Transfusion-related acute lung injury : etiological research and its methodological challenges

Middelburg, R.A.

Chapter 8

Prevalence of leukocyte antibodies in the Dutch donor population

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Abstract

Background
Donor leukocyte antibodies have been associated with transfusion-related acute lung injury (TRALI) and can be present in allo-exposed donors. Donor deferral policies aiming to exclude allo-exposed donors are increasingly implemented world wide. We aimed to assess leukocyte antibodies prevalence in different sub-groups of allo-exposed donors in the Dutch donor population.

Study design and methods
Consecutive donors were enrolled during routine whole blood donation. Donors filled out a questionnaire on allo-exposure history. Blood samples were tested for HLA (LifeScreen Deluxe and the Lifecodes LSA I/II assays) and granulocyte reactive (GIFT, GAT, and MAIGA) antibodies.

Results
6034 consecutive donors (60% male) were included. 2.5% reported a history of blood transfusions and 51% (of female donors) reported a history of pregnancy. In never allo-exposed donors the prevalence of granulocyte reactive antibodies was 2.0% (95% CI: 1.6 to 2.4%) and for HLA antibodies it was 7.0% (95% CI: 6.3 to 7.8%). In previously pregnant donors the prevalence of granulocyte reactive antibodies was increased to 3.0% (95% CI: 2.0 to 4.0%) and for HLA antibodies it was increased to 33% (95% CI: 30 to 36%). Prevalence of leukocyte antibodies of all types depended on transfusion history, number of pregnancies, time since last pregnancy, and pregnancy outcome.

Conclusion
14% of Dutch blood donors are allo-immunized against HLA or granulocyte antigens. Deferral of all self-reported allo-exposed donors will decrease this prevalence to 9%. Deferral of all female donors and transfused male donors will result in a similar prevalence among remaining donors but approximately twice as many deferrals.

Introduction

Transfusion-related acute lung injury (TRALI) is a clinical syndrome of respiratory distress that develops within six hours of transfusion of one or more blood products.[1-3] It has been shown that a substantial part of the TRALI cases are caused by antibodies directed against either human neutrophil antigens (HNA) or human leukocyte antigens (HLA) of both class I and class II.[1,4-9]. Therefore, deferral of donors with these antibodies is a logical preventive measure to reduce the incidence of TRALI.

Such deferral policies should naturally be based on adequate data of the relative prevalence of leukocyte antibodies in different donor groups. Leukocyte antibodies in the donor are caused by exposure to cells and tissues of another human being (allo-exposure). This allo-exposure may occur through pregnancy, through transfusion of blood or blood products and through transplantation of stem cells, tissues or organs. However, not all allo-exposure events lead to antibody formation (allo-immunization). The prevalence of allo-immunization increases with the number of allo-exposure events.[10-15] Further, it has been reported that the prevalence of leukocyte antibodies tends to decrease with time after last allo-exposure.[11,13,15]

Therefore, the aim of the current study was to asses the prevalence of all leukocyte antibodies in the Dutch voluntary, non-remunerated donor population and in subgroups of these blood donors, who received prior blood transfusions, had different numbers of pregnancies, different times since last pregnancy, and different pregnancy outcomes.

Methods

Donor recruitment

From July 2008 till August 2008 consecutive donors were recruited at four different blood collection facilities in the North Western part of The Netherlands. Donors were registered for whole blood donation in the usual way. During this registration, donors were asked to participate in the study. Relevant oral and written information concerning the study was provided. After consent, participating donors were asked to fill out a short questionnaire about transfusion and pregnancy history. Approval for this study was obtained from the ethical advisory board of Sanquin, the Dutch national blood supply organization.

Sample processing and leukocyte antibody testing

During the routine blood donation, blood from the diversion pouch was collected into a standard venous blood vacuum serum collection tube. Serum was stored at -80^{o}C until use.

HLA antibody testing

HLA class I and class II antibody screening and specificity determination was performed by means of the LifeScreen Deluxe and the Lifecodes LSAI/II assays (Tepnel, Stamford, CT) according to the manufacturer's instructions.

Screening for the presence of HLA antibodies was performed as follows: 5 µl of microbeads coated with purified HLA class I/class II glycoproteins was incubated with 12,5 µl of donorserum for 30 minutes. After extensive washing to remove unbound antibodies, the beads were incubated for 30 minutes with a phycoerythin conjugated anti-Human IgG antibody. Test samples were diluted and analyzed in the LifeMatch® Fluoroanalyzer. The signal intensity of each bead was compared to the signal intensity of the negative control bead which was included in the analysis to determine positivity or negativity for HLA antibodies. A positive result was defined according to the manufacturer's criteria as one or more bead sets positive for all three adjective values (Adj).

