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

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Transfusion-related acute lung injury

Etiological research and its methodological challenges

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Chapter 1

Introduction

Rutger A. Middelburg

When a patient who has just received a blood transfusion suddenly becomes short of breath to the point where it requires medical intervention, a diagnosis of transfusion-related acute lung injury (TRALI) should be considered. At present TRALI is thought to be the most frequent serious side effect of blood transfusions.[1-4] However, the temporal relation to a transfusion is often overlooked and the fluid built-up in the lungs, visible in chest X-rays, is frequently blamed on cardiac problems. Furthermore, if kept on adequate supportive care, such as mechanical ventilation, most patients recover within four days.[5-7] Therefore, TRALI can easily go unrecognized.[8] This likely contributed to the fact that, for such a serious problem, it has only relatively recently started to receive much attention in the literature.

Although TRALI was probably reported in the literature as early as the 1950s, those first reports were not specifically concerned with TRALI.[9,10] The first report of what was likely a patient with TRALI was of a single case in a series of four cases all suffering from different transfusion related hypersensitivity reactions.[9] The next publication that reported a likely TRALI case, mentioned this case only briefly while focusing more attention on the immunologic properties of leukocytes.[10] Furthermore, this report actually concerned a patient who seems to have suffered primarily from an anaphylactic reaction with TRALI as a possible secondary reaction only.[10] Both these publications likely concerned TRALI cases, but both lack the necessary information to be sure these cases would also be considered TRALI today and the authors certainly did not recognize TRALI as a distinct clinical entity.[9,10] In 1962 the first TRALI case was published with sufficient clinical details to verify that it would meet today's criteria.[11] This publication, however, compared this case mainly with previous reports of febrile reactions and again did not recognize TRALI as a distinct syndrome.[11] It wasn't in fact until the second half of the 1960s that the literature started showing evidence that TRALI was first recognized as a distinct clinical entity.[12,13] Subsequently the clinical syndrome that we now call TRALI was given a variety of names in case reports and small case series throughout the 1970s and the early 1980s.[14-22] Two decades after it was first recognized as a distinct transfusion-related complication the syndrome finally received the name we still use today.[23]

By that time it had already been suggested repeatedly that TRALI was caused by the passive infusion of leukoagglutinins.[10,11,14-19,21,22] These leukoagglutinins included antibodies against human neutrophil antigens (HNA) and human leukocyte antigens (HLA) of both classes I and II. These antibodies are now considered the most important risk factor for TRALI and are most commonly collectively referred to as leukocyte antibodies.[5,23-27] These antibodies are most prevalent in parous women and transfusion recipients (i.e. allo-exposed individuals).[28-33] Which has lead these groups of donors to be considered potentially more likely to cause TRALI.[34-38] In recognition of this fact and the increased risk of other complications associated with the transfusion of HLA antibodies, some local blood banks (e.g. Blood bank Leiden) excluded plasma from parous and transfused donors from use for transfusion or the suspension of platelets as early as the 1970s. However, in

the absence of a firm evidence base, this practice was abandoned later upon organizational fusions with other blood banks who did not previously take these measures.

In 1985 the publication of a large case series marked an important milestone in TRALI research.[5] This publication first suggested the acronym TRALI for transfusion-related acute lung injury, the name introduced two years earlier by the same authors.[23] It also suggested a definition that has changed remarkably little since then. Further, the first and arguably still most accurate estimate of the incidence of TRALI was made by closely monitoring all transfusion recipients at the Mayo Clinics in Rochester over a two year period. Finally, both donors and patients were tested for leukocyte antibodies and these were found in 89% of cases. There was no formal control group to ascertain the normal prevalence of leukocyte antibodies in the source population of donors and patients. Furthermore, most patients received transfusions from two or three donors, increasing the probability that at least one would test positive for leukocyte antibodies by chance alone. However, the authors clearly considered the observed number of positive cases to be higher than expected and concluded that leukocyte antibodies were an important risk factor for TRALI and this concept has dominated the TRALI literature ever since.[5,23-27]

The definition proposed in 1985 was largely confirmed by the Canadian consensus conference nearly two decades later.[39,40] The definition adopted by the conference and the European haemovigilance network (EHN) is based on the 1994 American-European consensus conference definition of acute respiratory distress syndrome (ARDS) and acute lung injury (ALI).[40,41] Next to the usual requirements for ALI (i.e. acute respiratory distress, with bilateral infiltrates in the chest X-rays, in the absence of circulatory overload) the definition of TRALI also states the first symptoms have to occur within six hours of the last transfusion. This differs only minimally from the 1985 definition which required a maximum of four hours since the last transfusion.[5] The chosen period of six hours is to some extend arbitrary and the rest of the definition held little surprises either. The main purpose of the consensus definition, however, was not to develop an entirely new definition. The consensus rather establishes a single, unambiguous definition to facilitate international research and communication. The biggest contribution of the consensus conference was probably the addition of the category of "possible TRALI" for patients who do meet the definition of TRALI but who also have other risk factors for ALI or ARDS.[39,40]

This category was necessary since TRALI is clinically indistinguishable from ALI or ARDS caused independent of transfusions. Even the pathophysiology of TRALI is almost identical to that of ARDS, with activated neutrophils damaging the pulmonary vasculature. Only the cause of neutrophil activation is different, making the marked difference in prognosis rather surprising. Mortality of TRALI is estimated to be between five and ten percent and the majority of patients recovers spontaneously and completely within 96 hours, on supportive care alone.[5-7]

With most of the research attention focused on leukocyte antibodies it took until 1997 before a serious alternative cause for TRALI was first suggested in the form of biological response modifiers (BRM).[42] These BRM can include biologically active lipids, peptides and any other substance, including antibodies, that can activate neutrophils.[42-44] This new theory finally made it possible to explain TRALI occurring when neither donor nor recipient had detectable leukocyte antibodies. However, in spite of this new theory, leukocyte antibodies and donors who have a high prevalence of them have remained the prime focus of the TRALI literature.

In their 2004 annual report the Serious Hazards Of Transfusion (SHOT; the haemovigilance organization of the United Kingdom) noted TRALI had become the leading cause of transfusion related serious morbidity and mortality in the United Kingdom. Therefore, they proposed to implement measures for the prevention of TRALI.[45] Though there was still no numerical evidence of a relation between TRALI and leukocyte antibodies, much less any indication of the strength of such a relation, the SHOT advised the exclusion of plasma from female donors from use for transfusion whenever possible.[45] This advice included the use of plasma from female donors for the suspension of platelets for transfusion. Both plasma and the plasma used for the suspension of platelets in the United Kingdom are now derived from male donors in over 95% of products. Unfortunately, the SHOT uses serological findings to score the imputability of reported TRALI cases and most analyses are restricted to the highest levels of imputability only.[45] Therefore, serological findings at least partly determine inclusion of a TRALI case in the analyses precluding any conclusions regarding the role of antibodies to be drawn from their data. Further, since they rely on passive reporting and TRALI is believed to be severely underreported, the number of reported TRALI cases is a poor indicator of the real number of TRALI cases. The effect of preventive measures on the occurrence of TRALI in the United Kingdom is therefore impossible to judge.

The TRALI literature until recently consisted almost exclusively of case series and case reports. Often investigations of leukocyte antibodies did not include all involved donors. A control group, to asses the normal prevalence of these antibodies in the source population, was never included. Further difficulties in using the literature to assess the role of leukocyte antibodies in the occurrence of TRALI arise from changes in blood products over time. For instance, the observation that six percent of TRALI is caused by leukocyte antibodies of the recipient reacting with transfused neutrophils[5] has obviously become irrelevant in the age of universal leukoreduction. Finally, publication bias likely favored publication of serologically positive cases, causing a false or falsely increased association in the literature. These factors all contribute to the fact that evidence from the literature, for a relation between leukocyte antibodies and TRALI, was until December 2007 largely impossible to asses and indirect at best (as shown in Chapter 2).

Other methodological issues, remaining largely unrecognized in the entire transfusion literature, include the problems of confounding by indication (Chapter 3) and the presence of innocent bystander transfusions, causing effect estimate dilution (Chapter 4). The problem of effect estimate dilution caused by multiple transfusions is not easily solved by conventional statistical methods, but several different specialized solutions are possible. In Chapter 4 we use simulation studies to demonstrate the surprisingly strong effect dilution, the complete inadequacy of conventional correction methods in this setting, and the validity of four newly proposed solutions to this problem.

In spite of the lack of numerical evidence and based primarily on the precautionary principle Sanquin (the Dutch blood supply foundation) has decided that all plasma donations intended for transfusion will be from never transfused male donors as of 1st October 2006. To lend scientific support to such preventive measures, we aimed to quantify the contribution of leukocyte antibodies, female donors, and allo-exposed donors to the occurrence of TRALI.

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Chapter 2

The role of donor antibodies in the pathogenesis of transfusion-related acute lung injury

A systematic review

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Abstract

Background

The majority of cases of transfusion-related acute lung injury (TRALI) are thought to be caused by the presence of leukocyte antibodies in the blood of the donor. We performed a systematic search of the literature to quantify the contribution of donor antibodies to the occurrence of TRALI.

Study design and methods

We conducted a systematic search of the PubMed and EMBASE databases. Retrieved articles were judged by three authors independently. Reference lists of all articles were subsequently screened for relevant references. All articles in English, German, French and Dutch, published at any time before December 2007 were eligible for inclusion.

Results

Of 77 articles, on leukocyte antibodies in donors involved in a case of TRALI, 14 articles contained sufficient data. These 14 articles reported leukocyte antibodies in 24 of 51 donors (47%) associated with 24 of 28 TRALI cases (86%). Of 15 articles that reported the prevalence of leukocyte antibodies in the general donor population, 2 articles reported a prevalence of 17% in (452) randomly selected donors. The odds ratio for developing TRALI was 15 (95% CI 5.1 to 45) for patients who received a transfusion from a donor who tested positive for leukocyte antibodies, compared to donors who tested negative. Leukocyte antibodies contributed to 80% (95% CI 51% to 92%) of all TRALI cases.

Conclusion

Leukocyte antibodies were more prevalent in donors involved in TRALI cases than among randomly selected donors. These findings suggest that donor antibodies contribute to four fifths of all TRALI cases.

Introduction

Transfusion-related acute lung injury (TRALI) is clinically indistinguishable from acute respiratory distress syndrome (ARDS) and develops during, or shortly after, transfusion of one or more blood products.[1] TRALI is currently recognized as the most common, severe side effect of blood components transfused.[2-5] It has an estimated incidence of 1 in 5000 transfusions and a mortality of 6%.[1] It is thought to often be caused by donor derived leukocyte antibodies, which can be directed either against the human leukocyte antigens (HLA) or against the human neutrophil antigens (HNA). These antibodies could activate the recipient's pulmonary neutrophils, which in turn damage the pulmonary endothelium. This causes pulmonary edema.[1,6,7]

Since these antibodies are most frequently found in parous women and are present primarily in plasma rich blood products,[8,9] it has been proposed to exclude parous women from donating plasma for transfusion.[10] In many countries, among others the United Kingdom and the Netherlands, plasma from female donors is no longer used for transfusion, when possible.[3] Although the situation does call for preliminary caution, other etiologies have also been suggested for TRALI.[11-15]

The importance of other potential causes and the contribution of leukocyte antibodies to the occurrence of TRALI is unclear. Therefore, it is not possible to estimate the benefits, expected to be gained, by measures directed at the elimination of products containing these antibodies from the blood supply.

Leukocyte antibodies do not always cause TRALI which is in part due to the heterogeneity of the HLA system. The presence of leukocyte antibodies in blood products is not particularly uncommon either,[88,9] which raises the possibility that their presence is merely a chance finding in some TRALI cases.

Therefore, to estimate the contribution of leukocyte antibodies to the occurrence of TRALI, the prevalence of these antibodies in a control group of randomly selected donors is needed, in addition to the prevalence in donors associated with a TRALI case. Unfortunately, this control group is generally not included in the TRALI literature, which consists mainly of case reports and case series.

Furthermore, evidence from the literature might be biased by circular reasoning and publication bias, causing only those cases where leukocyte antibodies were identified to be diagnosed and published as TRALI cases. Given that leukocyte antibodies are present by coincidence in a certain portion of TRALI cases, publication bias would ensure an overestimated association of these antibodies with TRALI cases.

To quantify the contribution of donor antibodies to the occurrence of TRALI, we compared the reported prevalence of leukocyte antibodies, in donors associated with cases of TRALI, with the reported prevalence in the general donor population.

Methods

We performed a literature based study into the role of donor leukocyte antibodies in TRALI. Case reports and case series from the literature were included to obtain data on antibody prevalence in donors associated with TRALI cases. These data were compared to a control group provided by reports on the prevalence of leukocyte antibodies in the donor population in general. Leukocyte antibody prevalences in these two groups were compared as described below in more detail.

To correct for differences between patients in the number of received transfusions, all prevalences determined in donors were recalculated to the number of patients that could potentially be transfused with antibody containing components, assuming all patients received the same number of transfusions. From this comparison we obtained the odds ratio, as an estimate of the relative risk for TRALI associated with the presence of leukocyte antibodies in transfused blood product.

Our question concerns the role of leukocyte antibodies in general and does not distinguish the relative contribution of anti-HNA or anti-HLA antibodies, nor does it focus on the role of antibodies that match with cognate antigens in the recipient. The available literature does not allow these additional comparisons to be made.

We performed an automated search of the literature and subsequently used predetermined criteria to include or exclude articles retrieved by this literature search. These criteria were chosen to select articles that would provide all data necessary for our comparison in the least biased way possible.

Search strategies

We conducted a systematic search of the PubMed and EMBASE databases, searching for articles mentioning either “lung” or “pulmonary” in combination with either “injury” or “edema” and mentioning “blood transfusion” while also mentioning either “antibodies” or “antigens” in any way. The complete search strategy is given in Appendix I. The resulting list of titles was judged for relevance by three authors independently and, of these selected articles, the abstracts were judged similarly. Exclusion criteria were: review (non-original data), animal study, *in vitro* study, stem cell transplantation or “different topic”. The reference lists of all articles, that were thus selected, were subsequently screened for relevant references, which had not been retrieved by the search.

An automatic search of the PubMed and EMBASE databases, for articles containing information on the prevalence of leukocyte antibodies in the general population, returned no articles with relevant information (see Appendix I for search strategy). Therefore, articles on this subject were selected solely from the reference lists of the articles selected

from the TRALI literature. Subsequently, we continued checking reference lists of referred articles until no relevant new references were found.

A flowchart of the selection process is presented in Figure 1. All articles in English, German, French and Dutch, published before December 2007 were eligible for inclusion.

Definitions

Articles that reported on measuring leukocyte antibodies in donors involved in TRALI cases (TRALI donors) were judged, in several steps, for the type of data available and the definition of TRALI used. A flowchart of the exclusion process is presented in Figure 2.

In each of these steps articles were excluded if they did not contain the information required for our analyses. In the successive exclusion steps, articles were excluded if they failed to report the number of TRALI cases tested, the number of donors tested, or the definition of TRALI used. Furthermore, articles were excluded if the presence of leukocyte antibodies was included in the definition of TRALI, the definition did not correspond to the clinical definition of TRALI that was agreed upon at the Canadian consensus conference in Toronto,[16,17] or if not all involved donors were tested.

In each successive step we documented the number of articles excluded during this step. For the remaining articles we assessed the number of TRALI cases reported, the number and percentage of TRALI cases with at least one donor tested positive for leukocyte antibodies, the number of donors tested, and the number and percentage of donors tested positive for leukocyte antibodies (Figure 2).

Articles that reported on the prevalence of leukocyte antibodies in the general population were included if the study population consisted of randomly selected donors. All articles reporting on specific subpopulations, such as female donors, (multi-)parous donors or previously transfused donors were excluded.

Analyses

The weighted average of the reported prevalences of leukocyte antibodies, among randomly selected donors, was calculated. This prevalence was used to estimate the number of donors with leukocyte antibodies that would be expected among donors not involved in TRALI cases. This expectation was corrected for the number of components transfused (Table 1, row 2), which was set as equal to that of the TRALI cases. The resulting expected number was subsequently compared to the observed number of donors with leukocyte antibodies among the donors that were involved in TRALI cases (Table 1, row 1).

Furthermore, the odds ratio (OR), and corresponding 95% confidence interval (CI), were calculated. The calculated OR was the ratio of the odds for developing a TRALI after being transfused with at least one product containing leukocyte antibodies, compared to the odds of developing a TRALI after being transfused only with products not containing

leukocyte antibodies, given that patients in both situations had an equal number of components transfused. This odds ratio is an estimation of the relative risk of developing a TRALI after being transfused with at least one product containing leukocyte antibodies, compared to the risk of developing a TRALI after being transfused as many products without leukocyte antibodies.

Finally, given the assumption that an observed association between leukocyte antibodies and TRALI was causal, the excess presence of antibodies could be used to calculate the fraction of TRALI cases explained by these antibodies. This was done by calculating the population attributable risk (PAR), i.e. the percentage of all TRALI cases that could be attributed to the presence of leukocyte antibodies, and the corresponding 95% CI. The OR and its variance were entered into the appropriate formulas to calculate the PAR and the corresponding 95% CI.[18]

Results

A total of 82 articles contained information on the prevalence of leukocyte antibodies in donors who had donated blood that was transfused to TRALI patients. A further 15 articles contained information on the prevalence of these antibodies in either the general population or a donor population. Figure 1 shows a flowchart of the selection process of these 97 articles. The complete lists of references for both searches are given in Appendix II.

Leukocyte antibodies and TRALI

Of 82 articles, eight were excluded because they failed to state the number of cases in which one or more donors tested positive for leukocyte antibodies. The 74 remaining articles showed 75% of 258 cases to involve at least one donor that tested positive. A total of 57 articles reported both the number of donors tested and the number of donors tested positive. From these the prevalence among 364 donors involved in 122 TRALI cases was estimated to be 32%. As shown in Figure 2, further stepwise exclusion of articles affected both these percentages.

Only 14 articles met all criteria for containing necessary data, reporting an average prevalence of 47% (24 donors positive of 51 tested), resulting in 86% of 28 cases in which at least one donor was tested positive for leukocyte antibodies (Table 1). Of these 14 articles eleven were case reports,[19-29] two reported on two cases each,[30,31] and one reported on 13 cases.[32] The average leukocyte antibody prevalence among the 13 articles reporting on 2 cases or less was 42%, while the prevalence reported in the case series of 13 cases was 62%. This corresponded to 93% and 77% of cases in which one or more donors tested positive for leukocyte antibodies, respectively. TRALI patients had an average of 1.8 (51/28) blood components transfused.

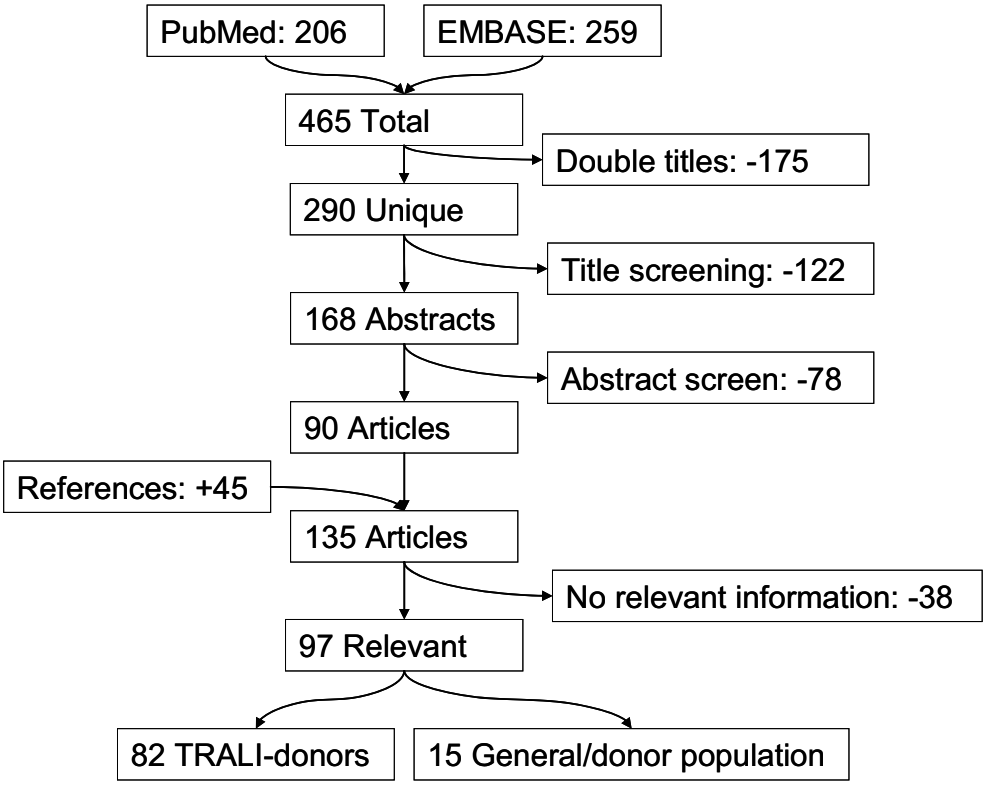

Figure 1: Flowchart of selection of relevant articles from the literature search. Values are numbers of articles. The literature search for leukocyte antibodies in the general population returned 309 articles. These were not included in this chart, since none contained relevant information. Articles on this subject were, instead, selected from reference lists only (see text for details).

