an HNA-1a specific antibody from a female donor. The role of IgM antibodies in TRALI is poorly elucidated with only a couple of cases reported including a case of a possible TRALI fatality associated with an IgM HNA-1a antibody in a male donor claiming no prior transfusions.[23,24] The mechanism by which pan-reactive or non-specific IgM antibodies might cause TRALI is unclear since granulocytes do not have Fc receptors for IgM antibodies. However, IgM antibodies are efficient at activating complement and complement activation with resultant activation of neutrophils is a mechanism that has been proposed for antibody mediated TRALI.[(23)] Although IgM antibody associated TRALI cases are undoubtedly rare, these case reports suggest this possibility and should be considered when no other explanation for a clear-cut TRALI case is identified. In the current approach to TRALI case workups such cases could be missed since not all laboratories test for HNA IgM antibodies.

We observed a rate of 0.7% granulocyte antibodies in our donor population. Three of 8 antibodies were HNA-1a specific, and one was HNA-4a specific. No HNA-3a antibodies, the neutrophil antibody associated with the majority of reported severe TRALI cases, were detected among 1171 donors (prevalence of 0.0% [95% CI, 0.0 - 0.31%]). There have been only a small number of studies of HNA antibodies in blood donors. Sachs et al. evaluated 229 sera from female blood donors with 3 or more pregnancies and found 91 to have anti-leukocyte antibodies. Twenty-three of the 91 had reactivity only with neutrophil but no HNA specificities could be assigned.[11] Reil and co-workers assessed 229 male and 5332 female (all previously pregnant) blood donors for HLA and neutrophil specific antibodies. No neutrophil antibodies were found in the male donors. Thirty three HNA antibodies (0.6%) were detected in the parous female donors. Ten of these had HNA-3a specificity.[12] We found no HNA-3a antibodies in our study of 783 parous females (prevalence of 0.0% [95% CI, 0.0 – 0.47%]). Given our relatively small sample size, this is not incompatible with the results of Reil et al. who identified HNA-3a antibodies at a rate of slightly less than 1 in 500 donors (0.2%). The importance of particular HNA specificities in the development of TRALI needs to be further elucidated.

There are a number of limitations to this study. We were able to evaluate only 1171 blood donors including 783 parous female donors for HNA antibodies. The detection of 3 neutrophil antibodies in the 388 non-transfused males tested may indicate that our transfusion histories were not accurate or may be an indication that these are true autoantibodies.[21,22] We used only the GIFT assay to screen for HNA antibodies including HNA-3a antibodies and did not use the GAT assay. A recent study has shown that a GIFT flow cytometry assay (similar to ours) has high sensitivity for HNA-3a antibody detection.[(25)] Although work from the Second and Fourth International Granulocyte Immunology Workshops suggests that the GAT assay is more sensitive than the GIFT assay for detecting the clinically important HNA-3a antibody these results likely evaluated the manual version of the GIFT assay.[26,27] Moreover, our testing laboratory participated in both workshops and performed well in detecting all antibodies including HNA-3a antibodies.

In conclusion, we showed that HNA antibodies occur with low frequency in the donor population. They occur in both male and female donors, with HNA specific antibodies present only in previously pregnant female donors, almost all of whom also had HLA antibodies. These data suggest that the current TRALI risk reduction policy of using plasma from male only or never pregnant female donors should have reduced but not eliminated HNA antibodies from transfused plasma. For those donors undergoing HLA antibody testing

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(AB plasma donors, apheresis platelet donors) the fact that HLA antibody occurred concomitantly with HNA antibody in the majority of HNA antibody positive female donors in our study indicates that the number of HNA antibody positive apheresis products transfused may have been reduced as a consequence of HLA antibody screening.

In a recent 2009 survey, there were no US blood centers performing routine donor HNA antibody testing. This is likely due, at least in part, to the limited availability of HNA antibody testing laboratories in the US and the lack of high throughput assays. Although our data are limited by the small size of our study, they do not create a compelling case for the need to urgently adopt a policy of HNA antibody screening of apheresis donors. However, it is possible that this situation will change as manufacturers design combined high throughput HLA and HNA antibody assays. Such assays will eliminate the need to use fresh neutrophils isolated from volunteer donors as a source of antigen. Moreover, the recent, exciting discovery of the molecular basis of the HNA-3a antigen, provides promise that a full complement of HNA antigens can be included in such assay systems.[15,28] Given the documented clinical relevance of HNA-3a antibody in TRALI, the issue of HNA antibody donor screening may warrant reconsideration if such high throughput assays are developed.

Acknowledgments

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Abbreviations

TRALI	Transfusion Related Acute Lung Injury
HLA	Human Leukocyte Antigen
HNA	Human Neutrophil Antigen
LAPS	Leukocyte Antibody Prevalence Study
U.S.	United States
GIFT-FC	Granulocyte Immunofluorescence Test, Flow Cytometry
MAIGA-FC	Monoclonal Antibody Immobilization of Granulocyte Antigen - Flow Cytometry
ELISA	Enzyme-linked Immunoassay

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Table 1

HNA Antibody Screening Results: Level 1 and Level 2 (post adsorption of known HLA antibodies)

HNA Antibody Type	Non-Transfused Males (n=388)	HLA Antibody Negative Females with 3 Pregnancies (n=390)	HLA Antibody Positive Females with 3 Pregnancies (n=393)	Total (n=1171)
IgG only	1 (0.3%)	4 (1.0%)	4 (1.0%)	9 (0.8%)
IgM only	2 (0.5%)	2 (0.5%)	2 (0.5%)	6 (0.5%)
IgG and IgM	0	0	0	0
Any HNA Antibody	3 (0.8%)	6 (1.5%)	6 (1.5%)	15 (1.3%)

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Table 2

HNA Antibody Screening Results: Level 3 Results

HNA Antibody Type	Non-Transfused Males (n=388)	HLA Antibody Negative Females with 3 Pregnancies (n=390)	HLA Antibody Positive Females with 3 Pregnancies (n=393)	Total (n=1171)
IgG only	1 (0.3%)	1 (0.3%)	3 (0.8%)	5 (0.4%)
IgM only	2 (0.5%)	0	1 (0.3%)	3 (0.3%)
IgG and IgM	0	0	0	0
Any HNA Antibody	3 (0.8%)	1 (0.3%)	4 (1.03%)	8 (0.7%)

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Table 3

HNA Ab Specificity Testing

Positive Donors	Gender	HLA Ab reactivity	HNA Ab Reactivity	HNA MAIGA	HNA Genotype
1	M	Neg	IgG Pan-reactive 4 of 4 +	Neg	NT
2	M	Neg	IgM Non-specific 5 of 8 +	Neg	NT
3	M	Neg	IgM Pan-reactive 6 of 6 +	Neg	NT
4	F	Class I and Class II	IgG HNA-1a 3 of 4 +	POS CD16	1b/1b
5	F	Class I	IgM HNA-1a 5 of 5 +	NT	1b/1b
6	F	Neg	IgG HNA-1a 3 of 6 +	POS CD16	b/1c
7	F	Class II	IgG HNA-4a 4 of 5 +	POS CD11b/18	4b/4b
8	F	Class I and Class II	IgG Non-specific 3 of 6 +	Neg	1a/1b 4a/4b 5a/5a