Sera of never allo-exposed donors which were considered positive in the Luminex Screen analysis were further analyzed for the specificity of the detected HLA class I and/or class II antibodies by means of the Lifecodes LSA I and II. Briefly, 40 µl of beads (each conjugated with a different single class I or II HLA glycoprotein) was incubated with 10 µl of donorserum for 30 minutes. After extensive washing to remove unbound antibodies, the beads were incubated for 30 minutes with a phycoerythin conjugated anti-Human IgG antibody. After which the test samples were diluted and analyzed on the LifeMatch® Fluoroanalyzer. The signal intensity of each bead was compared to the signal intensity of the negative control bead. An HLA specificity was considered positive as defined by the manufacturer's criteria as median fluorescent intensity (MFI) value of the first adjective (Adj) equal or higher than 2000 MFI.

Granulocyte reactive antibody testing

The presence of neutrophil specific antibodies of IgG or IgM class was tested by flow cytometry with the Granulocyte indirect Immunofluorescence Test (GIFT), based on the method of Verheugt et al.[16] with a panel of donor granulocytes typed for HNA-1a, 1b, 1c, 2a and 3a. The presence of neutrophil specific antibodies was further tested with the Granulocyte Agglutination Test (GAT),[17] with HNA-3a-positive and HNA-3a-negative donor granulocyte suspensions. Lymphocyte-reacting antibodies were examined by the Lymphocyte ImmunoFluorescence Test (LIFT) according to Décary et al.[18]

Donors were first screened with a panel of two typed granulocyte and lymphocyte suspensions in the GIFT and LIFT for the presence of IgG and/or IgM granulocyte reactive and HLA antibodies. Sera reacting in the LIFT were incubated with a pool of platelets to absorb the HLA class I antibodies before testing them in the GIFT and the GAT. If in the GIFT an aspecific granulocyte reactive antibody was detected the serum was also tested with an FcγRIIIb negative granulocyte suspension.

Finally, the detected antibodies were confirmed in the Monoclonal Antibody Immobilization of Granulocyte Antigens (MAIGA) assay, as previously described.[19] MoAbs against CD16 (238.7, kindly provided by Dr Brian Curtis, Blood Center of Southeastern Wisconsin, USA and 3G8, Medarex, inc, California, USA), CD177 (TAG4 and MEM166, kindly provided by Dr K.Taniguchi, Hiroshima, Japan and Dr V. Horesji, Praha, Czech Republic) and CD18 (IB4, Sanquin, Amsterdam) were used.

Statistical analyses

Descriptive statistics were used to explore the prevalence of different types of leukocyte antibodies in blood donors exposed to different risk factors. All point estimates are reported with 95% confidence intervals. The control group consisted of never allo-exposed donors, defined as: female donors without a history of either pregnancy or transfusion and male donors without a history of transfusion.

To explore changes in leukocyte antibody prevalence over time since the last pregnancy, the prevalence was corrected for the number of pregnancies, using standardization. To do this, we first calculated antibody prevalences observed after a certain time period since the last pregnancy in strata of women with the same number of pregnancies. Each of these observed prevalences at a given time since last pregnancy was weighted to calculate the 'pregnancy corrected' prevalence. The weights were the percentages of women with the same number of pregnancies, in the total group, irrespective of time since last pregnancy. Only women with one or more pregnancies were used to determine the weights, since women who have never been pregnant have no time since last pregnancy. This calculation gives the antibody prevalence that would have been expected if women in all categories of time since last pregnancy had the same number of pregnancies (i.e. if the number of pregnancies was independent of time since last pregnancy). Variance and confidence intervals for the standardized prevalence were calculated according to standard formulas.[20]

For the analyses of the changes in antibody prevalence in time after the last pregnancy a different (oppositely directed) effect was observed for women with one or two pregnancies compared to women with three or more pregnancies. Therefore, results for both groups are reported separately, with correction for differences in the number of pregnancies within those groups as described above.

Leukocyte antibody prevalence after only life births, only aborted (spontaneous or induced) pregnancies, and both life births and aborted pregnancies was corrected for the number of pregnancies. The antibody prevalence was standardized by weighting according to the percentage of women with a given number of pregnancies in the total group, irrespective of pregnancy outcome. Weights were based on women with two or more pregnancies, since the group with both life births and aborted pregnancies can contain only

women with two or more pregnancies. This standardization gives the antibody prevalence that would have been expected if the number of pregnancies was independent of the pregnancy outcomes. Variance and confidence intervals for the standardized prevalence were calculated according to standard formulas.[20]

All donors were tested for leukocyte antibodies, but self-reported variables had some missing values. We assumed missingness to be completely at random and therefore performed complete case analyses in all instances where missing values were encountered.

Results

Participation of 6034 consecutive eligible blood donors was 100%. Baseline variables of these donors are reported in table 1.