Leukocyte antibodies in the donor population

The literature search for leukocyte antibodies in the general population returned 309 articles. None contained relevant information. Articles on this subject were, instead, selected from reference lists only.

The prevalence of leukocyte antibodies in the general population or donor populations, as reported in the 15 articles included on this subject, ranged from 3% to 48%. Only two of these articles reported on 452 randomly drawn donors,[33,34] therefore representing the male-female-ratio found in those donor populations. The weighted average of the prevalence of leukocyte antibodies reported in these two articles was 17% (75 of 452; 95% CI 13% to 20%).

Transfusion of 452 products to control patients, each receiving 1.8 transfusions, would have resulted in the transfusion of 248 patients. Of these 248 control patients 70 would have been transfused with at least one product from a donor with leukocyte antibodies (Table 1).

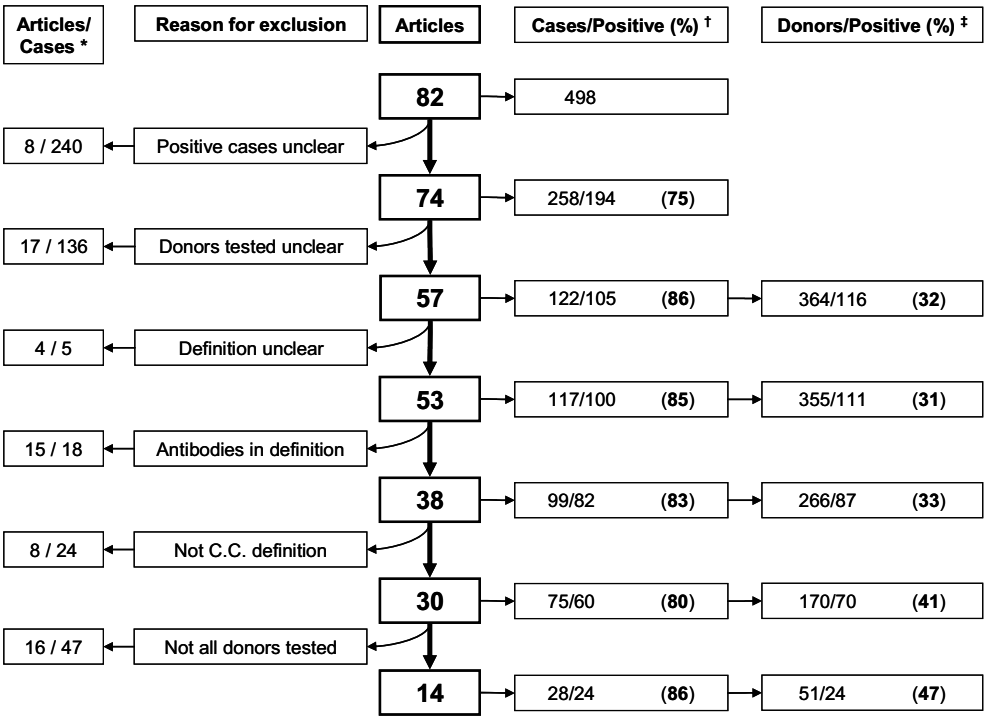

Figure 2: Flowchart of stepwise exclusion of 68 articles on TRALI donors in which insufficient information was reported. Values are numbers or percentages, as indicated below. The central column shows the number of articles decreasing stepwise from 82 to 14. *Articles/Cases: Numbers of articles and cases that were excluded in this step. †Case/Positive (%): Number of TRALI cases with information about the presence of leukocyte antibodies in one or more donors / Number of TRALI cases in which one or more donors were tested positive for leukocyte antibodies (TRALI cases in which ≥1 donor tested positive as percentage of all TRALI cases in which ≥1 donor was tested). ‡Donors/Positive (%): Number of donors tested / Number of donors tested positive (donors tested positive as percentage of total donors tested). C.C. definition: Canadian Consensus definition.

Comparison of observed and expected prevalences

The prevalence of leukocyte antibodies in donors involved in TRALI cases was higher than the prevalence in a group of randomly selected donors (47% versus 17%). Leukocyte antibodies were detected in 24 of 28 TRALI cases (86%). In each of these cases only one donor was tested positive, resulting in 24 of 51 donors (47%) testing positive.

The odds ratio for developing TRALI was 15 (95% CI 5.1 to 45) for patients who received a transfusion from at least one donor who tested positive for leukocyte antibodies, compared to patients who received an equal number of transfusions from donors who tested negative (Table 1). The population attributable risk was 80% (95% CI 51% to 92%).

Table 1: Number of TRALI cases and control patients with and without leukocyte antibodies present in at least one donor

	Leukocyte antibodies				
TRALI	+	-	Total	OR*	95% CI
+	24	4	28		
-	70†	178†	248†	15	5 to 48
Total	94	182	276		

*The OR has been calculated by the cross-product: (24*178)/ (4*70)=15

†Number of control patients that would have been transfused (in each group) with the 452 products from the randomly drawn donors, if each of them would have received 1.8 transfusions, the same number as received by the 28 TRALI cases.

Discussion

Our systematic review of the literature shows that the prevalence of leukocyte antibodies in donors involved in reported TRALI cases was higher than the prevalence reported among randomly selected donors. Our findings suggest four fifths of all cases of TRALI are explained by the presence of antibodies in donors.

Many articles on TRALI cases did not contain all the required information, or did not define TRALI according to the Canadian consensus definition.[16,17] Furthermore, to avoid the pitfall of circular reasoning, we excluded all reports in which the diagnosis of TRALI was made with knowledge of the antibody status of associated donors. This left us with only a minority of the TRALI publications. Data from only 28 of a total of 498 reported TRALI cases could be included in this review. Although this represents only a limited fraction of the total literature data, it gives the least biased estimate, due to systematic selection based on objectively predetermined criteria.

We could not include several large, well designed studies because they did not report on all data required for our analyses. One of these studies was the important publication by Popovsky and Moore in Transfusion, 1985.[1] Since this one study alone included a similar number of cases as the combination of all studies included in this review, we contacted the authors. Although the individual, case-specific data are no longer available after 22 years, averages could still be obtained. In 89% of cases one or more donors tested positive for leukocyte antibodies. In two or three of these cases more than one donor tested positive and

two to three donors were tested per case (personal communication, Mark A. Popovsky, august 2007). This leads to an estimation of the leukocyte antibody prevalence of between 32% and 49%, which is very similar to the results we obtained in our analyses.

Our study does not take into account antibody specificity, since our primary aim is to quantify the risk imposed by antibodies in the blood supply. From the perspective of the blood bank the presence of cognate antigens is not relevant, since it can not be known beforehand whether a future recipient will have the cognate antigen. The chance of antibodies causing TRALI can therefore be viewed as composed of both the chance of a recipient expressing cognate antigens and the chance of a recipient with cognate antigens being sensitive to developing TRALI. We use the prevalence of leukocyte antibodies in randomly selected donors as a control group. Therefore the calculated contribution of these antibodies to the occurrence of TRALI reflects the excess presence of antibodies, above the expected value. This excess presence can be explained only by biological significance, statistical variation and bias. Statistical variation is controlled for in the calculation of the 95% CI, which leaves only bias as an alternative to biological significance to explain our results. Possible causes of bias are further discussed below.

A large part of the literature reporting on TRALI cases is comprised of reports of one or two cases only, while larger case series remain relatively rare. Of the 14 articles that were included in this study only one reported on more than two cases. This article reported a lower fraction of positive cases (77%) than that reported in the case reports (93%). This is suggestive of publication bias in favor of reports of cases in which at least one donor was tested positive for these antibodies. Such bias may have lead to overestimation of the contribution of antibodies to the occurrence of TRALI in this systematic analysis.

Furthermore, it should be noted that other etiologies of TRALI have been suggested more recently[35] and may have a less severe clinical presentation.[7] Therefore, in these cases chest X-rays, which are required according to the Canadian consensus definition, may be performed less often. These studies would therefore be excludes from this review. Strict adherence to objectively predetermined criteria does result in the least biased estimate of the contribution of leukocyte antibodies to the occurrence of TRALI as defined according to the Canadian consensus definition.[16,17] However, less severe TRALI, which could still be clinically relevant, might not meet all criteria of this definition. Therefore, an etiological difference between less severe and severe TRALI can not be excluded based on our results.

The presented prevalence in the general donor population is based on only two studies, which could raise questions about the extrapolation of these results to other donor populations. Although there is not enough information to judge this in detail, one of these studies mentions 40% of the donors to be female. This does not seem a particularly unexpected percentage and thereby suggests the data from this study to be likely to apply to other donor populations as well.

The last possible source of bias is the presence of uncontrolled confounding. This would require there to be an unmeasured factor that is associated with the presence of leukocyte antibodies, but is causing TRALI by a mechanism unrelated to these antibodies. However, there seems no alternative biological mechanism readily identifiable that could convincingly explain the observed association of leukocyte antibodies with the occurrence of TRALI by means of confounding.

From this review the best estimate of the risk associated with the transfusion of leukocyte antibody containing blood products is a 15-fold increase in the odds of TRALI, compared to the transfusion of products not containing these antibodies. Of all TRALI cases, analyzed in this review, 80% are estimated to be attributable to donor derived antibodies. However, since the studies included in this review were not designed to investigate this specific question results could still be biased for several reasons, including publication bias. Therefore, new studies specifically designed to quantify the contribution of leukocyte antibodies to the occurrence of TRALI are necessary.

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Appendix I

Search strategy for TRALI and antibodies in PubMed

(“transfusion associated acute lung injury”[tiab] OR “transfusion related acute lung injury”[tiab] OR TRALI OR “transfusion associated respiratory distress”[tiab] OR ("Blood Transfusion/adverse effects"[Mesh] AND "Respiratory Distress Syndrome, Adult"[Mesh]) OR (“acute lung injury”[ti] AND transfusion[ti]) OR (“pulmonary reaction*”[ti] AND transfusion[ti]) OR “pulmonary transfusion reaction*”[tiab] OR (“pulmonary injury”[ti] AND transfusion[ti]) OR (“pulmonary edema”[ti] AND transfusion[ti]) OR (“lung edema”[ti] AND transfusion[ti]) OR (“pulmonary oedema”[ti] AND transfusion[ti]) OR (“lung oedema”[ti] AND transfusion[ti])) AND (Alloantibodies OR alloantibody OR Alloantigens OR alloantigen OR "Isoantigens"[MeSH] OR isoantigens OR isoantigen OR "Isoantibodies"[MeSH] OR isoantibodies OR isoantibody OR Alloantibod*[tiab] OR Alloantigen*[tiab] OR Isoantibod*[tiab] OR Isoantigen*[tiab] OR "Antibodies"[MeSH] OR antibody OR antibodies)

Search strategy for TRALI and antibodies in EMBASE

(((transfusion associated acute lung injury OR transfusion related acute lung injury OR TRALI OR transfusion-associated respiratory distress OR (acute lung injury AND transfus$)).ti,ab) OR exp Transfusion Related Acute Lung Injury/ OR (Acute lung injury/ AND exp Blood transfusion/) OR (exp Adult Respiratory Distress Syndrome/ AND exp Blood Transfusion/) OR (pulmonary reaction$.ti AND transfusion.ti) OR pulmonary transfusion reaction$.ti,ab OR (pulmonary injury.ti AND transfusion.ti) OR (pulmonary edema$.ti AND transfusion.ti) OR (lung edema$.ti AND transfusion.ti) OR (pulmonary oedema$.ti AND transfusion.ti) OR (lung oedema$.ti AND transfusion.ti)) AND ((alloantibod$ OR alloantigen$ OR isoantigen$ OR isoantigen OR isoantibod$ OR antibod$).ti,ab OR exp Antibody/ OR exp Alloantigen/)

Search strategy for prevalence of antileukocyte antibodies in PubMed

(Alloantibodies OR alloantibody OR Alloantigens OR alloantigen OR "Isoantigens"[MeSH] OR isoantigens OR isoantigen OR "Isoantibodies"[MeSH] OR isoantibodies OR isoantibody OR Alloantibod*[tiab] OR Alloantigen*[tiab] OR Isoantibod*[tiab] OR Isoantigen*[tiab] OR "Antibodies"[MeSH] OR antibody OR antibodies) AND ("anti-hla" OR "anti-hna" OR “anti-leukocyte” OR “anti-granulocyte” OR “anti-neutrophil”) AND ("Epidemiology"[MeSH] OR "Prevalence"[MeSH] OR "Incidence"[MeSH] OR prevalence [tiab] OR incidence [tiab])

Search strategy for prevalence of antileukocyte antibodies in EMBASE
((alloantibod$ OR alloantigen$ OR isoantigen$ OR isoantigen OR isoantibod$ OR antibod$).ti,ab OR exp Antibody/ OR exp Alloantigen/) AND (anti-hla OR anti-hna OR anti-leukocyte OR anti-granulocyte OR anti-neutrophil) AND (exp epidemiology/ OR prevalence.ti,ab OR incidence.ti,ab)

Appendix II

Complete reference lists for articles included in this review

Articles containing information on the prevalence of anti-leukocyte antibodies in the general population

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Chapter 3

Blood transfusions: good or bad?

Confounding by indication, an underestimated problem in clinical transfusion research

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Introduction

Confounding by indication is a serious potential problem in clinical observational research and can easily lead to unjustified conclusions, as has also been described previously.[1] In transfusion medicine such a conclusion could be "blood transfusions kill", since patients receiving more transfusions are almost invariably more likely to die. Even though most would agree that blood transfusions save lives, this erroneous conclusion did find its way into the literature. Though this example might seem overly obvious, confounding by indication can be indirect and much more subtle and hard to detect. It is therefore of the utmost importance to thoroughly understand the nature of confounding by indication, to be able to recognize it and avoid unjustified conclusions. We introduce the problem of confounding by indication with examples from clinical transfusion research and provide a general explanation to help identify this form of bias in the literature and in the every day clinical research settings (for guidelines on detection and handling, see Table 1).

Table 1: Guidelines on detection and handling of confounding by indication

1		Clearly define the outcome under study and identify known risk factors for this outcome
2		Define what aspect of transfusions is being investigated as a potential cause of this outcome (i.e. what is the exposure of interest)
3		Verify whether the exposure (step 2) depends on other transfusion parameters (e.g. male-only plasma recipients are less likely after more transfusions; the number of transfusions being the "other parameter" and male-only being the exposure)
4		Consider whether the exposure (step 2) or any of the other transfusion parameters (step 3) are related to risk factors for the outcome (step 1)
5	**A**	*When designing a study:* consider restricting the study to patients with identical risk factors for the outcome or gathering additional information on these risk factors, to allow for correction*
	B	*When reading a published study:* check whether the authors performed all of the above steps. If not, did they supply the necessary information for you to be convinced that confounding by indication is not a problem in their study?

***** Note: correction for confounding by indication is notoriously difficult, since patients get treated based on the risks perceived by the clinician and it is virtually impossible to capture all of the clinician's considerations in a statistical model. Furthermore, multiple transfusions can also give other types of bias, which can not always be corrected by conventional methods.[4]

Confounding by indication

Confounding by indication is a bias in clinical observational research that disturbs the association between a treatment and an outcome.[1-3] This bias occurs when studying the effect of a treatment, while the indication for the treatment causes the outcome. Patients with the indication are more likely both to receive the treatment and to experience the outcome, even if the treatment is not actually causing the outcome. This form of bias is called confounding by indication, since the association between the treatment and the outcome is confounded by the indication for the treatment. Sometimes this is also referred to as selection bias because the bias is caused by patients being selected for treatment. However, since the term selection bias is also commonly used to denote a multitude of other sources of bias, we prefer to call this type of bias confounding by indication.

Confounding by indication can be avoided by performing a randomized clinical trial (RCT).[2] In an RCT the allocation of treatment is independent of factors capable of causing the outcome (i.e. independent of indication). Many questions, however, can not be studied in an RCT, since this would be unethical or practically impossible. Therefore, much clinical research is observational and subject to the bias of confounding by indication. Unless adequately corrected for in the analyses, this bias prohibits any association between treatment and outcome from being interpreted as representing a causal relationship. Therefore, it is of the utmost importance to recognize and prevent or correct for confounding by indication.

Confounding by indication in transfusion medicine

In transfusion medicine the most common form of confounding by indication involves the number of transfusions received by a patient. Clearly, the sicker a patient is, the more transfusions will be indicated. Also, the sicker a patients is, the higher the risk that patient will have a poor outcome. Figure 1 illustrates how this causes a spurious association between receiving a high number of transfusions and experiencing a poor outcome, an association which should obviously not be interpreted as causal.

Indirect confounding by indication

A less easily recognized problem is “indirect confounding by indication”. In this case the indication still causes the outcome, but the relation with the treatment is indirect. If we again consider the example of a sicker patient receiving more transfusions, we can see that any other difference in treatment secondary to this higher number of transfusions will also become associated with poor outcome. For example we can imagine that patients receiving more transfusions are less likely to receive all transfusions from male donors (figure 2 A). Thus, comparing male-only to mixed-sex plasma transfusions will show a benefit for male-

only plasma where none exists. Importantly, the comparison of female-only to mixed-sex plasma transfusions should show a similar spurious association. An analogous situation with opposite bias arises for an analysis of the oldest unit transfused, which will on average be older in patients receiving more transfusions. A similar effect can be shown in the data by investigating the youngest unit transfused, which should be younger in patients with a poor outcome.

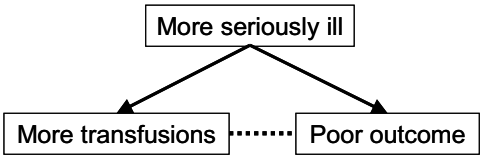

Figure 1: Cartoon of direct confounding by indication. Arrows indicate causal relationships and the dashed line denotes a spurious association. A more seriously ill patient is likely to receive more transfusions and experience a poor outcome. Therefore, a spurious association between receiving many transfusions and experiencing a poor outcome is created. This association is spurious because it is confounded by the indication for more transfusions (i.e. being more seriously ill). If in this example we were interested in the relation between the number of transfusions and a poor outcome (e.g. mortality) it would seem like transfusions are causing mortality, while this association is actually not causal.

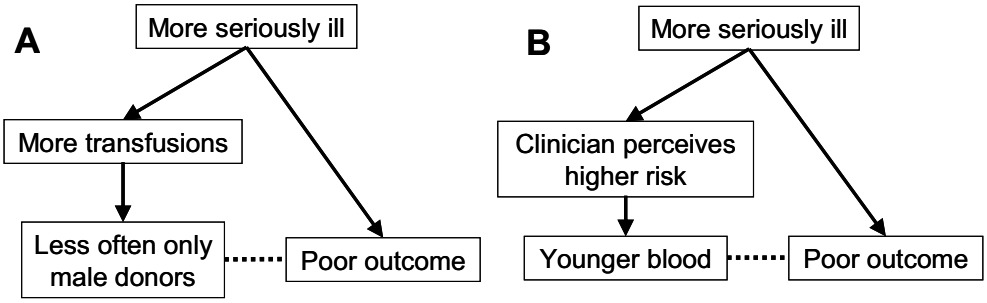

Figure 2: Cartoons of indirect confounding by indication. Arrows indicate causal relationships and the dashed lines denote spurious associations. **A**: If transfusions are allocated independent of donor sex, receiving more transfusions will reduce the probability of receiving all transfusions from male donors. Since the number of transfusions was (spuriously) positively associated with poor outcome (see figure 1), receiving all transfusions from male donors will become (spuriously) negatively associated with poor outcome. If in this example we were interested in the relation between donor sex and a negative outcome (e.g. TRALI) it would seem like receiving transfusions only from male donors is protecting against TRALI (and therefore like female donors are causing TRALI), while this association is actually not causal. **B**: If clinicians believe younger blood to be safer and therefore specifically reserve or order younger blood for more vulnerable patients with poorer prognosis, a (spurious) negative association between high product age and poor outcome will be created. If in this example we were interested in the relation between storage time and a negative outcome (e.g. mortality) it would seem like receiving younger blood causes mortality, while this association is actually not causal but created by the clinician.

Correction for confounding by indication

The association between receiving all transfusions from male or female donors and the number of transfusions is based on probability distributions, as is the association of the age of the oldest or youngest unit and the number of transfusions. Therefore, the strength of this association can be predicted based on the number of transfusions and a correction can then be applied. It should be noted however that a simple correction for the number of transfusions does not usually suffice.[4] Although the adequate correction is therefore not always straightforward to apply in practice, it does result in an unbiased estimate of the causal effect of donor sex or product age.

When no correction is possible

A problem that is generally beyond any hope of repair arises in the case where clinicians specifically ask for younger blood for their most vulnerable patients (figure 2 B). Young blood will then become spuriously associated with poor outcome and the strength of this spurious association can not be determined in any way. A similar problem, resulting in an association in the opposite direction, arises when blood banks specifically issue their oldest units for expected heavy bleeders. The blood bank only aims to reduce the chance of old units being returned to the blood bank near their expiration date, but it also ascertains that heavy bleeders who presumably have a poor prognosis get older blood. In both these examples the strength of the spurious association is not readily determined and correction is therefore not possible.

Implications

For an overview of a few simple steps that will help identify and deal with confounding by indication, see Table 1. Obviously, real causal relationships can be present alongside confounding by indication. However, these can not be quantified, or even qualitatively proven, and might in fact be completely obscured or even reversed, unless the bias of spurious associations is adequately corrected. It is therefore important to always realize which patient characteristics can both influence prognosis and are associated (directly or indirectly) with the treatment, or the secondary characteristics of treatment. Not all confounding by indication can be completely corrected for and adequate correction might require additional information. Therefore, it is vital to consider all these issues in the design phase of any study, when patient selection and data collection can still be adapted to avoid confounding by indication or allow for correction.

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Chapter 4

A solution to the problem of studying blood donor related risk factors when patients have received multiple transfusions

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Abstract

Background

A problem when studying adverse events of blood transfusions is that patients have usually received transfusions from several donors while only one of these donors is the actual cause. This will result in underestimation of the effect of donor related risk factors if not adequately corrected for. We encountered this problem when studying transfusion-related acute lung injury (TRALI) and describe four methods to overcome this problem.

Study design and methods

Simulated data are used to illustrate the results of six different approaches: not correcting for the number of donors, using standard correction methods, and four newly proposed methods. Donor sex is used throughout as an example. The first two new methods apply restriction of the study to cases who have received a transfusion from a single donor or from donors who are all of the same sex. In both restriction designs the sex of the causal donors is known and can be compared to the expected value from a reference population. The other two new methods apply statistical correction for the number of donors, either by standardization or by maximum likelihood methods.

Results

If not corrected for, or if corrected for by standard methods, increasing numbers of donors per patient result in decreasing estimates of the effect of risk factors. All four newly proposed methods yield valid estimates.

Conclusion

It is clear that the problem of multiple transfusions requires specialized correction methods. All four newly proposed methods yield on average good estimates of the underlying true value.

Introduction

Many adverse events associated with blood transfusions are due to one single transfusion, like transfusion transmitted infections, allo-immunization, most anaphylactic and allergic reactions, and transfusion-related acute lung injury (TRALI). The majority of transfusion recipients receive more than one transfusion. In most cases of adverse events it is not possible to identify the single causal transfusion. This complicates many studies that examine the association between donor related risk factors and transfusion reactions. We encountered this problem when studying TRALI.

TRALI is a form of acute respiratory distress syndrome (ARDS) that develops during, or within six hours after, the transfusion of one or more blood products.[1] TRALI is currently recognized as the most common of the severe side effects of blood transfusion.[2-5] It has an estimated incidence of 1 in 5,000 transfusions and a mortality commonly estimated to be between 5 and 10%, with the majority of patients spontaneously recovering within 96 hours, without long term sequelae.[1,6,7]

Both patient and donor related risk factors are thought to be involved in TRALI. Of the donor related risk factors, donor derived leukocyte antibodies are thought to be the most important.[1,8,9] Leukocyte antibodies are almost exclusively found in donors who have previously been exposed to allo-antigens and the large majority of allo-exposed donors are women who have been pregnant.[10,11] Therefore, donor sex is considered an important donor related risk factor for TRALI and several countries have excluded female donors from donation of plasma for transfusion. Since it is so important and can be determined so easily, donor sex will be used as an example throughout this paper.

Obtaining a quantitative estimate of the contribution of female donors to the occurrence of TRALI is complicated by the fact that in most cases TRALI patients have received transfusions from more than one donor before developing TRALI. Figure 1 gives a histogram of the number of donors involved in each case of a Dutch case series of 86 TRALI patients. In this representative case series 85% of TRALI patients have received transfusions of more than one donor in the six hours before the onset of symptoms. All donors of blood products transfused in this six hour window have to be considered as potentially causal. However, due to the low incidence of TRALI it can be assumed that the probability of having two causal transfusions is negligibly small. It is generally accepted that TRALI is caused by a transfusion from only one of the donors, the causal donor. Consequently, without identification of this causal donor, the crude quantitative estimate of the contribution of female donors to the occurrence of TRALI will be an underestimation.

The dilution effect resulting from transfusions from multiple donors can be quite substantial and conventional methods to correct for the number of transfusions (like stratifying by the number of transfusions or adding the number of transfusions in a

regression model) do not result in adequate correction. We discuss four approaches to obtain valid estimates of the contribution of female donors to the occurrence of TRALI. We simulated 1,000 data sets, each comprising 1,000,000 transfusion recipients, to describe the dilution effect of multiple transfusions, to illustrate the inadequacy of standard correction methods, and to compare the performance of the four newly proposed methods.

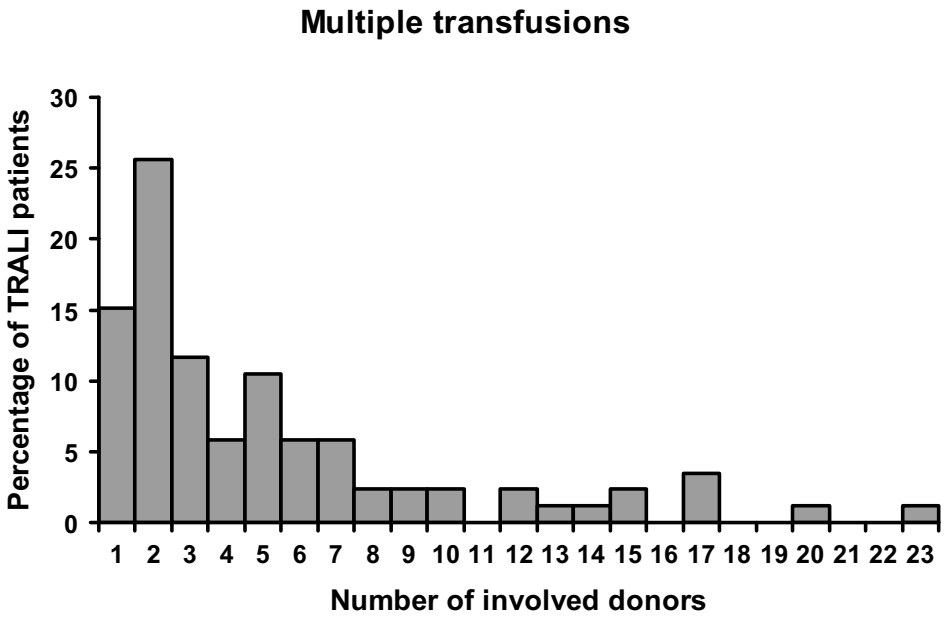

Figure 1: The number of donors involved in a series of 86 Dutch TRALI patients.

Materials and Methods

We will present here an informal description of the comparisons made between donors associated with TRALI cases and the source population of these donors. For a mathematical presentation of these comparisons we refer the interested reader to the Appendix. In the text we will also present simple numerical examples with each method. For the first three methods the actual calculations can be demonstrated in these examples; the last method (Correction by maximum likelihood estimation) requires specialized software to maximize the likelihood formula and the actual calculations can therefore not be presented. Thereafter, we will present the large sample simulations.

Assumptions and definitions

Two types of TRALI patients

We assume that TRALI can be caused by a women specific mechanism (i.e. antibodies as a consequence of pregnancies) or by unspecified other mechanisms (i.e. either antibodies due to other types of immunizing events, or other causes all together). Thus some TRALI cases are caused by female donors and could have been prevented by the exclusion of female donors. Such cases are caused by a women specific mechanism and will be referred to as "type I TRALI" cases. All other TRALI cases will be referred to as "type II TRALI". Amongst the type II TRALI cases there are also TRALI patients who have received blood from one or more female donors. However, in these cases the sex of the donor was a coincidence, rather than a causal prerequisite (i.e. either none of the female donors was causal, or one of the female donors was causal, but not due to a women specific mechanism). These type II TRALI cases were, therefore, caused independent of donor sex.

Problem of multiple donors

In standard etiological studies the exposure prevalence among cases is compared with that of the source population of the cases in a twofold table (table 1). All figures in table 1 represent numbers of patients, either with or without disease and with or without exposure to a female donor. If a TRALI patient (in the table referred to as TRALI +) received blood from only one donor and that donor was female, the patient is considered a female exposed case (A) and if the only donor was male the patient will be considered a female unexposed case (B). However, if the TRALI patient received more than one transfusion the causal transfusion is not known. Therefore, the values for cells A and B are not known and table 1 can not be composed directly. The four methods we propose describe different methods of dealing with this problem.

Table 1: Patients with TRALI, who have received transfusions from a single donor (TRALI +) and reference patients who also received a transfusion (TRALI -) according to the sex of one donor

TRALI	Female donor	Male donor	Total
+	A	B	N_1
-	C	D	N_2
Total	M_1	M_2	T

All figures represent numbers of patients.

Data assumed available or computable

We assume that for each TRALI patient with multiple donors we know the numbers of female and male donors. These observed numbers of female and male donors will need to be compared with expected numbers of female and male donors. The expected numbers can be estimated from the number of transfusions received and the fractions of female and male donations, as can be documented from the relevant donor population. Relevant in this context means: the donor population that represents the expected sex distribution for the donors involved in the TRALI case. The sex distribution of donors can be different between different countries or regions and between different product types and can also change over time. Therefore, the relevant donor population will, in practice, be a donor population donating the product type received by the TRALI patient, in the same geographical area, and during the same period as the TRALI occurred.

Definition of measure of effect

Our aim is to estimate the population attributable risk (PAR, the fraction of type I TRALI) as a measure of the contribution of female donors to the occurrence of TRALI. Estimation of the population attributable risk in the first two methods (restriction based methods) requires the relative risk (RR) to be estimated first. In the last two methods (correction based methods) the population attributable risk is estimated directly and the RR is calculated only for purposes of comparing the four methods' performance.

Proposed methods

1. Restriction to single donor cases

The first and simplest method to remove the diluting effect of multiple donors is to restrict the study to TRALI cases who have received transfusions from only one donor in the six hour period preceding the onset of symptoms. Since this one donor is by definition the causal one, the sex of this donor can be compared directly with the expected fraction of female donors. The expected value equals the fraction of blood products donated by female donors in the reference population. If the majority of TRALI cases would be caused by unspecified mechanisms (i.e. non-women specific mechanisms), the fraction of female donors among cases will be similar to that in the reference population and the relative risk (RR) will be close to unity.

Since the problem of multiple transfusions is effectively removed from the data, the population attributable risk can be calculated using standard formulas (Appendix, equations 1 and 2). The standard error and confidence interval for the estimated population attributable risk can be calculated using the delta method.[12]

For example, if the fraction of blood products donated by female donors is 0.35 and 20 patients have each received only a single transfusion before developing TRALI, 8 of which were from female donors. The RR would be 1.2 (i.e. [8x0.65]/[12x0.35]) and the population attributable risk would consequently be 8% (i.e. [0.35x1.2-0.35]/[0.35x1.2+0.65]).

2. Restriction to unisex cases

The second method also involves restriction of the study population to selected TRALI cases. In this case TRALI patients who have received transfusions either only from male donors or only from female donors (unisex cases) are included and compared to the reference population. If all donors involved in a TRALI case are of the same sex, this sex must be the sex of the causal donor. Therefore, the sex of the causal donor is known, even if the causal donor is not explicitly identified.

Since the sex of the causal donor is known, the relative risk of TRALI for a transfusion from a female donor versus a male donor can be calculated. However, the probabilities of receiving all transfusions either only from male donors or only from female donors are not necessarily equal to the fractions of donations made by female and male donors. Instead we should determine the relative probabilities of receiving multiple transfusions from only female and only male donors, given that we already know this TRALI patient to be a unisex case. These probabilities can easily be calculated if we know the number of transfusions received and the fractions of donations made by female and male donors (Appendix, equations 3 and 4). Further calculations of the RR and population attributable risk are identical to those used after restriction to single donor cases.

Consider the next example; if the fraction of blood products donated by female donors is again 0.35 and 20 patients have each received three transfusions, either only from male donors or only from female donors, before developing TRALI. Of these 20 patients 8 received all three transfusions from female donors. Since we have selected unisex cases we have to compare the number of cases caused by female and male donors to the number of unisex transfusion recipients, without TRALI, who received the same number of transfusions from female and male donors (Appendix, equations 3 and 4). Out of 20 unisex recipients receiving three transfusions each we would expect 2.7 to have received all three transfusions from female donors (i.e. $0.35^3/[0.35^3+0.65^3]$). The RR would be 4.3 (i.e. [8x17.3]/[12x2.7]) and the population attributable risk would consequently be 31% (i.e. [2.7x4.3-2.7]/[2.7x4.3+17.3]).

3. Correction by standardization

The third method applies *direct standardization*, according to the exposure in the reference population, to correct for the number of transfusions received by each TRALI patient. It consists of three steps. First, we estimate the total number of TRALI patients for whom the

causal transfusion was from a female donor (Appendix, equation 5). This number comprises all patients with a type I TRALI, i.e. those in which case the causal transfusion by definition has to be from a female donor, and a number of type II TRALI cases, i.e. in whom the causal transfusion is from a female donor by chance.

Second, from the fractions of female donors in the different reference populations (which can be different for each TRALI patient), we calculate a weighted average (Appendix, equation 6). This is the fraction of cases that is expected to be exposed. Multiplying this fraction by the number of cases will give the expected number of exposed cases, needed for the standardization.

Thus we have estimated both the observed number of exposed cases and the expected number of exposed cases. The third and final step is to use these numbers to calculate the standardized population attributable risk and RR (Appendix, equations 7 and 8)

To derive confidence intervals for the population attributable risk, the variance of the population attributable risk can be estimated as shown in the Appendix (equations 9-11). Another option would be to apply bootstrapping procedures. Furthermore, when either the fraction of female donors or the contributions of each stratum to the population attributable risk are reasonably homogeneous across strata, the normal non-weighted average of the fraction of female donors can be used directly, without the need to calculate a weighted average. Homogeneity of the contributions to the population attributable risk across strata is also referred to as absence of effect modification. Effect modification and its impact on the choice of method to use for correction will be considered in more detail in the discussion.

Consider again the example of 20 TRALI patients, each receiving three transfusions, while the fraction of blood products donated by female donors is 0.35. From all 60 involved donors 24 are again female, but we now assume these are randomly distributed across all patients. Therefore, four patients have received only transfusions from male donors, nine have received a single transfusion from a female donor, six have received two transfusions from female donors and a single TRALI patient has received all three transfusions from female donors. Since most patients have received transfusions from both female and male donors, it is impossible to tell which of these TRALI cases was caused by male or female donors. However, we can estimate that in 10 of our 20 TRALI cases the causal donor must have been female (Appendix, equation 5: 4x[0-3x0.35+0.35]+9x[1-3x0.35+0.35]+6x[2-3x0.35+0.35]+1x[1-3x0.35+0.35]). Note, though, that in 7 of these 10 cases, although the causal donor was female, the sex of the donor was a coincidence and the TRALI was not caused by a women specific mechanism. Since the fraction of blood products donated by female donors was constant for all cases the average (weighted or non-weighted) also equals 0.35. The population attributable risk is thus 23% (i.e. [10-20x0.35]/[20-20x0.35]) and the RR is 1.9 (i.e. [0.23/[0.77x0.35]]+1).

4. Correction by maximum likelihood estimation

The fourth method involves the use of a statistical model and maximum likelihood methods to correct for the number of transfusions received by each TRALI patient. The only data needed for this model is the sex of all the donors involved in each TRALI patient.

In this model there are two unknown parameters, which are estimated simultaneously. Firstly, the fraction of female donors in the reference population. Since this fraction is estimated from the available data on the donors of TRALI patients, this method does not require a reference population to be defined. Secondly, the population attributable risk is the fraction of TRALI cases preventable by the exclusion of all female donors.

For a patient with type I TRALI the causal donor is female. The number of female donors among the remaining donors of this patient follows a binomial distribution. For a patient with type II TRALI, the total number of female donors will follow a binomial distribution. The probability that a given TRALI is of type I is the population attributable risk (PAR) and the probability that it is of type II is (1-PAR). Together we can use this information to compose a likelihood formula for the probability of observing a given number of female donors out of a total number of donors for a person for whom the type of TRALI is unknown (Appendix, equations 12-15).

Maximizing this function with respect to the population attributable risk and the fraction of female donors in the reference population, yields the maximum likelihood estimates for both. Numerical methods are needed to maximize this function; we used the function "optim" of the statistical package R.[13] The second derivatives of the log-likelihood function can be used to estimate the standard errors of the estimated parameters, however in this particular case it is easier to calculate profile-likelihood based confidence intervals.[14]

Maximum likelihood methods can also be used when the expected fraction of female donors is obtained from population data, as was also the assumption for the other three methods. In this case the likelihood function has only one unknown parameter (i.e. the population attributable risk).

We consider again the previous example of 20 TRALI patients, each receiving three transfusions, while the fraction of blood products donated by female donors is 0.35. From all 60 involved donors 24 are again female and again four patients have received only transfusions from male donors, nine have received a single transfusion from a female donor, six have received two transfusions from female donors and a single TRALI patient has received all three transfusions from female donors. When we applied the standardization method (previous method), the result was a population attributable risk of 23%. If we determine the value of the population attributable risk that gives the maximum result from the likelihood formula (with a fraction female donations of 0.35) this would be almost identical at 24%.

Data simulation

We designed a simulation study in which we created 1,000 datasets with 1,000,000 patients each. We used the statistical package R to produce these data sets.[13]

1,000,000 patients were set to receive a random number of transfusions in the range from one to ten, using a uniform distribution. The fraction of donations by female donors in the total population of donors was set at 0.35, based on the Dutch donor population. The number of female donors amongst the number of received transfusions was subsequently drawn from a binomial distribution.

The overall probability of developing TRALI was set at 1/5,000 transfusions[1] and the relative risk associated with a transfusion from a female donor was arbitrarily set at 10. This implies that the probability of developing TRALI after a transfusion from a male donor equals 1/20,750 transfusions ([1/5,000]/[10x0.35+0.65]). The chance of developing TRALI after a transfusion from a female donor is consequently 10/20,750 transfusions. The true population attributable risk can be obtained using equation 1 and equals 75.90 % (i.e. [3.5-0.35]/[3.5+0.65]=3.15/4.15).

We had two primary effect measures of interest in our study: the fraction of TRALI cases preventable by excluding all female donors (population attributable risk, PAR) and the relative risk of TRALI after a transfusion from a female donor compared to a transfusion from a male donor. We applied all four proposed approaches to all 1,000 data sets. For each of the resulting eight different estimates the median and 5th and 95th percentiles of the estimates of the 1,000 simulations were determined. The median of 1,000 estimates gives an impression of the bias of that approach and the interval between the 5th and 95th percentiles about the precision.

R codes for running the four methods can be obtained from the corresponding author.

Results

True values

In our simulated data sets the true relative risk (RR) was 10 and the true population attributable risk (PAR, i.e. the fraction of TRALI cases preventable by the exclusion of female donors) was 75.90%.

Crude estimate and stratification by number of transfusions

Analyzing the simulated data without correction for the number of transfusions resulted in a crude RR of 1.3 (5th to 95th percentile: 1.3 to 1.4) and a crude population attributable risk of 10.87% (9.48% to 12.22%) (table 2).

When the RR was calculated within strata of the number of transfusions it decreased exponentially with each additional transfusion (figure 2). A conventional method of correction for the number of transfusions consists of pooling these stratum specific RR. Pooling resulted in an overall RR of 1.5 (1.4 to 1.5) and a population attributable risk of 13.82% (12.22% to 15.27%) (table 2), which was hardly better than the unadjusted RR and population attributable risk.