Table 1: Baseline variables of tested donors

Variable	Categories	Number*	(%)*
Sex	Male	3614	(60.7)
	Female	2341	(39.3)
Age (median and IQR†)		48 years	(35 – 57)
Pregnancies	0	1119	(48.3)
	1	209	(9.0)
	2	523	(22.6)
	3	313	(13.5)
	4	100	(4.3)
	>4	55	(2.4)
Transfusion history	Yes	148	(2.5)
	No	5719	(96)
	Unkown	91	(1.5)
Leukocyte antibodies	None	5165	(85.6)
	HLA Class I	377	(6.2)
	HLA Class II	521	(8.6)
	Any HLA	753	(12.5)
	Granulocyte reactive	137	(2.3)
	Any	869	(14.4)

Numbers of donors do not add up to 6034 in all categories, due to missing values for some variables. All 6034 donors were tested for leukocyte antibodies. However, due to double positivity of some donors, only the categories "None" and "Any" add up to a total of 6034.

* Unless otherwise indicated.

† IQR: Interquartile range

Never allo-exposed donors

The prevalence of HLA antibodies of any class was 7.0% (95% confidence interval (CI): 6.3 to 7.8%) among never allo-exposed donors and the prevalence of granulocyte reactive antibodies was 2.0% (95% CI: 1.6 to 2.4%) among never allo-exposed donors. Among 4531 never allo-exposed donors (1092 female, 3432 male, and 7 not reporting their sex) 318 tested positive for HLA antibodies (74 female, 243 male, and one not reporting his or her sex) and 137 tested positive for granulocyte reactive antibodies (64 female and 73 male). The prevalence of antibodies of all types among never allo-exposed donors is shown in table 2, according to the sex of the donors.

Table 2: Prevalences of different antibodies among never allo-exposed donors according to sex

Type of antibody	Male donors Prevalence n=3432 (95% CI)	Female donors Prevalence n=1092 (95% CI)	Total Prevalence n=4524 (5% CI)
HLA Class I	2.3% (1.8 to 2.8)	2.8% (1.9 to 3.8)	2.5% (2.0 to 2.9)
HLA Class II	5.2% (4.4 to 5.9)	4.6% (3.3 to 5.8)	5.0% (4.4 to 5.7)
Any HLA	7.1% (6.2 to 7.9)	6.8% (5.3 to 8.3)	7.0% (6.3 to 7.8)
Granulocyte reactive	1.9% (1.4 to 2.4)	2.4% (1.5 to 3.3)	2.0% (1.6 to 2.4)
Any leukocyte	8.8% (7.9 to 9.8)	8.9% (7.2 to 11)	8.8% (8.0 to 9.7)

Because of the unexpected high prevalence of HLA antibodies in never allo-exposed donors, the specificity of these HLA antibodies was further determined. For 108 of the 111 never allo-exposed donors who tested positive in the screening for HLA class I antibodies, we had enough material left to verify the results in the specificity analyses. In 34 of these 108 the presence or specificity of HLA class I antibodies could not be confirmed. For all but one of the 227 donors who tested positive in the screening for HLA class II antibodies, we had enough material left for such verification. Seventy-one of these 226 tested negative in the specificity analyses. Of the 318 donors who tested positive for HLA antibodies of any class we had enough material left for 315. Ninety-three of these 315 tested negative in the specificity analyses (27 female and 66 male). This corresponds to false positive rates in the screening test of 0.79% (95% CI: 0.52 to 1.0%) for the presence of HLA class I antibodies, 1.6% (95% CI: 1.3 to 2.0) for the presence of HLA class II antibodies, and 2.2% (95% CI: 2.8 to 2.6%) for the presence of antibodies against HLA of any class.

Among never allo-exposed donors this false positive rate applies to 95% of donors (i.e. the percentage of truly negative donors) resulting in 2.1% false and 5.0% true positivity in this group. Therefore the positive predictive value in this group is 70% (95% CI: 69 to

72%). In previously pregnant donors the prevalence of a positive test for HLA antibodies increases to 33% and the false positive rate therefore applies to only 69% of the population, resulting in 1.5% false and 31% true positivity and a positive predictive value of 95% (95% CI: 94 to 97%).