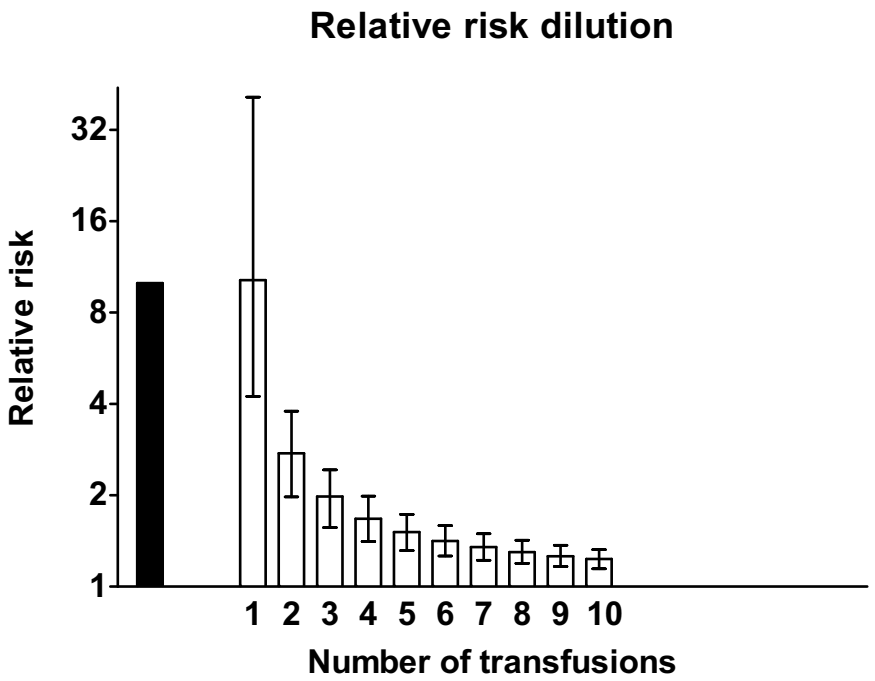

Figure 2: Dilution of the relative risk of female donors on TRALI with increasing number of transfusions, compared to the true value. The true value is represented by the filled bar. Error bars represent 5th and 95th percentiles of estimates from 1,000 simulations.

Proposed methods

Applying the four suggested methods to the simulated data resulted in good estimates of the population attributable risk and the RR (table 2). The main difference between the results was the width of the interval between the 5th and 95th percentiles of 1,000 simulations (table 2).

A median total of 1,100 TRALI cases (5th to 95th percentile: 1,044 to 1,150) was created per simulation. Of these cases a median of 20 cases (13 to 27) were single donor

cases and a median of 64 cases (51 to 77) were unisex cases. All single donor cases are inevitably also unisex cases. The two restriction based analyses were based on these cases. In the standardization and maximum likelihood analyses all cases could be included, resulting in more precise estimates of the RR and population attributable risk (table 2).

In the maximum likelihood analyses it was also possible to estimate the fraction of female donations in the reference population simultaneously with the population attributable risk. The median of the estimates of this fraction was 0.3496 (5^{th} to 95^{th} percentile: 0.3372 to 0.3634), while the true fraction of female donations in the simulated data was 0.3500 (0.3497 to 0.3504). Using the true fraction instead of estimating it simultaneously with the population attributable risk resulted in a further increase of the precision of the estimated population attributable risk, but had no material effect on the bias of the population attributable risk (table 2).

Table 2: Results of 1,000 simulations to compare the performance of the four different suggested approaches with the results after conventional correction and with the unadjusted result

Method of analysis	RR (p5-p95)*	PAR (p5-p95)*
True value	10	75.90
Unadjusted	1.3 (1.3-1.4)	10.87 (9.48-12.22)
Conventional adjustment †	1.5 (1.4-1.5)	13.82 (12.22-15.27)
Single donor	10.23 (4.2-41)	75.72 (52.00-91.90)
Unisex	10.13 (6.3-18)	76.16 (65.04-85.79)
Standardization	10.12 (6.7-17)	76.14 (66.42-85.15)
Maximum Likelihood	10.12 (7.5-15)	76.15 (69.32-82.60)
ML, *p* unknown ‡	10.07 (6.6-16)	76.15 (66.83-84.12)

* (p5-p95) represent 5^{th} and 95^{th} percentiles of estimates from 1,000 simulations.

† Conventional correction method using stratification according to the number of transfusions and subsequent pooling.

‡ Maximum likelihood method assuming the fraction of female donations (*p*) from the reference population to be unknown, therefore estimating the population attributable risk and *p* simultaneously.

RR: Relative risk. PAR: Population attributable risk.

Discussion

From the crude results obtained without correction it is clear that the problem of multiple transfusions can not be ignored when studying donor related risk factors for TRALI. Further, it is shown that conventional methods of correction for the number of transfusions do not solve the problem. All four proposed methods for dealing with this problem yield excellent estimates of the true fraction of TRALI cases preventable by the exclusion of female donors.

Conventional methods

Conventional methods (like stratifying by the number of transfusions or adding the number of transfusions in a regression model) take the weighted average of the relative risks shown in figure 2. Therefore, even if only a few patients have received more than one transfusion, the relative risk "corrected for the number of transfusions" by conventional methods will already be biased. If there are any patients at all who have received more than a single transfusion, one of the four proposed methods should always be used.

Restriction methods

The restriction methods are the most intuitively clear and computationally easy but, by definition, put a further restraint on the already limited number of TRALI cases available. Single donor cases are not only rare but any TRALI caused by the transfusion of pooled products (i.e. in some blood services either platelets, plasma, or both) can not be studied in this way.

Unisex cases occur more often, which likely contributed to the fact that this approach has been applied to real data, in a study of internationally gathered unisex cases.(*submitted*) However, for other donor related risk factors (i.e. parity) information is not available for all donors. This information has to be gathered specifically for all donors involved in TRALI cases to be able to identify cases receiving either all transfusions from parous donors, or all transfusions from nulli-parous donors. When known, this information can be used more efficiently by applying one of the other methods for correction.

Standardization

If information on the reference group is available, standardization can be used as a method for correction. This method uses the data a bit less efficiently than maximum likelihood estimation, but offers the advantage of relatively easy and straightforward calculations. Moreover, as with all standardization methods, it is the only valid summary measure in the case of effect modification. For instance if the risk associated with female donors of plasma rich products is different from the risk associated with female donors of red

cells.(*submitted*) In this case it will provide a summary measure which is the weighted average of stratum specific measures, weighted for the actual composition of the total donor population which was used for standardization. It is this measure that most accurately tells a blood bank which effect to expect from deferral of all female donors. However, it is only a valid estimate in the population it was determined in, or a population with very similar distribution of female donors across product types and very similar product type usage. In contrast, the results of the other three methods are often considered etiologically more relevant, since the RR estimated from these methods is less dependent of the population in which it was determined. However, since the population attributable risk is always dependent on the exposure prevalence in the population it does not share the RR's advantage of greater validity in other populations. The advantage belongs therefore to the RR only, while the population attributable risk is likely to be of greater interest to blood banks. However, the standardization method also has one drawback, common of all standardization methods. The reference population needs to be large enough to avoid estimates of the exposure fraction to equal zero or one hundred percent in even a single stratum. This is generally not a problem when studying donor related risk factors.

Maximum likelihood estimation

Maximum likelihood estimation uses the data most efficiently, resulting in the most precise estimate of the effect. Another advantage of the maximum likelihood method is that it doesn't necessarily require information on the reference population. The fraction of female donors in the reference population and the population attributable risk can be estimated simultaneous from information on donors involved in TRALI cases alone. Although estimation of two variables does decrease the precision of the estimate, this could be a worthwhile tradeoff in some situations. Especially when investigating other donor related risk factors, such as leukocyte antibodies. On such risk factors information might already be gathered for donors involved in TRALI cases, but could be expensive or cumbersome to collect for a large reference group.

Conclusions

In conclusion, all four methods can be used to study the contribution of donor related risk factors to the occurrence of TRALI. The unisex method is reliable and computationally easy, but can be difficult to apply to other donor related risk factors. Furthermore, since pooling across strata with different relative risks is only allowed with the standardization method, this will often be the only method to provide one summary measure. In these instances the standardization method provides the most relevant estimate of the effect on TRALI incidence that can be expected from implementing new donor deferral policies,

especially if these policies will be implemented on the same population used in the standardization.

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Appendix: Mathematical presentation of the four proposed methods

Data assumed available or computable

In standard etiological studies the exposure prevalence among cases is compared with that of the source population of the cases in a twofold table (table 1). Appendix table 1 instead represents these data for a single patient in a twofold table relating the sex of donors to the occurrence of TRALI. In this table *a* and *b* represent observed numbers of female and male donors and *c* and *d* are expected numbers of female and male donors for a patient with an equal total number of transfusions. These expected values are estimated from the fractions of female and male donations from the relevant donor population and the number of transfusions. In fact *c* and *d* can also be interpreted directly as expected fractions of female and male donors, instead of as expected numbers. For convenience this interpretation will be used throughout since it allows for consistent and short notations in all further definitions and equations.

For an analysis with N strata (i.e. N TRALI cases) the stratum specific values for the i^{th} stratum (i=1, i=2, ..., i=N) are given by:

a_i = the number of female donors (Appendix table 1).

b_i = the number of male donors (Appendix table 1).

n_{1i} = the total number of donors (a_i+b_i; Appendix table 1).

p_i = the fraction of female donors in the reference population.

From this, the following can be calculated:

c_i = the expected fraction of female donors for a given TRALI patient.

d_i = the expected fraction of male donors for a given TRALI patient.

Furthermore:

A_i = the (not always directly observed) contribution that this case will make to cell A of table 1 of the main paper.

Definition of effect estimator

Our aim is to estimate the population attributable risk (PAR, the fraction of type I TRALI) as a measure of the contribution of female donors to the occurrence of TRALI. It can be shown[12] in a population with a fraction *p* of female donors that the PAR equals:

$$\text{PAR} = \frac{pRR - p}{pRR + (1 - p)} \qquad (1.)$$

With RR the relative risk of developing a TRALI after a transfusion from a female donor, compared to a transfusion from a male donor.

Appendix table 1: Numbers of donors involved in one case of TRALI (TRALI +) and reference donors from the relevant donor population (TRALI -) according to sex

TRALI	Female donor	Male donor	Total
+	a	b	n_1
-	c	d	n_2
Total	m_1	m_2	t

All figures represent numbers of donors, but c and d can also be interpreted directly as the fractions of female and male donors in the relevant donor population. In this case n_2, m_1, and m_2 also loose their interpretation as numbers of donors. Since n_2, m_1, and m_2 are not necessary for any of the subsequent calculations, this distinction can be ignored.
Each TRALI patient has his or her own table. For an analysis with N strata (i.e. N TRALI cases) the stratum specific values for the i^{th} stratum (i=1, i=2, ..., i=N) are given by a_i, b_i, n_{1i}, c_i, and d_i.

Restriction to single donor cases

In this situation, the RR needed for equation 1 can be directly estimated using a Mantel Haenszel estimate (RR_{MH})[15] calculated, as given by:

$$RR_{MH} = \frac{\sum_{i=1}^{N} A_i d_i}{\sum_{i=1}^{N} B_i c_i} \qquad (2.)$$

With a_i=A_i, b_i=B_i, p_i=c_i and d_i=1-c_i. By estimating p by $p = \Sigma p_i/\Sigma n_{1i}$ (the average of p_i) across all strata the population attributable risk of female donors can be calculated by completing equation 1.

Restriction to unisex cases

In this situation, we need to estimate the probability of receiving all transfusions either only from male donors or only from female donors. This means we should determine the probabilities of receiving transfusions from female or male donors only, given that we already know this TRALI patient to be a unisex case. We know p_i to the power n_{1i} gives the probability of receiving all n_{1i} transfusions from female donors and (1-p_i) to the power n_{1i} gives the probability of receiving all n_{1i} transfusions from male donors. Adding these probabilities gives the probability of being a unisex case (with either male or female donors). Dividing either of the previous two probabilities by the probability of being a unisex case, gives the probability of receiving all transfusions from female or male donors, conditional on being a unisex case:

$$c_i = \frac{p_i^{n_{1i}}}{p_i^{n_{1i}} + (1-p_i)^{n_{1i}}} \quad (3.)$$

$$d_i = \frac{(1-p_i)^{n_{1i}}}{p_i^{n_{1i}} + (1-p_i)^{n_{1i}}} = 1\text{-}c_i \quad (4.)$$

Since all donors are of the same sex and only one donor per stratum is considered causal A_i and B_i can be estimated by: $a_i/n_{1i}=A_i$ and $b_i/n_{1i}=B_i$.

Correction by standardization

The third method applies direct standardization, according to the exposure in the reference population, to correct for the number of transfusions received by each TRALI patient. It consists of three steps. First, we estimate A (equation 5): the number of exposed TRALI patients in table 1. Second, we calculate the weighted average of p_i (p^*: equation 6). This is the fraction of cases that is expected to be exposed. Multiplying this fraction by the number of cases (N_1) will give the expected number of exposed cases, needed for the standardization. Third, the observed and expected numbers are used to calculate the standardized PAR (equation 7) and RR (equation 8).

Estimation of the number of exposed TRALI patients (A in table 1 of the main paper) can be achieved by:

$$A = \sum_{i=1}^{N} A_i = \sum_{i=1}^{N} a_i - n_{1i}p_i + p_i \quad (5.)$$

Which equals the sum of all stratum specific contributions to A (A_i). These contributions are defined by the observed number of transfusions from female donors (i.e. a_i) minus the expected number of female donors in all non-causal transfusions. The expected number of non-causal transfusions from female donors is rewritten as $n_{1i}p_i+p_i$, which equals $(n_{1i}\text{-}1)p_i$ (i.e. the number of non-causal transfusions multiplied by the fraction of female donors). Since his method sums the total number of female donors and subtracts the number of female donors among non-causal transfusions, it effectively sums the number of female donors among causal transfusions (i.e. A in table 1).

Summation of p_i into the weighted average p^* is given by:

$$p^* = \frac{\sum_{i=1}^{N} \frac{(a_i - n_{1i}p_i)}{p_i(1-p_i)} p_i}{\sum_{i=1}^{N} \frac{(a_i - n_{1i}p_i)}{p_i(1-p_i)}} \quad (6.)$$

The expected fraction of exposed cases (p^*) is needed to estimate the observed departure from the expected. The weight should therefore reflect the contribution that each

stratum makes to this departure. The numerator of the weight (a_i-$n_{1i}p_i$) is the actual contribution that each stratum makes to the departure from the expected. The denominator has two properties. First, by dividing by (1-p_i) the weight is corrected for the maximum possible departure this stratum could have contributed, give p_i. Second, dividing by p_i corrects for p_i itself. This gives a weight proportional to the contribution that this stratum would have made to the departure from the expected if everybody in that stratum were exposed. In the event of effect modification this standardization procedure is the most appropriate because the contribution of the different strata among the exposed remains unchanged.[16]

The observed and expected measures defined in equations 5 and 6 can be used to calculate the PAR. This is done by dividing the deviation of the observed from the expected by the maximum possible deviation, as given by:

$$\text{PAR} = \frac{A - N_1 p^*}{N_1 - N_1 p^*} \qquad (7.)$$

Where N_1 is the total number of TRALI cases. By rearranging equation 1 it can be shown that the estimate of the relative risk (RR) defined in terms of the population attributable risk (PAR) can be found as:

$$\text{RR} = \frac{PAR}{(1 - PAR)p^*} + 1 \qquad (8.)$$

To derive confidence intervals for the PAR, the variance of the PAR can be estimated by the variance of A, which is the sum of the variances of a_i (see below). Another option would be to apply bootstrapping procedures. Furthermore, when either p_i or the contributions of each stratum to the PAR are reasonably homogeneous across strata, the normal non-weighted average of p_i can be entered directly into equations 7 and 8, without the need to calculate p^*.

Variance estimation for the PAR

The variance of the standardized PAR, as given in equation 7, can be estimated by:

$$Var(\text{PAR}) = Var\left(\frac{\sum_{i=1}^{N}(a_i - n_{1i}p_i)}{N - \sum_{i=1}^{N} p_i}\right) = \frac{\sum_{i=1}^{N} Var(a_i)}{\left(N - \sum_{i=1}^{N} p_i\right)^2} \qquad (9.)$$

The variance of a_i (i.e. $Var(a_i)$) depends on the probability that the i^{th} case is a type I TRALI, which is given by:

$$\Pr(\text{type I}) = I_i = \frac{(a_i - n_{1i}p_i)}{(1-p_i)} \quad (10.)$$

This probability can be used to calculate the weighted sum of the variance of a_i in the case of a type I TRALI (variance of a binomial(n_{1i}-1, p_i) distribution) and the variance of a_i in the case of a type II TRALI (variance of a binomial(n_{1i}, p_i) distribution). Weighting these variances according to the probability given in equation 10 gives the variance of a_i:

$$Var(a_i) = I_i p_i (n_{1i}-1)(1-p_i) + n_{1i}p_i(1-I_i)(1-p_i) \quad (11.)$$

Correction by maximum likelihood estimation

In this model there are two unknown parameters, which are estimated simultaneously. Firstly, p is the fraction of female donors in the reference population. Secondly, PAR is the fraction of TRALI cases preventable by the exclusion of all female donors.

For a patient with type I TRALI the causal donor is female. The number of female donors among the remaining (n_1-1) donors follows a binomial distribution. The probability that we observe a female donors out of a total of n_1 donors is therefore given by:

$$\Pr(a_i=a|\text{type I}) = \binom{n_1}{a} p^a (1-p)^{n_1-a} \frac{a}{n_1 p} \quad (12.)$$

For a patient with type II TRALI, the total number of female donors will follow a binomial distribution. The probability that we will observe a female donors out of a total of n_1 donors is therefore given by:

$$\Pr(a_i=a|\text{type II}) = \binom{n_1}{a} p^a (1-p)^{n_1-a} \quad (13.)$$

The probability that a given TRALI is of type I is the PAR and the probability that it is of type II is (1-PAR). This yields, for a person for whom the type of TRALI is unknown, that the probability of observing a female donors out of n_1 total donors is:

$$\Pr(a_i=a) = \text{PAR} \times \Pr(a_i=a|\text{type I}) + (1-\text{PAR}) \times \Pr(a_i=a|\text{type II})$$

$$= PAR\binom{n_1}{a} p^a (1-p)^{n_1-a} \frac{a}{n_1 p} + (1-PAR)\binom{n_1}{a} p^a (1-p)^{n_1-a} \quad (14.)$$

The likelihood function for the data of all N TRALI cases is given by:

$$L(p,PAR) = \Pr(a_{i1}=a_1, a_{i2}=a_2, \ldots, a_{iN}=a_N,)$$

$$= \prod_{i=1}^{j} PAR\binom{n_{1i}}{a_i} p^{a_i} (1-p)^{n_{1i}-a_i} \frac{a_i}{n_{1i} p} + (1-PAR)\binom{n_{1i}}{a_i} p^{a_i} (1-p)^{n_{1i}-a_i} \quad (15.)$$

Maximizing this function with respect to PAR and p, yields the maximum likelihood estimates for PAR and p. Numerical methods are needed to maximize this function; we used the function "optim" of the statistical package R.[13] The second derivatives of the log-likelihood function can be used to estimate the standard errors of the estimated parameters, however in this particular case it is easier to calculate profile-likelihood based confidence intervals.[14]

Chapter 5

Female donors and transfusion-related acute lung injury

A case-referent study from the International TRALI Unisex Research Group

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Abstract

Background

Although quantitative evidence is lacking, it is generally believed that the majority of cases of transfusion-related acute lung injury (TRALI) are caused by female blood donors. We aimed to examine the relation between female donors and the occurrence of TRALI.

Study design and methods

We performed an international, multi-center case-referent study. TRALI patients who were diagnosed clinically, independent of serology or donor sex, and had received transfusions either only from male donors or only from female donors (Unisex cases) were selected. The observed sex distribution among the donors of these TRALI patients was compared to the expected sex distribution, based on the relevant donor populations.

Results

83 clinical TRALI cases were included; 67 cases received only red cells, 13 only plasma rich products and 3 both. Among red cell recipients the relative risk of TRALI after a transfusion from a female donor was 1.2 (95% confidence interval: 0.69 to 2.1) and among plasma rich product recipients the RR was 19 (1.9 to 191). The p-value for the difference between red cells and plasma was 0.023.

Conclusion

Our data support the notion that plasma from female donors is associated with an increased risk of TRALI, while red cells from female donors are not.

Introduction

Transfusion-related acute lung injury (TRALI) is currently recognized as the most important of the severe side effects of transfusions.[1-4] TRALI is characterized by the development of acute respiratory distress within six hours after the end of a transfusion, in the absence of circulatory overload.[5,6] It is clinically indistinguishable from acute respiratory distress syndrome (ARDS), but it is rarer and has a better prognosis. The estimated incidence is 1:5000 transfusions and the mortality is estimated to be between 5 and 10%.[7-9] Treatment is mostly supportive and in the majority of cases (80%) recovery is rapid and complete.[7-9] Different etiologies for TRALI have been suggested, but most research has been focused on the role of donor leukocyte antibodies,[7,10-12] as summarized in several recent reviews.[13,14]

Leukocyte antibodies are induced by previous exposure to allo-antigens. Such allo-exposures occur either through pregnancies or through blood transfusions and organ or stem cell transplantation. As a consequence leukocyte antibodies are much more prevalent in female than in male donors.[15-18] Since the UK first started to exclude plasma from female donors for transfusion in 2004, several other countries have also implemented or are considering to implement this policy to prevent TRALI.[1,19,20] Although some encouraging data on the effects of such measures have been published,[19,21] the evidence does not allow quantitative estimation of the effect of excluding plasma from female donors. Furthermore, the question arises whether for blood products that contain only small volumes of plasma, female donors also confer an increased risk of TRALI.[22]

To obtain a quantitative estimate of the contribution of blood products from female donors to the occurrence of TRALI is complicated because most TRALI patients have received transfusions from female as well as from male donors. However, some TRALI patients have received transfusions only from female or only from male donors, which we called Unisex cases. The ratio of female to male donors among these Unisex TRALI cases can be compared directly to the expected value calculated from the fraction of female donors in the respective total donor populations. We set out to quantify the association of female donors with the occurrence of TRALI by studying TRALI patients who had received transfusions either from female donors only or from male donors only.

Design and Methods

Design and study population

We performed a case-referent study consisting of TRALI patients who had been diagnosed clinically, without knowledge of serology or donor sex. Case-referent study is essentially synonymous to case-control study, but it is considered a more appropriate name in some

situations.[23] In the current study the TRALI case-patients were compared to a reference value from the complete donor population, rather than to control-patients without TRALI, thus rendering the name case-referent study more appropriate.

Since TRALI is a rare complication and TRALI patients who have received transfusions only from female or only from male donors are inevitably even rarer, no single research group or country is likely to be able to collect enough of these cases to perform a meaningful study. To overcome this problem we performed an international collaborative project.

We included only TRALI patients defined on clinical criteria alone, because TRALI that is defined by serological criteria (i.e. on the basis of presence of antibodies in donor blood), has the problem of circularity in reasoning since the diagnosis demands the presence of antibodies that are more frequent among female donors.[24] We contacted groups who had previously published TRALI cases defined on clinical criteria alone, independent of serology or donor sex, and asked them to join the International TRALI Unisex Research Group.

Measurements

TRALI cases

We asked each contributing group to identify all TRALI patients from their records. From all patients previously recorded as TRALI patients we further asked the collaborating groups to verify the sex of the donors of all products transfused within six hours before the onset of symptoms. Only those patients receiving all transfusions from donors of a single sex were eligible for inclusion in the present study. For these patients the presence of the other inclusion criteria for this study was checked retrospectively. The selection criteria were that the patient had presented with acute dyspnea (as a clinical sign of hypoxemia), within six hours after transfusion, without evidence of circulatory overload. For these patients, which we call “clinical TRALI” patients, we recorded the number of transfusions, the types of transfused products, and the sex of the involved donors. Furthermore, we collected data on all criteria of the definition of TRALI according to the Canadian consensus conference; these criteria were acute dyspnea, within six hours after transfusion, without evidence of circulatory overload, in the presence of new or worsening bilateral lung infiltrates in chest X-rays, and the absence of other risk factors for acute lung injury (ALI) or acute respiratory distress syndrome (ARDS).[5,6] Finally, specifications of blood products were recorded and all products containing 250 mL or more of plasma (all plasma and platelet products) were classified as plasma rich, while all other products (red cells, always leukoreduced and always containing less than 50 mL of plasma) were classified as plasma poor.

Reference population

Each collaborating group also reported fractions of donations made by female donors as registered in their donation databases. For each TRALI patient we documented a unique fraction: the fraction of female donors of the specific blood product, in the country or region of the reporting group, at the date of occurrence of the TRALI.

Statistical analyses

Our analysis follows the line of reasoning of one of the methods that we have proposed earlier,[25] which we briefly and informally recapitulate here. For each TRALI patient we first calculated that patient's individual probability of receiving all transfusions from a female donor. This probability was equal to the individually matched fraction of donations made by female donors in the relevant donor population, raised to the power of the number of these products received by that patient. For example, a TRALI patient receiving three units of red cells from a donor population in which 40% of red cells are donated by female donors has a probability of receiving all three units from female donors of $(0.40)^3=0.064$. For patients who received different product types the probabilities were first calculated for the different product types separately and then those probabilities were multiplied. The probability of receiving all transfusions from male donors was calculated in the same way (in the example $(0.60)^3=0.216$). Adding these two probabilities gives the probability of receiving all transfusions from donors of the same sex (in the example 0.064+0.216=0.28), which is the probability of being a Unisex case. We then calculated an expected fraction of Unisex cases caused by a female donor, by dividing each probability of receiving all transfusions from female donors by the probability of being a Unisex case (in the example 0.064/0.28=0.229).

The odds ratio and the corresponding 95% confidence interval (CI) were calculated with a matched analysis. The observed value for each individual case (i.e. 1 or 0, for all female or all male donors) was matched to the fraction of cases expected to be caused by female donors, as calculated for that individual case. In this matched analysis the size of the reference group, which was based on national registration data, was relatively so much larger than the number of cases (one per stratum) that the contribution of the reference group to the variance of the odds ratio was treated as negligible. The odds ratios are interpreted as relative risks (RR) throughout.

To estimate the population attributable risk (PAR) we calculated the average of the fractions of female donations from the different donor populations, by weighting for the number of TRALI patients contributed by each population. It can be shown[25,26] that, for an average fraction p of female donors, the PAR equals:

$$\text{PAR} = \frac{pRR - p}{pRR + (1 - p)}$$

Where the RR is estimated by the OR from the matched analysis. The odds ratio and its variance (both from the matched analysis) were then used to calculate the population attributable risk and the corresponding 95% CI, according to standard formulas.[26]

Data were analyzed according to whether the transfused products were red cells or "plasma rich" (i.e. either plasma or platelets). Effect modification by product type was quantified by calculation of a ratio of relative risks (RRR) and corresponding 95% CI, according to standard formulas.[27]

All analyses were repeated among the subgroup of patients of whom we had sufficient information to assess whether the diagnosis was conform to the Canadian consensus criteria[5,6]: patients who had bilateral infiltrates proven in chest X-rays and who had no other risk factors for ALI/ARDS (i.e. excluding "possible TRALI"). In this way we could compare the results in all clinical TRALI patients with those patients that had TRALI according to the Canadian consensus definition.

Results

Population characteristics

Based on a previous literature study,[24] we identified 43 different research groups from 52 publications, describing clinically defined TRALI patients. All groups for whom email addresses could be retrieved were contacted. Apart from the Netherlands, six more groups had the relevant data available and were interested in collaborating on this study. Collected data pertained to cases occurring between June 1991 and October 2007.

A total of 83 clinical TRALI patients were included, all presenting with acute dyspnea, without evidence of circulatory overload, within six hours after a transfusion. Of these patients 67 (81%) had received only red cells, 13 (16%) had received only plasma rich products (7 plasma, 6 platelets) and 3 (3.6%) had received both red cells and plasma rich products. On average the TRALI patients had received 1.8 transfusions (range 1-8) in the six hours preceding the onset of symptoms.

Of 67 cases caused by a transfusion of red cells 23 had another risk factor for acute lung injury, and in 17 no chest X-rays were available (3 patients had both). Therefore, of the cases caused by a transfusion of red cells a total of 30 (45%) were classified as TRALI patients according to all criteria of the Canadian consensus definition. Of 13 cases caused by transfusion of a plasma rich product 2 had another risk factor for acute lung injury, while in 1 (8%) a chest X-rays was not available, and the remaining 10 (77%) were classified as TRALI patients according to all criteria of the Canadian consensus definition.

Table 1: Distribution of patients according to product type, donor sex, and geographical location

	Red cells		Plasma		Platelets	
Source of patients	TRALI patients (♀/♂ donors)	Reference group (percentage ♀ donors)	TRALI patients (♀/♂ donors)	Reference group (percentage ♀ donors)	TRALI patients (♀/♂ donors)	Reference group (percentage ♀ donors)
Denver, CO, USA	5/3	45%	N.A.	N.A.	N.A.	N.A.
Netherlands	5/16	41%	-/2	10%	-/1	41%
Poland	1/19*	22%	-/1*	22%	-/2	2%
Rochester, MN, USA	6/5*	43%	3/1*	47%	2/-	47%
Spain	4/3	50%	N.A.	N.A.	1/-	50%
Finland	2/-	51%	1/-	51%	N.A.	N.A.
United Kingdom	1/-*	50%	2/-*	50%	N.A.	N.A.

Values are numbers of patients and percentage of donations from female donors in the corresponding reference group. Where changes in fractions donated by female donors occurred over time the represented fractions are weighted averages, weighted for the number of TRALI patients in each period.

N.A.: Not applicable (i.e. no Unisex TRALI cases associated with this product type in this country or region, n=0)

* Patients receiving both plasma and red cells were counted in both categories (only in this table). This occurred three times, once in Poland, once in Rochester and once in the UK.

The distribution of patients, according to product type and geographical location, with numbers of cases associated with male and female donors and corresponding percentage of female donors in the reference group are given in Table 1. For both red cells and plasma the fraction of products donated by female donors ranged from 0.22 in Poland to 0.51 in Finland, while for platelets it ranged from 0.02 in Poland to 0.50 in Spain (Table 1).

Female donors and TRALI risk

Among 67 red cell recipients the relative risk (RR) of clinical TRALI after a transfusion from a female donor was 1.2 (95% CI 0.69 to 2.1) in the matched analysis; among 13 recipients of plasma rich products (plasma or platelets) the RR was 19 (1.9 to 191) (Table 2). After restricting the analyses to cases who had proven bilateral infiltrates in chest X-rays and no other risk factors for ALI/ARDS (i.e. Canadian consensus definition), the RR for 30 red cell recipients remained similar at 0.86 (95% CI 0.37 to 2.02) while the RR for 10 recipients of plasma rich products increased to 66 (1.3 to 3465) (Table 2).

The ratio of the relative risks of red cell and plasma rich product recipients was 16 (1.5 to 170), the p-value for the difference in relative risks between these groups was 0.023. After limiting to the Canadian consensus definition the ratio became 77 (1.3 to 4410) and the p-value for a difference between the groups became 0.046.

The percentage of cases preventable by the exclusion of female donors (population attributable risk, PAR) was 7.0% (-17% to 26%) among red cell recipients, and 86% (17 to 98%) among recipients of plasma rich products (Table 3).

Table 2: Relative risk for developing TRALI after a transfusion from a female donor

Product type	All cases		Canadian consensus*	
Red cells	1.2	(0.69 to 2.1)	0.86	(0.37to 2.0)
Plasma rich	19	(1.9 to 191)	66	(1.3 to 3465)

Values are relative risk and (between parentheses) 95% confidence intervals.
* Only those cases defined completely according to the definition of the Canadian consensus conference.[5,6]

Table 3: Percentage of TRALI cases preventable by the exclusion of female donors

Product type	All cases		Canadian consensus*	
Red cells	7.0	(-17 to 26)	-5.9	(-45 to 23)
Plasma rich	86	(17 to 98)	96	(-126 to 100)

Values are percentages of population attributable risk (PAR) and (between parentheses) 95% confidence intervals. Negative PAR values can only be interpreted as indicative of some protective effect, but not of any size of that effect.

* Only those cases defined completely according to the definition of the Canadian consensus conference.[5,6]

Discussion

The risk of TRALI was increased among recipients of plasma rich products from female donors, but not among recipients of red cells from female donors. A strong association of female donors with the risk of TRALI was expected because, according to the literature, most TRALI cases are caused by donor leukocyte antibodies [24] and the prevalence of these antibodies in female donors is several times higher than in male donors.[15-18]

A unique feature of this study was the restriction to Unisex TRALI cases: patients who had received transfusions either only from male or only from female donors. Most patients who develop TRALI have received transfusions from several donors of either sex, and the one donor causing the TRALI can not be directly identified; therefore the sex of the causal donor remains unknown. In our study, since only patients with donors of a single sex were included, the sex of the causal donor was known even if the causal donor was not identified. Our approach solves the problem of attenuation caused by transfusions from multiple donors.[25]

Due to the international collaborative effort of this study TRALI patients were selected from several different centers or countries with different sized background populations. It is therefore not possible to compare the selected patients with the unselected part of the total population of TRALI patients, since there is no single identifiable background population. However, since all TRALI patients were originally diagnosed independently of donor sex and serology this can not have biased our results with respect to donor sex as a risk factor for TRALI. The separate effect estimates for red cells and plasma rich products are therefore valid in any population, but remain specific for those products. To apply them to a different population all that is needed is to know the relative contribution of the different product types in that population.

The main limitation of this study, pertaining only to the results for plasma rich products, is the limited number of cases caused by these products. The selection of Unisex cases causes an indirect selection of cases with few transfusions, who in turn will have rarely received only transfusions of plasma rich products. Although the distribution of product types among the patients in our study may well be different from the background population, no bias will be introduced by the selection. Firstly, since we analyzed red cells and plasma rich products separately, the fraction of TRALI cases caused by each product type in the background population is irrelevant. Secondly, the lesser number of transfusions received by TRALI patients in our study, in comparison to other published series, should not cause bias either. The mechanism by which TRALI is caused is considered to be an immunologic reaction to a single transfusion[25] - which is independent of the number and type of the other transfusions received by the patient. In spite of the small number of cases caused by plasma rich products, a strong association of plasma rich products from female donors with an increased risk of TRALI was observed, while no such association was observed for red cells.

The most surprising finding was this lack of association of female donors and the risk of developing a TRALI in red cell recipients. To appreciate this finding we considered an alternative explanation: if not all included cases were really TRALI patients the effect of donor sex would be diluted, obscuring a true association. One source of such misdiagnosis could be the patients of whom we did not have all information to be certain that the diagnosis was conform to the Canadian Consensus conference. However, the exclusion of these patients did not support the notion that the effect was diluted by their inclusion among the clinical TRALI patients. In this analysis increasingly stringent selection criteria reduce the number of potentially misclassified patients. Misclassified patients would contribute donors to the analyses who did not actually cause a TRALI case. These donors would therefore follow the sex distribution of the reference group, thus causing the TRALI group to become more similar to the reference group. Excluding those patients would therefore increase the difference between the TRALI group and the reference group. However, no such increase was observed in red cell recipients who, if anything, showed an inverse association with female donors after exclusion of clinical TRALI patients who did not fulfill all criteria of the consensus definition. Therefore, misclassification of TRALI patients does not seem a likely explanation for the lack of association between donor sex and the risk of TRALI in red cell recipients. Only for recipients of plasma rich products did restriction to consensus definition cases cause an increase in relative risk – which indicates that the association might even be stronger.

Another possible source of misclassification could be transfusion associated circulatory overload (TACO). In accordance with the Canadian consensus definition the exclusion of TACO was based on the criterion of “no evidence of circulatory overload”, which does not specify the type of evidence of which the presence should be excluded. The

absence of circulatory overload is therefore mainly based on clinical judgment, which makes this criterion the most subjective in the definition. However, to explain our findings in red cell recipients almost complete misclassification of these patients would be necessary. Even with the subjective nature of this clinical judgment, it seems unlikely that nearly all observed TRALI patients related to red cells would be misclassified TACO. This is especially unlikely since a strong association with donor sex was observed in recipients of plasma rich products, indicating those patients were not misclassified. Furthermore, Unisex cases have on average received only few transfusions, which also reduces the risk of TACO.

To compare our findings with what was known from the literature, we performed a systematic review of the literature to summarize the direct evidence of the relation between female donors and TRALI- see Appendix for methodology and selection criteria. We found 6 such studies: 4 with a contemporary control group and 2 with a before/after comparison (Table 4).

None of these 6 publications investigated the difference between plasma rich and plasma poor products. Publications that make before/after comparisons (i.e. before and after introduction of a male-only plasma measure) run the risk of clinical suspicion or reporting bias. Only a small portion of TRALI patients are reported, either through lack of clinical suspicion/recognition or through poor reporting. The fraction of TRALI patients that is reported is inconsistent and highly variable over time and is likely to change strongly after well publicized and dramatic measures for the prevention of TRALI (i.e. the exclusion of female donors). Therefore, a difference in the number of reported TRALI patients before and after implementation of this preventive measure does not necessarily correspond to a real difference in the number of TRALI patients.

Of the 4 publications with a contemporary control group 1 only included six cases and 1 included only three cases. The remaining 2 did not correct for a difference in the number of transfusions (Table 4). TRALI patients have on average received more transfusions than other patients which are used as control patients in these studies. Both the chance of receiving male-only plasma and the amount of female plasma received depend on the total number of transfusions. A higher number of transfusions is strongly related to a higher risk of TRALI. Without correction this precludes quantitative conclusions from an observed difference in either the prevalence of TRALI between male-only and mixed plasma recipients, or a difference in the amount of female plasma received between TRALI patients and control patients.[24,25]

Considering the limitations of previous studies, their quantitative conclusions are uncertain. The methodology which we advocate here and elsewhere,[25] is aimed at overcoming these potential shortcomings. Furthermore, our study makes a clear distinction in the analyses between plasma rich products and red cells and shows a striking difference between the associations of female donors with TRALI caused by these products.

Table 4: Six publications investigating the relation between female donors and TRALI

Publication	Quantitative interpretation limited by	Description
Gajic 2007 [36]	Difference in number of transfusions	Amount of female plasma compared between TRALI patients and controls
Sanchez 2007 [37]	Statistical power	Only six cases (pilot study)
Imoto 2007 [38]	Statistical power	Only three cases
Wright 2008 [19]	Before/after comparison	Number of reported cases before vs. after male-only plasma measure
Chapman 2009 [21]	Before/after comparison	Number of reported cases before vs. after male-only plasma measure
Nakazawa 2009 [39]	Difference in number of transfusions	Risk of TRALI compared between male-only and mixed plasma recipients

Several countries have implemented policies excluding female donors from the donation of plasma, to prevent TRALI.[1,19,20] Our findings suggest that the vast majority of the TRALI cases caused by plasma rich products are indeed preventable by the exclusion of female donors. However, to estimate the overall effect on the occurrence of TRALI we also need to estimate the relative contribution of plasma rich products to the occurrence of TRALI, which can not be estimated directly from our data. The literature gives estimates of the contribution of red cells to the occurrence of TRALI varying from one third to more than 90%.[28-32] Based on the literature and our own previously published experience[29] we assume that on average approximately half of all TRALI cases are caused by transfusion of red cells alone. Therefore, exclusion of female donors from donation of plasma rich products might prevent roughly half of all TRALI cases.

In TRALI caused by red cell transfusions our data indicate the role of female donors to be negligible. This suggests that current red cell preparation procedures, by reducing the amount of plasma in the product, already suffice to effectively reduce the risk posed by donor leukocyte antibodies in these products. Therefore, removing the small amount of remaining leukocyte antibodies from red cells is likely to have only limited effect. This is in agreement with current thinking about the pathogenesis, which suggests that red cells may cause TRALI by different mechanisms.[30,33-35]

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Appendix: Systematic review of the literature

To compare our results with what was known in the literature, we performed a systematic review. On December 24 2009 we searched the PubMed database for all publication on TRALI and donor sex using the search strategy: ("transfusion related acute lung injury"[All Fields] OR TRALI[All Fields]) AND (("female"[MeSH Terms] OR "female"[All Fields]) OR ("sex"[All Fields] OR "sex"[MeSH Terms]) OR ("male"[MeSH Terms] OR "male"[All Fields]) OR "gender"[All Fields]) AND ("donor"[All Fields] OR “donors”[All fields]).

We retrieved 125 publications, 100 contained original data, of which 86 had TRALI as their primary focus. Of these 86, only 22 actually investigated donor sex as a risk factor, while most only mentioned donor sex in relation to antibody testing in a case report or case series. Only 4 of the 22 remaining publications included a contemporary control group and two made a before/after comparison (Table 4). This left only six publications that actually made the comparison we were interested in. The evidence available, from the selected publications, for a relation between female donors and TRALI risk was summarized (Table 4).