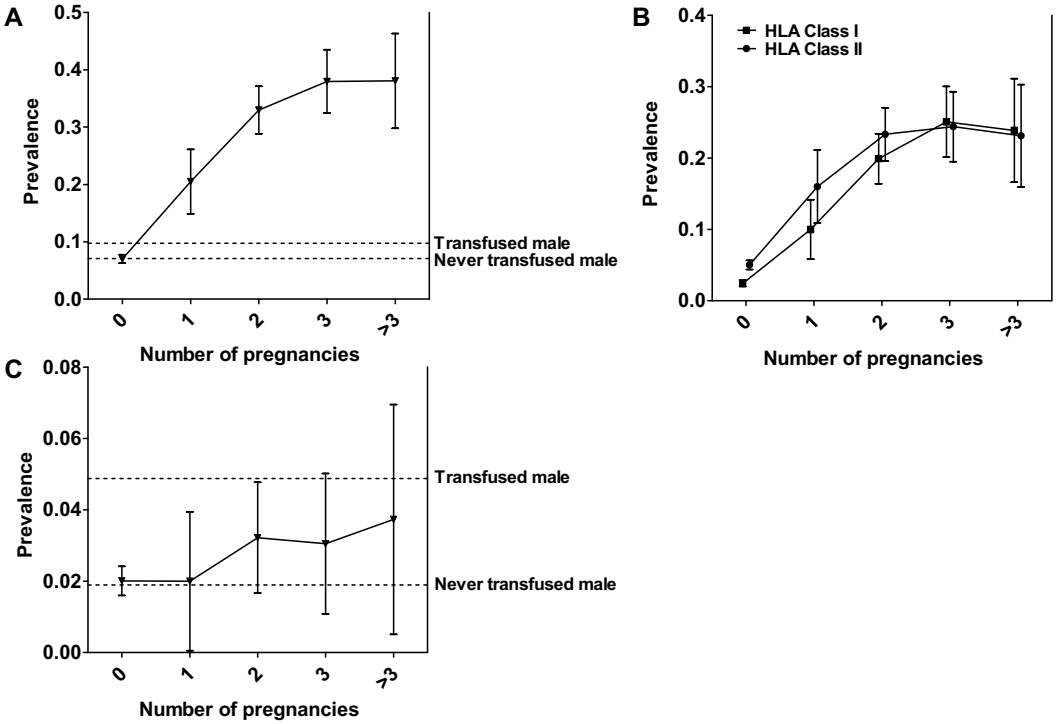

Figure 1: Prevalence of HLA Class I and II and granulocyte reactive antibodies, according to number of pregnancies. **A**: Prevalences of HLA of any class, **B**: HLA Class I (squares) and II (circles) separately or **C**: granulocyte reactive antibodies are shown in relation to the number of pregnancies. All prevalences were determined among never transfused donors, with never transfused male donors added to the category of zero pregnancies.

Number of pregnancies

Figure 1 shows the prevalence for the different antibodies according to the number of pregnancies for never transfused female donors, irrespective of pregnancy outcome. The prevalence of HLA antibodies increases to 38% (95% CI: 30 to 46%) after three or more pregnancies. Of 2215 never transfused female donors for whom the pregnancy history was known, 1094 had previously been pregnant (irrespective of number or outcome of

pregnancies) and 1121 had never been pregnant. On average previously pregnant, never transfused female donors have a prevalence of HLA antibodies of 33% (95% CI: 30 to 36%). For granulocyte reactive antibodies the prevalence among previously pregnant, never transfused female donors was 3.0% (95% CI: 2.0 to 4.0%).

Time since last pregnancy

Figure 2 shows the prevalence of HLA antibodies at different times after the last pregnancy, corrected for the number of pregnancies and stratified according to the number of pregnancies. Less than 10 years after the last pregnancy the standardized prevalence of HLA antibodies was 35% (95% CI: 29 to 40%). Between 10 and 20 years after the last pregnancy the prevalence was 32% (95% CI: 27 to 38%), between 20 and 30 years it was 34% (28 to 40%), and after more than 30 years it was 29% (95% CI: 23 to 36%). Less than 10 years after one or two pregnancies the prevalence was 36% (95% CI: 30 to 43%) and after more than 30 years this decreased to 22% (95% CI: 14 to 29%). Less than 10 years after three or more pregnancies the prevalence was 32% (95% CI: 23 to 40%), and after more than 30 years it was 41% (95% CI: 30 to 53%).

The difference in prevalence between the group with one or two pregnancies and the group with three or more pregnancies (both corrected for the number of pregnancies) was -4.6% (95% CI: -15 to 6.0%) after less than 10 years after the last pregnancy, 6.9% (95% CI: -3.8 to 18%) between 10 and 20 years, 20% (95% CI: 7.5 to 32%) between 20 and 30 years, and 20% (95% CI: 6.2 to 33%) after more than 30 years.

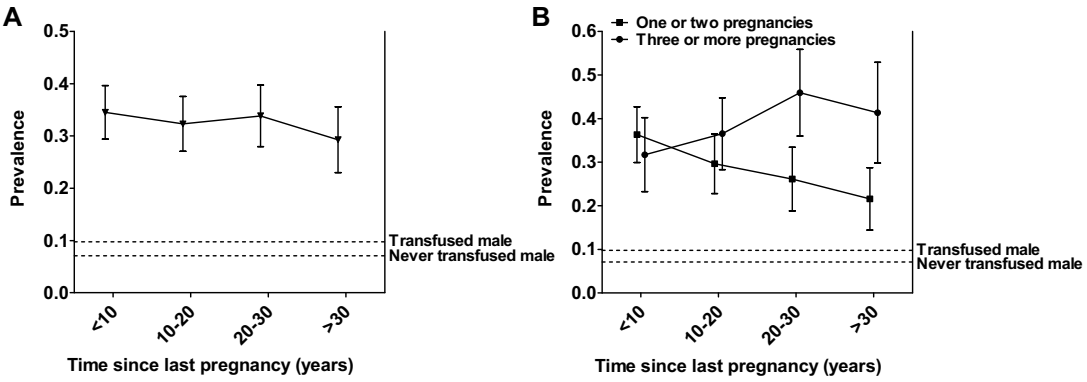

Figure 2: Prevalence of HLA antibodies, according to time since last pregnancy. **A**: The prevalence was standardized for the number of pregnancies and **B**: stratified according to the categories of "One or two pregnancies" (squares) and "Three or more pregnancies" (circles). Within the strata of "One or two pregnancies" and "Three or more pregnancies" the prevalence was further standardized for the number of pregnancies. All prevalences were determined among never transfused donors with at least one pregnancy.

Pregnancy outcome

Aborted pregnancies (either spontaneous or induced) reduced the prevalence of antibody formation, compared to life births (figure 3). However, this prevalence reduction was absent in women with both life births and aborted pregnancies, even after correction for the number of pregnancies.

The difference between the group with life births only and with aborted pregnancies only was 18% (95% CI: 3.9 to 31%) for HLA antibodies of any class and 1.3% (95% CI: -3.9 to 6.6%) for granulocyte reactive antibodies. The difference between the group with life births only and the group with both life births and aborted pregnancies was -8.5% (95% CI: -19 to 1.7%) for HLA of any class and 0.70% (95% CI: -5.1 to 3.7%) for granulocyte reactive antibodies.

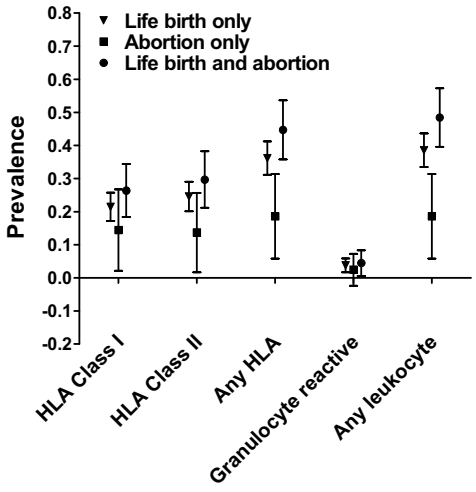

Figure 3: Prevalence of different types of antibodies, according to pregnancy outcomes. Prevalences are compared for women with only life births (triangles), only abortions (spontaneous and induced; squares), and both life births and abortions (circles). All prevalences were determined among never transfused donors with at least two pregnancies and standardized for the total number of pregnancies.

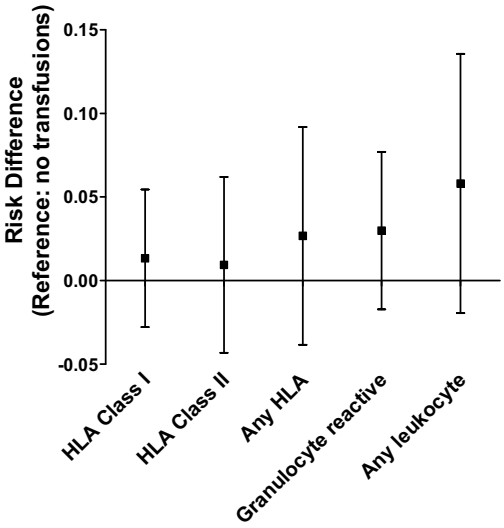

Figure 4: Risk difference of different types of antibodies after a transfusion.

Blood transfusions

A positive transfusion history showed a positive association with all types of antibodies (figure 4). The strongest association was observed for granulocyte reactive antibodies with a risk difference of 3.0% (95% CI: -1.7 to 7.7%) and the weakest for HLA Class II antibodies with a risk difference of 0.94% (95% CI: -4.3 to 6.2%). For HLA Class I antibodies the risk difference was 1.3% (95% CI: -2.8 to 5.4%) and for HLA of any class it was 2.7% (95% CI: -3.8 to 9.2%). The overall risk difference for any kind of leukocyte antibodies after transfusion was 5.8% (95% CI: -2.0 to 14%).