Chapter 6

No association of allo-exposed blood donors with transfusion-related acute lung injury after transfusion of plasma poor products

A case-referent study

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Abstract

Background

Donor leukocyte antibodies are thought to increase the risk of transfusion-related acute lung injury (TRALI). Leukocyte antibodies can be present in blood products from donors who have been allo-exposed, mostly through pregnancies. Allo-exposed donors are increasingly excluded from donating plasma. Plasma poor products are still donated by allo-exposed donors while possible differences between different product types have not been studied. We aimed to quantify the contribution of allo-exposed donors to the occurrence of TRALI for different blood product types.

Study design and methods

We performed a case-referent study including all TRALI patients reported by Dutch hospitals and all Dutch blood donors. Data on allo-exposure status of donors of all TRALI cases reported between January 2004 and October 2008, in the Netherlands, were compared to information on the total donor population.

Results

Allo-exposure status of all 223 involved donors was compared to the expected status. The overall percentage of TRALI cases that could have been prevented by the deferral of all allo-exposed donors (i.e. population attributable risk; PAR) was 51% (95% confidence interval (CI): 14% to 88%). In 19 recipients of exclusively plasma-poor products (mostly red cells) allo-exposure of the donors was not associated with TRALI While in 28 recipients of both plasma-poor and plasma-rich products (>200 mL plasma) the PAR was 94% (95% CI: 34% to 100%).

Conclusion

Allo-exposed donors conferred an increased risk of TRALI in recipients of plasma-rich products, but not in recipients of plasma-poor products. Although leukocyte antibodies are an important risk factor for TRALI, amongst red blood cell recipients another risk factor must be more important.

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.[1,2] With an estimated incidence of 1:5000 transfusions TRALI is one of the most common serious side effects of blood transfusions.[3] As a form of acute respiratory distress syndrome it has a relatively mild prognosis with a mortality commonly estimated to be between 5 and 10% and the majority of patients spontaneously recover within 96 hours, without long term sequelae.[3-5] However, due to the widespread use of blood transfusions, total morbidity and mortality associated with TRALI poses a considerable problem.[6-8]

Since the publication of the first large case series,[3] it has been suggested that TRALI can be caused by antibodies directed against either human neutrophil antigens (HNA) or human leukocyte antigens (HLA) of both class I and class II.[3,9-13] These leukocyte antibodies arise from exposure of the immune system to allogeneic cells and tissues (allo-exposure).[14,15] This allo-exposure can occur through pregnancy, transfusion of blood or blood components and transplantation of stem cells, tissues or organs.

As a consequence parous donors and donors who have received blood transfusions are more likely to possess leukocyte antibodies.[14-19] The prevalence of these antibodies increases from below 5% in subjects without known allo-exposure, to 10-15% after blood transfusions or a single pregnancy, to well over 30% after three or more pregnancies.[14-19] Allo-exposed donors are therefore considered to be at increased risk of causing TRALI in recipients of their blood.[20,21] These donors are thought to confer this increased risk primarily through the plasma-rich products made from their blood, since these contain the highest quantities of antibodies. Therefore plasma from female donors is now excluded from use for transfusion in an increasing number of blood services.[6,22-25] In some instances these measures also include other products considered to be plasma-rich (some types of platelet products) and sometimes also male donors with a history of blood transfusion.[6,24]

However, the evidence remains largely circumstantial and a quantitative estimation of the expected benefit of these measures is therefore not possible. This was also confirmed in a recent review of the literature on the contribution of female donors to the occurrence of TRALI, which was published in conjunction with an international collaborative case-referent study on the same subject.[26] In the absence of such quantification, these measures are based on the precautionary principle. The main obstacle to the quantification of the preventable number of TRALI cases is methodological complexity. Most patients have received transfusions from more than one donor before developing TRALI. Both ignoring this problem and applying conventional methods to correct for the number of transfusions result in severely biased effect estimates.[27] Therefore, previous estimates of the role of donor related risk factors, such as donor sex, parity, transfusion history, and presence of

leukocyte antibodies cannot be used to predict the expected benefits of measures directed at removing these risk factors from the blood supply.

Furthermore, the question has now arisen how much leukocyte antibody containing plasma is necessary to cause TRALI.[28] If the small amount of plasma present in red cells is sufficient, this could have the obvious implication of excluding allo-exposed donors from all forms of blood donation. On this subject, only anecdotal evidence exist to date and further investigation of differences between product types are therefore necessary.[28]

We applied new statistical methods, which have been shown to adequately correct for the number of transfusions received,[27] to quantify the contribution of allo-exposed donors to the occurrence of TRALI caused by plasma-poor and plasma-rich products, in all reported TRALI cases between January 2004 and October 2008 in the Netherlands.

Design and methods

Study design

Ethical approval was granted by both the medical ethical committees of the Leiden University Medical Center and the Sanquin Blood Bank.

We performed a case-referent study for which we used the prospectively collected data on all TRALI patients reported in the Netherlands from January 2004 till October 2008. For each included TRALI patient donors of transfused blood components were identified and their allo-exposure status was determined (see below for details). Donors were considered allo-exposed if the donor had received one or more blood transfusions, if the donor had been pregnant at least one time (including terminated pregnancies), or both.

Allo-exposure status of donors associated with TRALI patients was compared to the allo-exposure status of a reference group of donors (see below for details). These control donors donated blood for products that represent the source population of the blood components from which the components transfused to TRALI patients were randomly drawn.

The allo-exposure status of the donors of each TRALI patient was matched to the allo-exposure status that would have been expected, based on the allo-exposure status of the reference group (as described below).

TRALI patients: definition, reporting, verification

TRALI was defined, according to the Canadian consensus definition, as acute respiratory distress with new or worsening bilateral infiltrates in the chest radiograph in the absence of evidence of circulatory overload, within six hours after completion of a blood transfusion.[1,2] Also in accordance with the consensus definition, a distinction was made between TRALI and “possible TRALI”, the latter being clinically diagnosed TRALI in the presence of other

risk factors for acute lung injury.[1,2] All further mention of TRALI will refer to the complete group of all TRALI patients, including "possible TRALI". When "possible TRALI" is excluded, this is stated explicitly.

Suspected TRALI cases were reported to Sanquin (the national blood supply organization in the Netherlands) and TRIP (Transfusion Reactions In Patients, the national haemovigilance office in the Netherlands). Reports from hospitals are made by either the hospital's haemovigilance staff or the responsible physicians.

Reports to Sanquin were verified by physicians of Sanquin's clinical consultation service and reports to TRIP were independently verified by TRIP physicians. Physicians from both organizations received additional clinical information from the reporting hospitals, as required for verification of the TRALI case. All cases were verified on clinical criteria alone, without any knowledge on the donor's sex or allo-exposure status. Records of Sanquin were then compared to those of TRIP for further verification. All confirmed TRALI patients were further classified as TRALI without other risk factors for acute lung injury or "possible TRALI".

Donors of TRALI patients: identification, allo-exposure verification

For each included TRALI patient all blood components transfused within six hours before the onset of symptoms were identified by a physician of the reporting hospital. All donors of these components were then identified in the database of the national blood supply organization in the Netherlands.

Since allo-exposure variables like parity and transfusion history are not routinely collected, we contacted all donors to obtain this information. Donors were sent a questionnaire by post, if necessary they received a reminder (with the same questionnaire included), and if they did not return the questionnaire they were also contacted by telephone. The questionnaire included questions on the donor's history of transfusions and pregnancies. Donors were considered allo-exposed if they reported either one or more pregnancies, one or more blood transfusions, or both.

For donors for whom the allo-exposure status could not be ascertained the average allo-exposure status from the other donors of the same TRALI patient was used. There is no reason to assume causal donors are more or less likely to have missing information than non-causal donors, especially since it is unknown which donors are causal. Therefore, for some TRALI patients the missing donor will be causal and for some it will be one of the innocent bystander donors. In the first case the total allo-exposure of all donors for that patient will be underestimated. In the second case the total allo-exposure of all donors for that patient will be overestimated. It can be shown mathematically that, in the applied analyses, these effects will cancel each other out perfectly and lead to a valid effect estimate.[27]

Reference subjects: allo-exposure status

Male and female donors have different allo-exposure prevalences and the fractions of donations from male and female donors changed during the study period. Amongst the most important changes was the decision to exclude all plasma from female donors, donated after October first 2006, from transfusion. To correct for changes in fractions of donations from male and female donors, we first determined these fractions for each year and product type separately (as described below; for plasma donated in 2006 we also distinguished between donations before October first and donations on or after October first).

We then determined the allo-exposure prevalence for male and female donors separately (as described below). Subsequently we calculated the fraction of donations from allo-exposed male donors by multiplying the year-of-donation-and-product-type-specific fraction of donations by male donors with the fraction of allo-exposure among male donors. The fraction of donations from female allo-exposed donors was calculated in the same way and these two fractions were added to estimate the total fraction of donations from allo-exposed donors (i.e. which is also year of donation and product type specific).

The fraction of products from female donors was determined from complete records of all blood donations in the Netherlands. For each product type the national blood supply organization records were used to determine the exact numbers produced from donations by male and female donors. From these data the fraction of each product type donated by male and female donors was calculated, specified by year of donation. This included an average of 558,716 whole blood donations per year for red cells and buffy coat derived platelets and an average of 51,472 plasmapheresis donations per year for fresh frozen plasma. The fraction was matched by donation date rather than transfusion date, to allow for the large variations in storage time of fresh frozen quarantine plasma.

History of blood transfusion and pregnancy were determined as part of the Donor InSight study. This study was conducted by Sanquin Blood Bank, between April 2007 and April 2009, to gain insight into characteristics and motivation of the Dutch donor population. Donors who were not permanently deferred at time of invitation for the Donor InSight study were eligible to take part in the study. About 50,000 randomly selected whole blood and plasma donors were invited to participate. Each month a random sample of active donors was selected from the donor population and invited. Donors received an information brochure and questionnaire by regular mail. Donors who agreed to participate in the Donor InSight study were asked to return the completed questionnaire by mail. The questionnaire also recorded information on blood transfusion and pregnancy history. The Medical Ethical Committee Arnhem-Nijmegen in The Netherlands approved the study.

The present report is based on data collected from April first 2007 until March 31st 2008. During this year, a total of 24,179 donors were invited to participate, of which 15,249 returned the questionnaire and gave informed consent for participation (response 63.1%). A random sample of 1,500 donors was drawn from these 15,249 donors who returned the questionnaire. Donors were considered allo-exposed if they reported either one or more pregnancies, one or more blood transfusions, or both.

Of the 1,500 randomly drawn donors 279 had not donated blood in the last year and were not included in further analyses, since their donation frequency was zero. Amongst the remaining 1,221 a further 181 were excluded because their only donation was for safety testing purposes (n=10), because they only donated plasma for fractionation purposes (n=132), or because based on the donation code they could be identified as specifically selected to be non-transfused male donors (n=39). Allo-exposure status among 1,040 donors was therefore used in the analyses. These 1,040 donors are a random sample of all normally donating donors and therefore represent the average allo-exposure status among donors donating products transfused to TRALI patients who only received products from routine donations.

Sanquin records were used to link the allo-exposure status of each individual donor to the donation frequency of that donor. Numbers of donations from allo-exposed donors were calculated by multiplying the numbers of allo-exposed donors by their donation frequency. The fractions of products from allo-exposed donors were then calculated for male and female donors separately. The average of these male and female specific fractions was subsequently calculated, weighted for the fractions of donations from male and female donors according to product type and year of donation (determined as described above).

Blood products

Transfused blood products were classified as either plasma-poor or plasma-rich. Platelet concentrates derived from multiple donors were treated as multiple products in all analyses.

Plasma-poor products were defined as all products containing less than 40 mL plasma. This included red cells, the platelets from donors supplying only platelets (i.e. not plasma) for a pooled platelet product (i.e. including 4 of every 5 donors for platelets in plasma and all donors for platelets in platelet additive solution II (PAS II)).

Plasma-rich products were defined as all products containing more than 200 mL plasma. This included fresh frozen plasma (FFP) and the platelets (and plasma) from the donor supplying both platelets and plasma for pooled platelets in plasma.

Plasma measure

Since October first 2006 all plasma donated for transfusion in the Netherlands is from never transfused male donors. In September 2007 the first TRALI patient receiving plasma

donated after October first 2006 was reported. Therefore, all TRALI cases occurring since September 2007 are considered "post-plasma-measure". For the primary analyses only TRALI cases occurring before September 2007 are included. However, the sub-group of patients receiving only plasma-poor products could not have been affected by the plasma measure. Therefore, an additional analysis was performed including all TRALI patients receiving only plasma-poor products from January 2004 till October 2008.

Statistical analyses

We aimed to estimate the contribution of allo-exposed donors to the occurrence of TRALI. This contribution was expressed as a population attributable risk (PAR; the fraction of TRALI cases that could have been prevented by the exclusion of all allo-exposed donors).

As previously described, standard statistical correction methods are inadequate to correct for the number of transfusions received by each TRALI patient.[27] We therefore used an adapted form of standardization that has been shown in simulation studies to give a valid estimate of the contribution of donor related risk factors to the occurrence of TRALI.[27]

Briefly, the difference of the observed number of allo-exposed donors of each TRALI patient from the expected number for that same TRALI patient was calculated. These differences were used to estimate the number of TRALI patients in whom the causal transfusion was provided by an allo-exposed donor. The difference of this number from the number of TRALI patients expected to be caused by allo-exposed donors was considered the excess number of TRALI patients caused by allo-exposure of donors. The maximum excess number was the total number of TRALI patients minus the number expected to be caused by allo-exposed donors. Dividing the excess number by the maximum excess number gives the population attributable risk (PAR; the fraction of TRALI cases that could have been prevented by the exclusion of all allo-exposed donors).

We first performed these analyses for all TRALI patients, giving an estimate of the effect of exclusion of all allo-exposed donors from donations of any type. The analyses were repeated, selecting patients who had received only plasma-poor product, only plasma-rich products or mixed product types (both plasma-poor and plasma-rich products). Finally, separate analyses were performed for all groups by repeating all analyses after exclusion of the "possible TRALI" cases.

Results

TRALI patients

From January 2004 till September 2007 a total of 50 TRALI cases were reported in the Netherlands. Of these, 11 also had other risk factors for acute lung injury and were therefore classified as "possible TRALI". Table 1 shows the numbers of donors and

different product types involved separately for all 50 TRALI cases, 39 TRALI cases excluding all "possible TRALI", and in 11 "possible TRALI".

From September 2007 till October 2008 another 21 TRALI cases were reported, of which 11 (including four "possible TRALI") received only plasma-poor products. These 11 patients were included in the additional analysis presented in table 2. All other analyses are restricted to the 50 TRALI patients reported before the plasma measure became effective.

Of 288 donors involved in the total of 61 included TRALI cases data on pregnancy and transfusion history could be gathered for 283 (98.3%).

Table 1: Numbers of TRALI patients, transfusions, and involved donors, according to product types and classification as TRALI and "possible TRALI"

	TRALI	Possible TRALI	Total
Number of cases	39	11	50*
Number of transfusions	179 (4.6/case)	32 (2.9/case)	211
Number of donors	223 (5.7/case)	44 (4.0/case)	267
Red cells	110 (49%)	23 (52%)	133
Platelets†	55 (25%)	15 (34%)	70
FFP	58 (26%)	6 (14%)	64

* Table 1 represents only TRALI cases occurring prior to September 2007, showing a representative composition of the population of TRALI patients before the plasma measure became effective.

† Platelets are mostly pooled concentrates of buffy coat derived platelets from five donors in the plasma of one of those donors. Three TRALI cases were reported after receiving pooled concentrates of buffy coat derived platelets from five donors in PAS II. The reported 70 platelet donors represent 14 platelet transfusions: 11 for 10 TRALI cases and 3 for 2 "possible TRALI" cases.

Allo-exposure in reference subjects

A final number of 1,040 donors with known allo-exposure status were used to determine the expected allo-exposure status of donors involved in TRALI cases. This included 528 female donors and 512 male donors. Pregnancy was reported by 352 donors and a history of blood transfusion by 24. Of these donors 13 reported both previous pregnancies and blood transfusions. Of the resulting total of 363 allo-exposed donors 354 (98%) were female. Allo-exposed donors constituted 68% of all female donors.

The average number of donations in 2007 was 2.67 for male donors and 1.84 for female donors. Allo-exposure status was not associated with the number of donations. It was 1.86 for non allo-exposed female donors and 1.84 for allo-exposed female donors.

Table 2: Population attributable risk of allo-exposed donors, according to product types

		N in analyses		Population attributable risk; allo-exposed donors	
		Patients‡	Donors		
Overall*		50	267	51%	(14% to 88%)
Plasma-poor*	Before†	19	38	-10%	(-52% to 31%)
	Total†	30	59	-3.5%	(-36% to 29%)
Plasma-rich*		3	6	24%	(-56% to 100%)
Mixed*		28	223	94%	(34% to 100%)

* Overall: all reported TRALI patients. Plasma-poor: patients receiving only products containing less than 40 mL plasma per donor. Plasma-rich: patients receiving only product containing more than 200 mL plasma per donor. Mixed: patients receiving both plasma-rich and plasma-poor products.

† Before: before the measure; only patients before the plasma measure became effective (September 2007), these are from the same period as the other groups (i.e. Overall, Plasma-rich, and Mixed). Total: also including 11 patients, receiving plasma-poor products only, who were reported between September 2007 and October 2008.

‡ Includes all patients until September 2007, except for the third row where, as indicated, patients were included until October 2008.

A negative PAR value can only be interpreted as indicative of some protective effect, but not of any size of that effect.

Allo-exposed donors and TRALI risk

The expected percentage of allo-exposed donors among 267 donors, involved in 50 TRALI cases was 27%; the observed percentage was 66%. After exclusion of all "possible TRALI" among the remaining 223 donors, involved in 39 cases, the expected percentage was 27% and the observed was 65%.

Table 2 represents the percentage of cases preventable by the deferral of allo-exposed donors (population attributable risk; PAR). The overall PAR of receiving a transfusion from an allo-exposed donor was 51% (95% confidence interval: 14% to 88%). There were only three patients who had received exclusively plasma-rich products. For patients who had received both plasma-rich and plasma-poor products the PAR of receiving a transfusion from an allo-exposed donor was 94% (34% to 100%) (table 2). In 19 patients who had received only plasma-poor products (mostly red cells) allo-exposure of the donors was not associated with TRALI.

Exclusion of "possible TRALI" cases

The findings were similar after exclusion of "possible TRALI" cases (figure 1): The PAR for the total group was 60% (17% to 100%). For plasma-poor product recipients the PAR

was -28% (-86% to 30%). For recipients of both plasma-rich and plasma-poor products the PAR was 100% (44% to 100%).

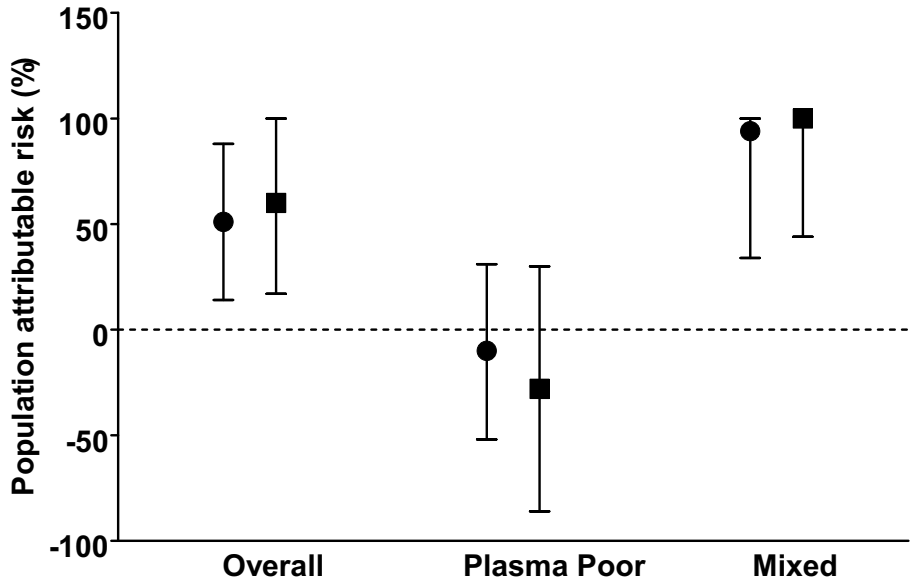

Figure 1: Population attributable risk of allo-exposed donors for all 50 TRALI patients and for 39 TRALI patients, excluding all "possible TRALI". Circles represent the PAR for all 50 TRALI patients and squares represent the PAR for 39 TRALI patients after excluding all cases of "possible TRALI". "Overall" indicates the estimate for all patients, regardless of product mix. "Plasma-poor" indicated the estimate for patients receiving only plasma-poor products (<40 mL plasma per donor). "Mixed" indicated the estimate for patients receiving both plasma-poor and plasma-rich products (i.e. excluding patients receiving either only plasma-poor or only plasma-rich, as opposed to "Overall" which includes both these groups and "Mixed".) Bars represent 95% confidence intervals. The dashed line indicates the level of null-effect. A negative PAR value can only be interpreted as indicative of some protective effect, but not of any size of that effect.

Discussion

Among recipients of only plasma-poor blood products allo-exposure of donors was not associated with an increased risk of TRALI. However, among recipients of both plasma-rich and plasma-poor blood products allo-exposure of the donors was a major risk factor for TRALI. This suggests firstly that allo-exposure of the donor is an important risk factor for

TRALI when plasma rich components are transfused, and secondly that the plasma rich products are more likely to have caused the TRALI in recipients of both plasma rich and plasma poor products.

We used allo-exposure of the donors as a marker for the increased prevalence of leukocyte antibodies. Although only a minority of allo-exposed donors actually develops leukocyte antibodies, nearly all leukocyte antibodies are found in allo-exposed donors. We assumed there is no other reason, besides leukocyte antibodies, why allo-exposed donors can increase the risk of TRALI. It can then be shown that the percentage of TRALI cases preventable by the exclusion of allo-exposed donors equals the percentage preventable by the exclusion of all donors with leukocyte antibodies. This can be understood since, firstly, exclusion of allo-exposed donors also excludes donors with leukocyte antibodies and therefore prevents the cases caused by those antibodies. Secondly, it does not prevent any other cases than those caused by leukocyte antibodies since allo-exposed donors do not cause TRALI through any other mechanism. This is one of the major advantages of using the population attributable risk (PAR) as the effect estimate, since it removes the need to actually determine the presence or specificity of leukocyte antibodies.

Beyond the distinction between TRALI and "possible TRALI", we ignored all other data on potential patient risk factors. All diseases can be considered multi-causal and TRALI is no exception in this respect.[29] However, this was a study of donor related risk factors. We consider these risk factors more interesting, since they are relatively easy to control, while the patients predisposition for developing TRALI is usually not readily influenced. By the analyses in which the allo-exposure status of a patient's donors was matched to the probability of receiving those transfusions from allo-exposed donors, we created the statistical equivalent of matching a TRALI patient to an otherwise identical patient without TRALI. Therefore, all patient related risk factors become irrelevant (i.e. are fully corrected for).

An assumption necessary for the used analyses to be valid is that the calculated probability of receiving a transfusion from an allo-exposed donor should really represent this probability at the moment of issuing of the product transfused to the TRALI patient. For this to be true the reference group has to be representative for a non-selected, random sample of all actively donating donors. We have no reason to assume that in the few years of our study the average allo-exposure status of the Dutch female or male changed substantially. Furthermore, we corrected for any changes in female to male ratio among donations.

Although we report one of the largest known case series of TRALI patients, a major limitation of our study was still the size. The limited size precluded analyses by product type and forced us to lump several product types together into plasma-rich and plasma-poor products. Even so, we still did not find enough recipients of only plasma-rich products (i.e. only three patients) to report an effect estimate for this group. Obviously patients receiving

only plasma-rich products are rare and so is TRALI. The combination is therefore even rarer and difficult to study. However, since plasma-poor products show no association with allo-exposed donors, any association seen in the patients receiving both must be due to plasma-rich products. Since the population attributable risk in this group is close to one, it is suggested that nearly all the cases in this group are caused by plasma-rich products. Further, the population attributable risk for these plasma-rich products must also be close to one. This is further supported by the differences between the recipients of plasma-poor products and the total group (the latter reflecting the weighted average of all plasma-rich products and all plasma-poor products).

TRALI is known to be underreported and this underreporting may be selective for plasma-rich of plasma-poor products. This could influence our conclusion that plasma-rich products are more likely to cause TRALI, but none of our other conclusions. Only if reporting was selective for the presence of leukocyte antibodies or allo-exposed donors, this could cause bias in the conclusions that TRALI after plasma-poor products is not associated with allo-exposed donors and that TRALI after plasma-rich products is. However, since this information was not available to the reporting physicians, this reporting bias can not be a problem in our study.

Our results indicate that half of all TRALI cases may be preventable by the exclusion of all allo-exposed donors, which is in close agreement with a previous estimate of the population attributable risk of female donors.[26] Furthermore, our findings confirm that allo-exposure of the donor is the dominant determinant for TRALI in patients receiving plasma-rich products, while for plasma-poor products other risk factors must be more important. This is in agreement with the characteristics of a limited number of TRALI cases reported in the 2008 annual SHOT (Serious Hazards Of Transfusion) report.[25] Of 17 TRALI cases described in this report, 11 involved the transfusion of red cells (six received only red cells). In none of the ten completely investigated cases were concordant leukocyte antibodies found in donors of the transfused red cells. For all five cases in which leukocyte antibodies were identified, the implicated products were either fresh frozen plasma (three cases) or platelets (two cases).[25]

We also repeated all analyses after exclusion of all “possible TRALI” cases. Some of these “possible TRALI” cases were probably not TRALI, but rather acute lung injury caused independently of transfusion. Therefore, they should not show any association with risk factors for TRALI and cause some dilution of the estimated effect. Consequently, excluding these cases can be expected to increase any observed association. Only minor increases were observed. The estimate for the total group (overall estimate) reflects a weighted average of effects exerted through plasma-poor and plasma-rich product. Changes therefore reflect the weighted average of changes in different directions (i.e. simultaneous increase of a negative association for plasma-poor products and a positive association for plasma-rich products) and can not be interpreted directly.

In conclusion, our findings confirm the increased risk of TRALI associated with allo-exposed donors, which are used as a proxy for leukocyte antibodies. However, this association was only observed for plasma-rich products. Allo-exposed donors are almost exclusively female and female donors are increasingly being excluded from donation of plasma-rich products. Therefore, the contribution of plasma-rich products from allo-exposed donors to the occurrence of TRALI is dwindling and the relative importance of red cell transfusions is steadily growing.

Already nearly half of the reported TRALI cases in this study were caused by red cells alone. At present little is known about risk factors for TRALI related to red cells. With the growing contribution of red cells to the occurrence of TRALI these risk factors need to be identified.

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Chapter 7

Male-only fresh frozen plasma for TRALI prevention

Before-and-after comparative cohort study

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Abstract

Background

TRALI is one of the most serious complications of blood transfusion. It can be caused by incompatible leukocyte antibodies in transfused plasma. The objective of this study was to quantify the reduction of TRALI following introduction of male-only plasma for transfusion as a preventive measure, which took effect in 2007.

Study design and methods

In the Netherlands all cases of TRALI are reported to the national haemovigilance office. All reported cases of TRALI from 2002 to November 2009 were considered for inclusion. Those meeting the Canadian consensus clinical definition were included and subdivided according to whether or not the patient had received quarantine FFP (Q-FFP) in the six-hour period before the reaction. The numbers of TRALI cases involving plasma donated before the measure and of those involving plasma donated after the measure were compared to TRALI cases that did not involve Q-FFP in order to adjust for reporting bias.

Results

110 cases were included in the analysis. Of 68 cases before the measure, 36 involved Q-FFP. 31 cases occurred after the measure of which 8 involved Q-FFP. Eleven occurred in the transitional period, of which 4 involved Q-FFP. The population attributable risk of pre-measure plasma among TRALI cases occurring before the measure was 0.33 (95% CI: 0.09 to 0.51).

Conclusion

In the Netherlands the male-only Q-FFP measure was associated with a 33 percent reduction of TRALI cases.

Introduction

Transfusion-related acute lung injury (TRALI) is one of the most serious transfusion reactions and one of the top three causes of transfusion-related mortality in most haemovigilance registries.[1,2] According to the Canadian consensus criteria, respiratory distress, hypoxia, increased airway resistance and frothy sputum in ventilated patients arise within six hours of transfusion and are associated with (new) infiltrates showing on X-ray. This is assumed to be due to neutrophils entering the pulmonary interstitium and fluid loss into the alveoli.[3,4] TRALI has been attributed to incompatibility between donor leukocyte antibodies (HLA class I and II antibodies as well as anti-granulocyte antibodies) in transfused plasma and recipient leukocytes.[5,6] However, in many cases no leukocyte incompatibility is found. In the postulated two-hit mechanism of TRALI, a first hit consists of neutrophil priming or initial triggering of endothelium in the pulmonary vascular bed. The second hit can be the transfusion of leukocyte antibodies incompatible with the recipient or other factors that arise during storage of blood products.[4]

The proportion of TRALI cases which are deemed to be caused by leukocyte incompatibility has been estimated at up to 89%.[3] Leukocyte antibodies are mainly induced by pregnancy or blood transfusion.[7] Therefore several countries where fresh frozen plasma (FFP) is used for transfusion have introduced FFP preferentially or exclusively derived from male donors who have never received a blood transfusion with the aim to reduce the number of TRALI cases. In the UK, analysis of ten years of TRALI registration within "SHOT" (Serious Hazards of Transfusion) the national haemovigilance office shows that implementation of preferential male-only FFP has led to a near-disappearance of TRALI associated with leukocyte incompatibility following plasma transfusion.[2] However this may be partly a consequence of the SHOT method of assessing "imputability", the likelihood that the clinical picture of TRALI is related to transfusion. SHOT grades imputability of TRALI reports higher in the presence of patient-incompatible leukocyte antibodies. The international consensus definition for TRALI does not include leukocyte incompatibility as a criterion.[8,9]

The male-only measure became effective in The Netherlands for all quarantine plasma (Q-FFP; henceforth in this article we will refer simply to "plasma") distributed to hospitals since 1st July 2007. The aim of the present study was to quantify the reduction of TRALI cases, as defined by the international consensus definition, following implementation of male-only plasma.

Design and Methods

Design and study setting

We performed a cohort study among all patients who had a diagnosis of TRALI in the Netherlands from 2002 to 2009 with the aim of comparing the incidence of TRALI before and after the male-only plasma measure became effective. In the Netherlands all suspected cases of TRALI are reported to TRIP (Transfusion Reactions in Patients), the national haemovigilance system which became fully operational in 2003. The reports are submitted on a paper or digital reporting form; additional information is requested from hospitals if necessary for standardized classification. TRIP also receives information on reported TRALI cases from the blood service. Inclusion was terminated on 15th November 2009, when a further measure was introduced in the production of platelet concentrates.

Patients

TRALI case definition

TRALI cases had to conform to the criteria of the international consensus definition of TRALI: a patient was included in the cohort if there were clinical findings of hypoxia with bilateral infiltrates on the chest X-ray, starting within 6 hours of the transfusion of a labile blood component; circulatory overload had to be excluded as a (more likely) cause.[8,9] Information on the clinical condition of the patient was evaluated for known risk factors for acute lung injury or other possible causes of hypoxia with a temporal relationship to the respiratory distress.

All reports were reviewed by a panel of transfusion experts and assessed on clinical information without considering results of leukocyte serological investigation, which in most cases were not available to the reviewing committee. If the patient had a risk factor for acute lung injury (e.g. aspiration, toxic inhalation, lung contusion, near-drowning, cardiopulmonary bypass, pneumonia, acute pancreatitis, sepsis) the case was flagged as a "possible TRALI" according to the consensus definition.[8,9] Cases were excluded if there were other more likely causes for the respiratory problems. All blood components received by the patient up to 6 hours before onset of respiratory symptoms were recorded.

Transfusional setting and analysis periods

In the Netherlands plasma for transfusion is prepared from apheresis plasma which is released after the donor has been retested for infectious diseases after a minimum of six months. From October 2006 all plasma collected for Q-FFP and from July 2007 onwards all plasma distributed to the hospitals was from male never-transfused donors. Units distributed before 1st July 2007 were not recalled from the hospitals and were transfused

from the hospital inventory over the following months. Cryosupernatant plasma is occasionally used for refractory TTP and prepared on demand from Q-FFP.

Since 1988 all platelet products and since 2002 all red cell components have been leukoreduced by prestorage filtration (<1x 10^6 leukocytes per unit). Plasma for transfusion meets the same specification. Red blood cell concentrates are stored in SAGM additive solution and contain less than 20 ml of residual donor plasma. Over 90% of platelet concentrates are prepared from five pooled buffy coats and resuspended in either 200 ml of plasma from one of the donors (approx. 70% of total platelet units) or platelet additive solution with residual circa 85-100 ml plasma consisting of <20 ml of plasma from each buffy coat. Apheresis platelets are collected in a volume of 150 to 400 ml donor plasma and are used for special indications such as HLA-matched platelets, Parvo B19 or CMV-safe products. During the study years the total number of blood components distributed to the hospitals annually was approximately 700,000 units.

For TRALI cases reported after June 2007 the donation date of transfused plasma was checked. Reports where any plasma had been transfused were classified according to the donation date of the plasma as occurring with products from before or after the measure. TRALI cases involving no plasma were assigned to the same period as any plasma-associated TRALI in that month. The three analysis periods were: before the measure (2002 – June 2007), the transitional period during which cases were associated with plasma both from before and after the measure (July – November 2007) and after the measure (December 2007 – 15 November 2009). Plasma-associated cases during the transitional period were assigned according to the date of donation of the plasma and the cases without plasma were assigned half to before and half to after the male-only measure for purposes of calculation.

Statistical analysis

We compared the number of reported TRALI cases from before introduction of the male-only measure with the number after it had become effective. If the measure was effective a reduction will be seen in the number of TRALI patients who received one or more units of plasma, with or without other blood components, when only male plasma was available for transfusion. The number of reported cases where the patient had not been transfused with plasma reflects the overall sensitivity of TRALI detection and reporting in any period. This number was used to correct for changes in this sensitivity.

We expected that after the measure became effective there would be a drop in the proportion of TRALI reports after transfusion of plasma against the total number of reported TRALI cases. The drop represents the population attributable risk (PAR) for female plasma as available prior to the measure, and corresponds to the fraction of TRALI prevented by the implementation of male-only plasma. An additional sensitivity analysis

was performed, calculating the PAR separately for the ramp-up phase of reporting to TRIP (2002 – 2004) and for the plateau phase (2005 – July 2007). The main result was recalculated with the omission of reports from the interim period as an additional verification.

The formula used is:

$PAR = (R_B - R_A)/R_B = 1 - \text{risk after/risk before}$

with R_B the risk of TRALI in transfusion recipients before the measure and R_A the risk in transfusion recipients after the measure.

During the reporting period there was little change in numbers of blood components distributed in the Netherlands,[10] so stable proportions of patients transfused with different types and combinations of types of blood component are assumed. The number of TRALIs (N) reported in a given period is

$N = XfY$

in which X is the "true" incidence rate of TRALIs (number per year), f is the proportion detected and reported and Y the follow-up period (years).

$PAR = 1 - (\text{risk after/risk before}) = 1 - X_A/X_B = 1 - (N_A/(Y_A f_A))/(N_B/(Y_B f_B))$

For TRALIs where no plasma was transfused the "true" rate cannot have changed since the measure was introduced so

$X_{B,\text{ no plasma}} = X_{A,\text{ no plasma}}$

Since we collected TRALIs with and without plasma concurrently we can also assume that f at any time is the same for TRALI with and without plasma. This allows the proportion $Y_A f_A/(Y_B f_B)$ (for all cases) to be estimated by $N_{A,\text{ no plasma}}/N_{B,\text{ no plasma}}$. Thus the PAR was calculated as

$PAR = 1 - ((N_A/N_B)(N_{B,\text{ no plasma}}/N_{A,\text{ no plasma}}))$

simply using the observed numbers of reported TRALIs.

A confidence interval for the PAR was calculated using

$Var[\ln(1-PAR)] = 1/N_{B,\text{no plasma}} - 1/N_B + 1/N_{A,\text{ no plasma}} - 1/N_A$.[11]

Results

Characteristics of the study population

The study population comprised 110 patients with TRALI approved by expert review as complying with the TRALI definition. Figure 1 shows the numbers of all suspected TRALIs per year from 2002 to 2009 according to the types of blood component(s) received by the patient.

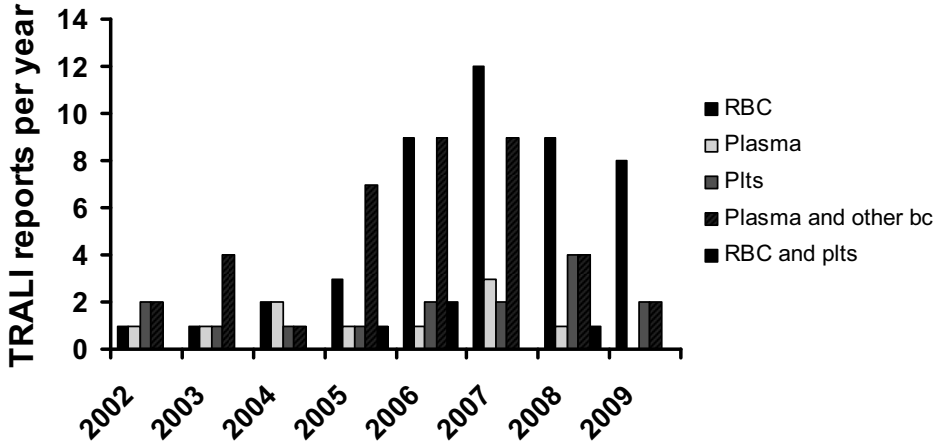

Figure 1: Reports of suspected TRALI and associated blood components, 2002-2009. RBC: red blood cells, plts: platelets, bc: blood component(s).

TRALI before and after the male-only plasma measure

The earliest TRALI involving one or more plasma units from after the measure occurred in July 2007, the last case where one or more plasma units dated from before the measure occurred in November 2007. Thirty-one of the TRALI cases were designated as "possible TRALI" according to the consensus definition because one or more other risk factors for acute lung injury (ALI) were present.

Outcomes and estimation

The annual number of reports of TRALI rose for all types of blood component between 2002 and 2007, which can be attributed to increased awareness of TRALI. The initial rise in total annual number of reports to the new haemovigilance reporting system had leveled off in 2005. A total of 68 cases of TRALI occurred before the male-only plasma measure of which 36 involved plasma, with or without other types of blood components. From December 2007 there were 31 cases of which 8 involved plasma. Four of the eleven cases in the transitional period were associated with plasma, two with plasma donated before the measure. Table 1 summarizes the numbers of reports with and without plasma per analysis period. The overall PAR was 0.33 (95% CI: 0.09 – 0.51) for all TRALI. After exclusion of "possible TRALI" it was 0.37 (95% CI: 0.06 – 0.58). In the sensitivity analysis comparing the separate periods of 2002 – 2004 and 2005 – July 2007 to that after the measure the PAR was comparable though with a wider confidence interval: PAR 0.41 (95% CI: -0.07 – 0.67); and 0.31 (95% CI: -0.02 – 0.54) respectively.

Table 1: TRALI cases and transfused blood components per analysis period

TRALI	Before the measure Jan 2002-July 2007	Transitional period* July 2007-Dec 2007	After the measure Dec 2007–15 Nov 2009	PAR $1 - ((N_A/N_B)(N_{B, \text{no plasma}}/N_{A, \text{no plasma}}))$
All TRALI	68	11	31	1-((36.5/73.5) (35.5/26.5)) = 0.33 (95% CI: 0.09 – 0.51)
with plasma	36	2 before 2 after	8	
without plasma	32	7	23	
Excluding "possible TRALI"	48	8	23	1-((27/52) (22.5/18.5)) = 0.37 (95% CI: 0.06 – 0.58)
with plasma	28	2 before 1 after	7	
without plasma	20	5	16	

* If cases in the interim period are left out of the calculation the PAR becomes: 1 – ((31/68)(32/23)) = 0.37 (95% CI: 0.12 – 0.54) and 0.40 (95% CI: 0.08 – 0.61) if also excluding "possible TRALI".

Discussion

The male-only plasma measure was associated with a 33 percent reduction of TRALI in the Netherlands, a reduction totally driven by lower numbers of cases where plasma had been transfused in combination with red blood cells and/or platelets. The finding implies that against the average number of approximately 20 reports per year before the measure, some 7 of the previously reported cases annually may have been avoided by the measure. Moreover, since the plasma measure can only prevent TRALI caused by plasma, this size of effect means that the majority of TRALI cases where plasma had been transfused prior to the measure were in fact caused by female plasma. The figures in Table 1 show that TRALI cases where plasma had been transfused are in the majority in the period before the measure and that this is reversed after the measure.