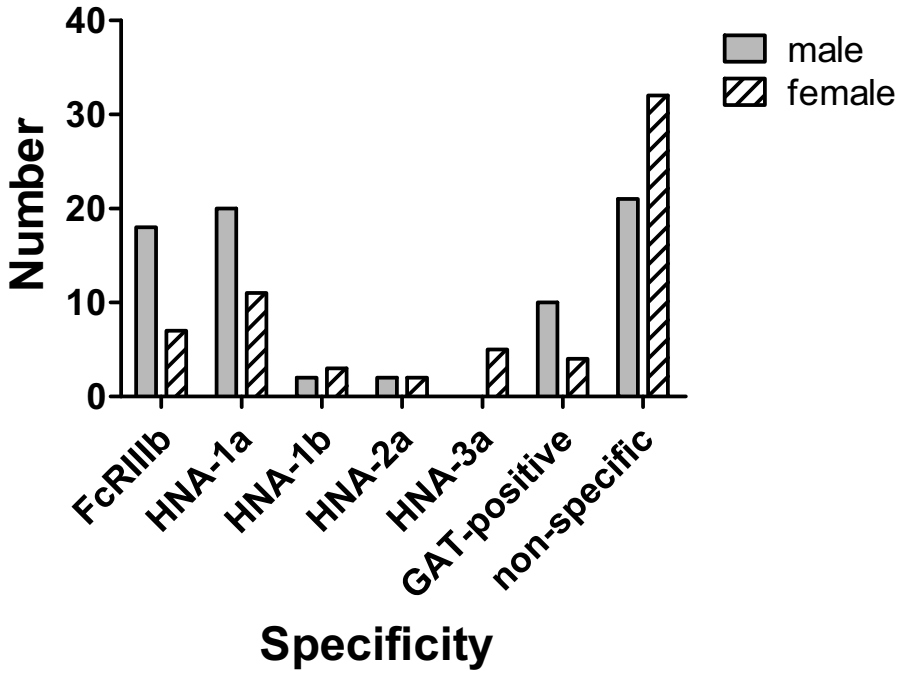

Figure 5: Specificities of 137 granulocyte reactive antibodies, in 3614 male and 2341 female donors, according to sex of the donor.

Granulocyte reactive antibodies

Specificities of granulocyte reactive antibodies are shown in figure 5. The overall prevalence was higher in women 2.7% (95% CI: 2.1 to 3.4%) than in men 2.0% (95% CI: 1.6 to 2.5%), with a risk difference of 0.71% (95% CI: -0.090 to 1.5%). This difference was most pronounced for antibodies of which no further specificity could be determined beyond

their known specificity for granulocytes. For these antibodies the prevalence in women was 1.5% (95% CI: 1.0 to 2.0%) and in men 0.86% (95% CI: 0.56 to 1.2%), with a risk difference of 0.68% (95% CI: 0.10 to 1.3%). For antibodies with confirmed specificities against either HNA or FcRIIIb the prevalence in women was 1.2% (95% CI: 0.81 to 1.5%) and in men 1.2% (95% CI: 0.76 to 1.6%), with a risk difference of 0.034% (95% CI: -0.53 to 0.60%) (see table 3A).

The overall prevalence was also higher in allo-exposed donors 3.2% (95% CI: 2.2 to 4.1%) than in never allo-exposed donors 2.0% (95% CI: 1.6 to 2.4%), with a risk difference of 1.2% (95% CI: 0.12 to 2.2%). The difference between allo-exposed and never allo-exposed donors did not vary with the specificity of the antibodies (see table 3B).

Table 3A: Numbers of donors tested positive for different specificity granulocyte reactive antibodies, according to donor sex

Antibody specificity	Female n=2341	Male n=3614	Total
FcRIIIb	7	18	25
HNA-1a	11	20	31
HNA-1b	3	2	5
HNA-2a	2	2	4
HNA-3a	5	0	5
GAT positive	4	10	14
non-specific	32	21	53
Total	64	73	137

Table 3B: Numbers of donors tested positive for different specificity granulocyte reactive antibodies, according to donor allo-exposure status

Antibody specificity	Positive n=1297	Negative n=4531	Total
FcRIIIb	5	19	24
HNA-1a	7	22	29
HNA-1b	3	2	5
HNA-2a	2	2	4
HNA-3a	5	0	5
GAT positive	4	10	14
non-specific	15	36	51
Total	41	91	132

Deferral of all allo-exposed donors

Deferral of all allo-exposed donors would exclude 1297, or 22% (95% CI: 21 to 23%) of 5828 donors with known allo-exposure status. Allo-exposure was unknown for 206, or 3.4% (95% CI: 3.0 to 3.9%) of 6034 donors, leading to a maximal total deferral of 1503, or 25% (95% CI: 24 to 26%).

Deferral of all donors who were either allo-exposed or for whom allo-exposure status is unknown would lead to the exclusion of 468 antibody positive donors, out of a total of 869, which corresponds to 54% (95% CI: 51 to 57%). However, since the total donor pool

would also be reduced by 25%, the percentage of antibody positive donors would only decrease from 14% (95% CI: 14 to 15%) to 8.9% (95% CI: 8.0 to 9.7%).

Deferral of all female or transfused donors

Deferral of all female donors and all transfused male donors, as is currently the practice for the donation of plasma for transfusion in the Netherlands, leads to the exclusion of 65% (95% CI: 62 to 68%) of all donors carrying antibodies. However, since 43% (95% CI: 42 to 44%) of the donor population is deferred, the total donor pool is also reduced. Therefore, the prevalence among remaining donors only decreases too 8.9% (95% CI: 7.9 to 9.8%).

Discussion

The prevalence of leukocyte antibodies of any type increases with increasing numbers of pregnancies, but the increase levels off after three pregnancies. By comparison, the effect of blood transfusions is much smaller. In women with one or two pregnancies the prevalence decreases with increasing time since the last pregnancy, but this decrease is very limited. Women with only aborted pregnancies also have a lower prevalence of leukocyte antibodies, but this prevalence is still substantially higher than in never allo-exposed donors and also higher than in transfused donors.

Blood transfusions and few, aborted, or older pregnancies are associated with less leukocyte antibodies than many recent life births, but all pregnancies are an important risk factor for leukocyte antibodies and blood transfusions are also associated with a minor increase in leukocyte antibody prevalence. Although interesting differences in prevalence between different groups of allo-exposed donors are observed, these differences are very small. Furthermore, most previously transfused donors are already deferred to decrease the risk of prion transmission. Finally, only a relatively limited number of donors could be preserved by selectively not deferring their specific subgroup of allo-exposure. Therefore, there seems to be little justification to selectively exclude only part of the allo-exposed donors, since all types of allo-exposure increase the prevalence of leukocyte antibodies to some extend. Provided it poses no serious threat to the continuity of the blood supply, questionnaire based deferral measures should therefore be directed at all allo-exposed donors. However, it should also be noted that the clinical relevance of the detected antibodies has not been confirmed. Therefore, any deferral measure based on the (predicted) presence or absence of such antibodies is based on the precautionary principle. Consequently these measures should only be considered if they pose no threat to the blood supply.

Alternatively, deferral measures based on testing of donors for leukocyte antibodies could be considered. In our population 14% of donors would have to be deferred due to

allo-immunization, against 25% deferral in the questionnaire based scenario. Testing based deferral would of course also have the added advantage of a total removal of all leukocyte antibodies from the blood supply, but the financial cost of such measures should be carefully weighted against the practical benefits and the potential risks associated with the antibodies remaining after deferral of all allo-exposed donors.

Although deferral of all allo-exposed donors would remove half of the leukocyte antibodies from the blood supply, the prevalence would be reduced by only a third, because the donor pool would also be reduced in size by a fourth. By also excluding many allo-exposed donors without antibodies, the antibodies of never allo-exposed donors become relatively more important. Allo-immunization rates were comparable between male and female never allo-exposed donors (according to self reported pregnancy and transfusion history), indicating no reason to exclude women reporting no previous pregnancies. The prevalence of leukocyte antibodies in the remaining donor pool would be comparable to selective exclusion of allo-exposed donors only. However, excluding all female donors would result in almost twice as much donor deferral.

The leukocyte antibody prevalence in never allo-exposed donors was higher than previously reported for both HLA antibodies[11,13-15] and granulocyte reactive antibodies.[14] To rule out non-specific antibodies the specificities of HLA antibodies of never allo-exposed donors were determined. Verification of the specificities of all granulocyte reactive antibodies showed almost half to be non-specific for known granulocyte antigens, but confirmed all to be granulocyte reactive. Determination of HLA antibody specificities showed the false positive rate to be so low that it could not materially influence our conclusions. For the high prevalence of HLA antibodies found in never allo-exposed donors another possible explanation could be the presence of antibodies against epitopes on HLA molecules that are exposed in the test kit but not on cells that have a natural conformational structure. However, it is unlikely that the prevalence of these antibodies would change dramatically after pregnancy. Therefore, the prevalence of these clinically irrelevant antibodies would be expected to be a constant low percentage which would not influence our conclusions. Furthermore, when considering possible donor deferral strategies, use of the bead-based assay is preferable due to higher sensitivity and greater ease of use in large scale screening. In this light it is also important to note that the possible unnecessary deferral of probably less than a percent of donors is likely to be preferable over erroneously failing to defer a similar or even larger percentage of donors with potentially dangerous antibodies. The same arguments would apply to granulocyte reactive auto-antibodies. If a low percentage of detected granulocyte reactive antibodies are indeed auto-antibodies, which can be present in the donor without causing any symptoms, this percentage will likely not change with allo-exposure and the clinical relevance for TRALI could not be excluded. Therefore, further distinguishing granulocyte reactive antibodies into auto-antibodies and allo-antibodies would not be informative.

Due to continuously improving methods for the detection of leukocyte antibodies it is impossible to name a single method as a gold standard with which to compare all others. Since we used a relatively new and very sensitive assay, for the detection of HLA antibodies, the primary concern should be for false positive results. As detailed above, using manufacturer recommended cut off values did produce some false positive results. However, adapting the cut offs would require specific information about which antibodies are considered clinically relevant and which are not. For TRALI, this is at present not possible. Therefore, we consider the very low false positive rate preferable to a similar, or even higher, false negative rate.

Several studies have previously investigated the association of pregnancies and blood transfusions with the occurrence of leukocyte antibodies.[10-15] However, techniques for the detection of leukocyte antibodies are continuously improved, leading to increased sensitivity. Furthermore, due to changes in composition of blood products, the risk of developing leukocyte antibodies after receiving a blood transfusion also changes. Recent studies have been done in populations in North America,[13,15] where the ethnical composition of donor populations is very different from the Western European situation. Since the ethnical background is associated with different frequencies of HLA and granulocyte antigen genotypes, this could also influence the prevalence of leukocyte antibodies. However, the observed leukocyte antibody prevalence after pregnancies was comparable to two recent North American studies[13,15] and, as might be expected, slightly higher than an older study.[11] Remarkably the observed prevalence were substantially higher than a previous German study.[14] Even disregarding the granulocyte reacting antibodies that were non-specific for known granulocyte antigens, the difference with this German study is still bigger than would be expected by chance variations. Any attempt to explain this difference must remain purely speculative. It might well be possible that in the Dutch population immigrants from different backgrounds have throughout the centuries contributed to a more diverse array of HLA and granulocyte antigen genotypes. This would increase the chances of an antigen mismatch between a pregnant woman and her child. This can, however, not explain the difference in prevalence in male donors. Which highlights the importance of independently screening seemingly similar donor populations, since unknown differences between populations can apparently have a substantial influence on antibody prevalence.

The most surprising result was the marked difference, in the change of antibody prevalence with time since last pregnancy, between women with one or two and three or more pregnancies. The observed decrease in prevalence after one or two pregnancies is in accordance with previous studies.[11,13,15] However, the increase with time after three or more pregnancies has not previously been reported. Since there is no plausible biological mechanism that could cause antibody prevalence to really increase several decades after the last exposure, it seems likely there has been an additional pregnancy related risk factor for

antibodies development in the Netherlands that has been removed between 10 and 20 years ago. Donors who had their last pregnancy more than 20 years ago would have been exposed to this risk factor, while donors who had their last pregnancy less than 10 years ago would not have been exposed. This effect has likely been present in donors with one or two pregnancies as well, but due to lower persistence of antibodies after fewer immunizing events it is completely counteracted by the natural decrease in antibody prevalence in time.

We also showed aborted pregnancies to have a lower risk of inducing leukocyte antibodies, probably due to reduced exposure to allo-antigens. This risk reduction was not observed in women with both life births and aborted pregnancies. This may be due to the fact that those women mostly have had more than two pregnancies and would therefore, even based on their life births alone, be likely to be in the plateau of high antibody prevalence after two or more pregnancies. In most previous studies no distinction was made between different pregnancy outcomes (life born, stillborn, miscarriage, abortus provocatus).[14,15] The type of pregnancy outcome could influence both the degree of exposure of the mother to paternal HLA or granulocyte antigens and the extent of tissue damage and related inflammation involved in this exposure, which together influence the probability of developing antibodies.

A possible concern regarding the ascertainment of information on the history of blood transfusions and pregnancies could be that self-reported histories lack the necessary accuracy. This could especially be expected for aborted pregnancies. However, the rate of aborted pregnancies compared to the rate of life births as reported in our study corresponded well with the national average. Assuming the number of life births to be reported reasonably accurately, this suggests that under reporting of aborted pregnancies was not a problem in our study.

In conclusion, 14% of Dutch, non-remunerated, volunteer blood donors has been allo-immunized against HLA or granulocyte antigens. Amongst self reported never allo-exposed donors, the prevalence of leukocyte antibodies is 9%. Consequently, the deferral of all allo-exposed donors (i.e. 25% of all donors) will remove only half the leukocyte antibodies from the blood supply, reducing the prevalence by only a third. Deferral of all female and all transfused male donors (i.e. over two fifths of all donors) will result in a similar decrease in antibody prevalence.

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