We observed a higher attributable risk when cases of "possible TRALI" were excluded. In some cases where other risk factors for ALI were present, ALI was probably not induced by the transfusion. Inclusion of some such cases leads to dilution and underestimation of the effect of the measure. The higher attributable risk after exclusion of "possible TRALI" is probably more valid and provides further support that there is a true reduction.

Strengths and limitations

The strength of this analysis lies in its inclusion of all reported patients meeting the standardized criteria for TRALI in a whole country, with as little as possible interference from awareness of the results of leukocyte serology testing. Reporting of such a serious complication as TRALI to TRIP and/or the blood service is expected to be nearly complete. An important advantage is that we use the number of TRALIs not associated with plasma to correct for variability in detection and reporting behavior. The fact that a similar effect is found in the sensitivity analyses of the sub-periods supports our use of these cases as a comparator.

A limitation of the study is its observational nature and reliance on spontaneous reporting of cases. A recent analysis has shown that bias may operate in the decision whether to report a reaction as suspected TRALI.[12] If any interpretation bias operated it could be expected to favor reports of TRALI associated with FFP and to have most strongly influenced TRALIs where FFP was the sole product transfused. However the present findings do not support this. Also, since most clinicians in The Netherlands are not aware of the plasma measure this reporting preference is unlikely to have changed and therefore could not have biased our analyses.

The overall blood use and the proportions of type of blood component remained largely stable over the study period, except for a slight (less than 10%) drop in the number of both RBC and plasma units distributed to the hospitals between 2002 and 2004. Thus a relative reduction of the use of plasma as compared to cellular blood components has not contributed to a lower incidence of TRALI. The assumption of unchanged risk associated with RBC and platelet transfusion could also be challenged if female plasma donors returned to whole blood donation. In fact however female donors continued to donate plasma for fractionation.

The overall incidence of reported TRALI appears to show a downward trend after the year 2007 (figure 1). Analyses by TRIP show that there have been increased reports of transfusion-associated circulatory overload and other transfusion reactions, suggesting that the diagnosis of TRALI is assigned more critically.[1] As explained above the calculated drop in TRALI is based on the ratio of TRALI cases where plasma was (one of blood components) transfused, to cases without plasma, and would be valid despite a reduced trend in the overall level of TRALI detection and reporting.

Consistency with prior findings

A reduction by 33% is slightly higher but in the same order of magnitude as suggested by the findings of leukocyte serology as reported recently from our country.[13] The reduction is comparable to observational pre- and post intervention data on ALI in ruptured abdominal aneurysm repair from a single UK center (0.39, 95% CI: 0.16 – 0.90).[14] An American study of TRALI fatalities in 2003–2005 found that 18 out of 38 probable TRALI fatalities (47%) were associated with female antibody-positive fresh frozen plasma and might be avoided by limiting transfusion of leukocyte antibody-containing FFP.[15] This proportion is again similar although the relative contribution of allo-immune TRALI associated with FFP would not necessarily be the same among cases with fatal outcome. A recent overview of probable TRALI (including nonfatal cases) reported by the American Red Cross describes a drop from 30 cases associated with plasma transfusion in 2006 to 10 cases in 2008 after implementation of male-predominant plasma for transfusion.[16]

In the United Kingdom reports to SHOT of TRALI associated with FFP containing patient-incompatible leukocyte-reactive antibodies dropped from 10 in 2003 to none in 2004–2007 since implementation of preferential use of male plasma. This suggests that, if supply of exclusively male plasma is achieved, this measure could prevent most or all TRALI caused by plasma. As explained above, SHOT assesses the likelihood that a suspected TRALI is indeed transfusion-related partly on the basis of the finding of concordant HLA antibodies in the transfused unit(s). The overall rate of reported TRALI (assessed as highly likely, probable or possible) before the change in the UK was 1.9 per 100,000 units, compared with 2.6 per 100,000 in 2005-2006 in our registry. In The Netherlands, the expert assessors were blinded to the results of serological investigation from 2007 onwards. Prior to that year they were not consistently blind to the results but these were not used for the clinical definition of TRALI. The calculated reduction in The Netherlands is remarkably similar to the effect in the UK despite the important difference in the assessment of cases; this is in line with the hypothesis of TRALI cases being prevented by elimination of patient exposure to incompatible leukocyte antibodies in plasma from female donors.

Meaning of the study, implications for clinicians and policymakers

Not in all countries are donors excluded if they have been recipients of transfusion. Plasma from male donors who have (ever) been transfused should logically also be excluded, although it has been established that pregnancy-related HLA antibodies persist for longer than antibodies developed following blood transfusion. In The Netherlands it was possible to implement the measures for no significant costs and without serious threat to the blood (plasma) supply. We adopted the use of male-only plasma for the plasma added to platelet

pools in mid November 2009. A further safety improvement will be obtained if this achieves a comparable risk reduction for the platelet concentrates preserved in plasma.

Some blood services have implemented antibody screening for all female donors, with repetition of the screening following pregnancy.[17] This should have comparable efficiency in preventing TRALI, while resulting in fewer donor deferrals, but is associated with increased costs. Other countries (e.g. France, Ireland, Norway, and Finland) use pooled solvent-detergent (S/D) virally inactivated plasma and report that TRALI is not seen in association with this product. Reduction in non-infectious transfusion complications (both TRALI and allergic reactions) was included as an important aspect in a recent review of cost-effectiveness aspects of this product.[18]

Conclusion

In conclusion, our findings suggest that in the Netherlands the male-only plasma measure has led to a reduction of TRALI cases of about 33 percent.

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Chapter 8

Prevalence of leukocyte antibodies in the Dutch donor population

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Vox Sanguinis; Epub ahead of print

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%).

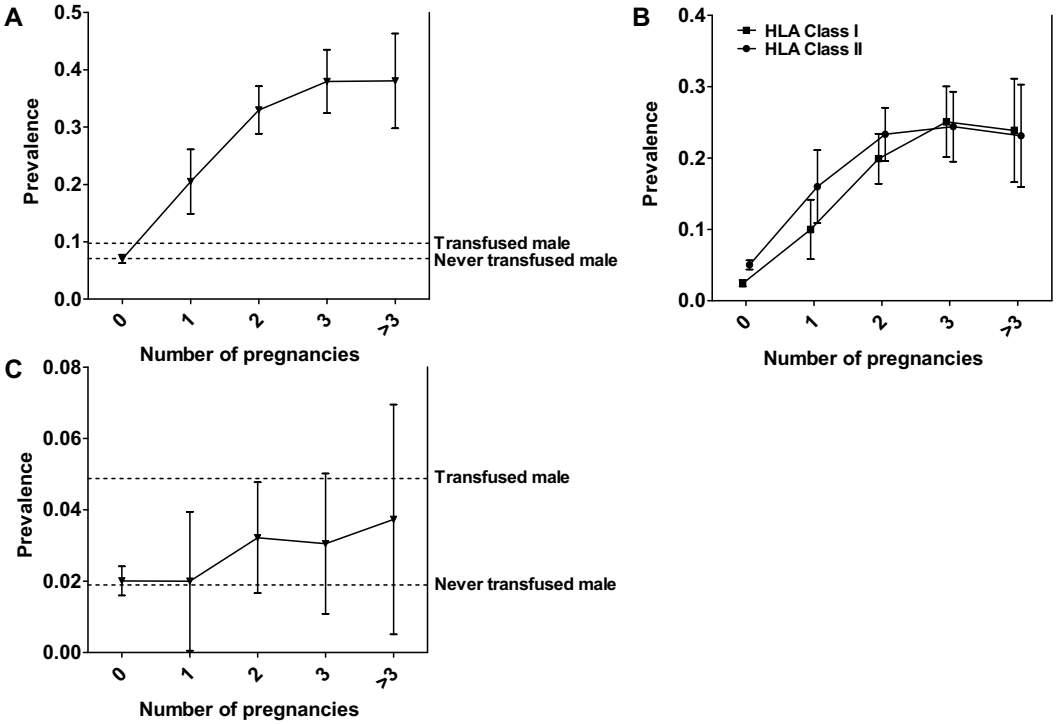

Figure 1: Prevalence of HLA Class I and II and granulocyte reactive antibodies, according to number of pregnancies. **A**: Prevalences of HLA of any class, **B**: HLA Class I (squares) and II (circles) separately or **C**: granulocyte reactive antibodies are shown in relation to the number of pregnancies. 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.

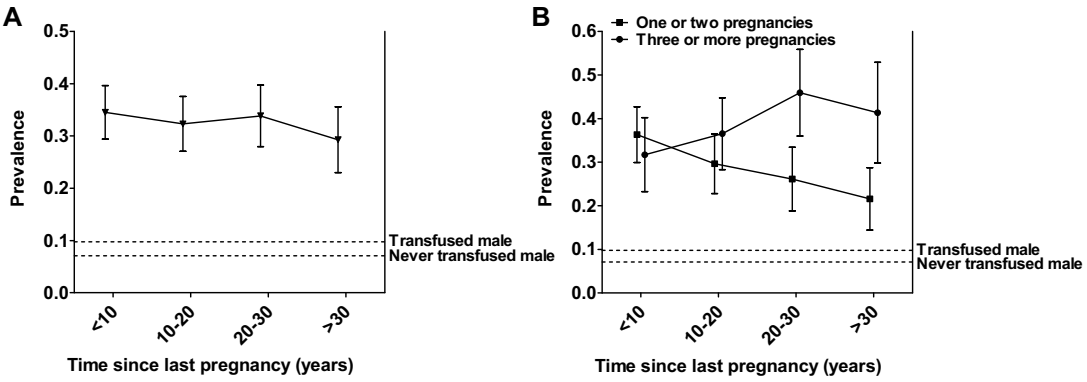

Figure 2: Prevalence of HLA antibodies, according to time since last pregnancy. **A**: The prevalence was standardized for the number of pregnancies and **B**: stratified according to the categories of "One or two pregnancies" (squares) and "Three or more pregnancies" (circles). Within the strata of "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.

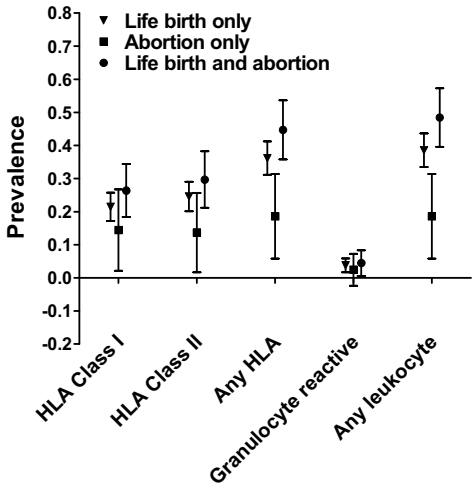

Figure 3: Prevalence of different types of antibodies, according to pregnancy outcomes. Prevalences are compared for women with only life births (triangles), only abortions (spontaneous and induced; squares), and both life births and abortions (circles). All prevalences were determined among never transfused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

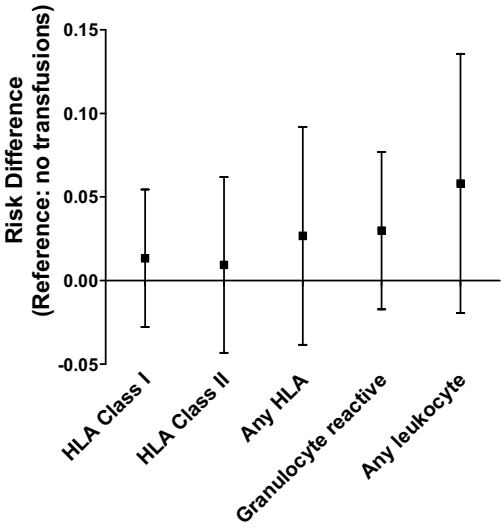

Figure 4: Risk difference of different types of antibodies after a transfusion.

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%).

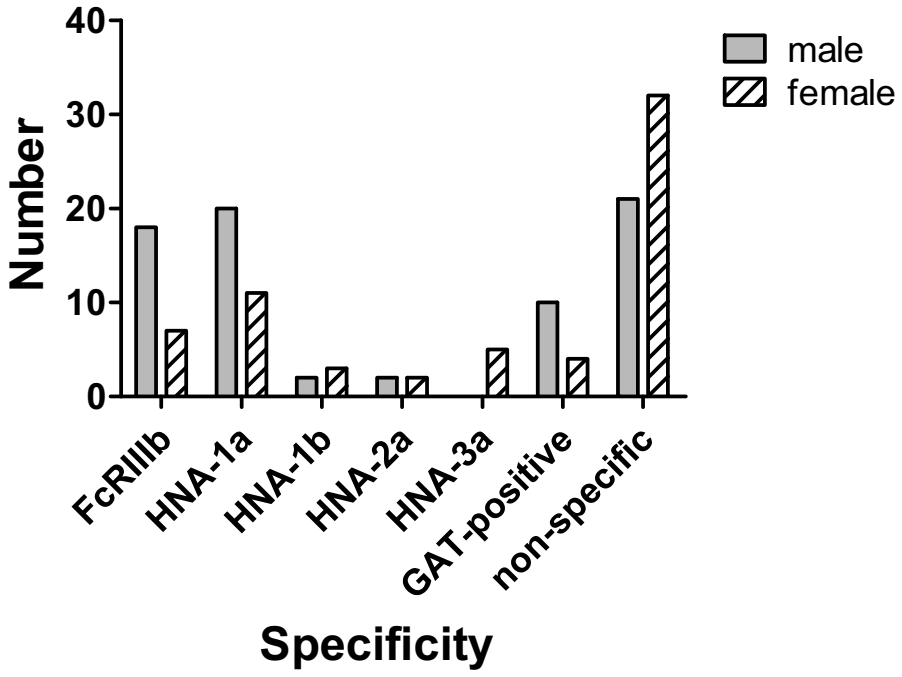

Figure 5: Specificities of 137 granulocyte reactive antibodies, in 3614 male and 2341 female donors, according to sex of the donor.

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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Chapter 9

Discussion

Rutger A. Middelburg

The research presented in this thesis aimed primarily to quantify the contribution of female and allo-exposed donors to the occurrence of transfusion-related acute lung injury (TRALI). We considered these specifics groups of donors since they have a relatively high prevalence of leukocyte antibodies,[1-6] which the literature suggests are an important risk factor for TRALI.[7-16]

Leukocyte reactivity of antibodies is rarely caused by auto-immune disorders or naturally occurring antibodies with cross-reactivity against leukocytes. Leukocyte antibodies most often occur after allo-exposure and the most common form of allo-exposure is during pregnancy. Therefore, it can be assumed that the overwhelming majority of leukocyte antibodies will be found in female (parous) donors. Further we assume there is no other mechanism, of any quantitative importance, by which female donors confer a higher risk of TRALI than male donors. It can then be shown that all three population attributable risks (PAR), for TRALI caused by leukocyte antibodies, allo-exposed donors, and female donors, will be approximately the same. The intuition for this is that excluding all female donors will exclude (almost) all donors with leukocyte antibodies and therefore prevent all TRALI cases caused by leukocyte antibodies. If, furthermore, (almost) no other TRALI cases than those caused by leukocyte antibodies are prevented by the exclusion of female donors, exclusion of female donors prevents (approximately) the same TRALI cases as exclusion of leukocyte antibodies.

We therefore started by quantifying the evidence from the literature for the role of leukocyte antibodies in the etiology of TRALI. In Chapter 2 we selected published TRALI cases that were diagnosed independent of donor sex or serological findings but were fully serologically investigated after diagnosis (i.e. including all involved donors). We estimated that four fifths of these TRALI cases are caused by leukocyte antibodies. In the absence of any major distortion by publication bias we would expect a similar proportion of TRALI cases to be caused by allo-exposed donors and female donors.

Multiple transfusions

In a series of Dutch TRALI patients 85% of patients received transfusions of more than one donor. Due to the low incidence of TRALI the probability of having two causal transfusions is negligibly small. Furthermore, even if two transfusions were both individually capable of causing TRALI in a given patient, only the first of the two could really be considered causal. If the first transfusion caused a patient to be selected as a case all subsequent transfusions become irrelevant, since they can no longer contribute to this selection. It is this selection that leads a causative exposure to be overrepresented in cases, compared to the reference value obtained from the source population or a valid control group. Therefore, once this selection is made, the difference in exposure prevalence between the cases and the

reference value is fixed and all other transfusions become irrelevant. As a consequence it can be assumed that TRALI is caused by a transfusion from only one of the donors, the causal donor.

As shown in Chapter 4, without identification of this causal donor the crude quantitative estimate of the contribution of donor characteristics to the occurrence of TRALI will always be an underestimation. One solution to this problem is to select only TRALI patients who have either received all transfusions from donors with a certain characteristic (e.g. female donors) or who have received all transfusions from donors without that characteristic (e.g. male donors). In the case where we compare patients with only transfusions from female donors to patients with only transfusions from male donors we call these TRALI patients unisex cases (Chapter 5).

Risk factors for TRALI

A first analysis of internationally gathered unisex cases showed less that one fifth to be caused by female donors. However, this apparent lack of effect could partly be explained by the selection of unisex cases. This selection indirectly also selects for patients who received only few transfusions and therefore also for patients who have received red cells, rather than other, more plasma rich products (platelets and fresh frozen plasma). Separate analyses of patients receiving different product types revealed female donors not to confer any increase in risk in recipients of red cells. Conversely, of all TRALI caused by plasma rich products at least four fifths were estimated to be preventable by the exclusion of female donors. It is therefore suggested that in the previous analyses of published TRALI cases there was a publication bias favoring cases caused by plasma rich products. This favoring could have occurred either directly, or due to the association of plasma rich products with leukocyte antibodies, an association which was likely absent from TRALI caused by plasma poor products.

As expected, similar results as those observed in the unisex analyses were found for analyses of the contribution of allo-exposed donors to the occurrence of Dutch TRALI cases (Chapter 6). This also confirmed the marked difference between plasma rich and plasma poor products. Furthermore, previous findings suggest approximately half of the Dutch TRALI cases to be caused by fresh frozen plasma (FFP).[17] Therefore, it can be estimated that the exclusion of all allo-exposed donors (i.e. all female and transfused male donors) from the donation of plasma for transfusion (i.e. the Dutch plasma measure, effective as of 1st October 2006) should prevent approximately two fifths of all TRALI cases in the Netherlands.

This estimate was also confirmed by an evaluation of the shift in the relative contributions of different product types, to the occurrence of TRALI, after implementation

of the plasma measure (Chapter 7). From this comparison it was estimated that nearly half the number of TRALI cases occurring before the plasma measure were prevented by implementation of the plasma measure.

Effect measures for etiological inferences

All the above estimates are population attributable risks (PAR; i.e. preventable fractions). The PAR depends heavily on the exposure prevalence, since at lower exposure prevalence fewer cases can be caused by exposure, while the number of exposure independent cases remains the same, causing the fraction of cases caused by exposure to be lower. For example, after implementation of the plasma measure, the PAR for female donors in recipients of FFP must logically become zero. Its dependence on exposure prevalence is often considered a shortcoming of the PAR, causing it to be considered inferior to the relative risk (RR) as a measure of effect in etiological research. However, the RR is not the absolute biological constant it is often erroneously claimed or believed to be either.

Before the plasma measure approximately half of the reported TRALI cases were associated with the transfusion of plasma rich products. However, only one fifth of all released blood products can be considered plasma rich. Assuming negligible bias due to differential reporting of TRALI for patients receiving different product types, this would suggest an estimated RR for TRALI after receiving plasma rich products of about five. After the plasma measure, the RR of plasma rich products compared to plasma poor products will have changed to unity (i.e. by the prevention of four fifths of TRALI from plasma rich products and none of the TRALI from plasma poor products). Thus the RR, obviously, is not the more stable effect measure it is often claimed to be.

So, the RR can also change dramatically in response to changes in prevalence of (other) risk factors. Stability of the effect measure can therefore not be an argument for favoring the RR over the PAR and we must reconsider the value of both. As in all scientific inquiry and comparisons, also in the comparison of effect measures, we must first answer the question of what exactly we want to know.[18] In etiological research we aim to understand the contribution of different risk factors to the occurrence of a particular disease. We would like to produce an effect measure that reflects an underlying biological truth and which is therefore applicable to any population of human beings at any point in history. This, unfortunately, is not possible. As the prevalence of other risk factors changes, the number of cases caused by biological interactions between the risk factor under study and these other risk factors also changes. Therefore, the best we can hope for is an effect measure that will be constant and valid in any population of human beings at any point in history, given that the prevalence of all other risk factors that interact biologically with this risk factor are comparable to the prevalence in the study population. Within this restriction

we would like to estimate how many individuals are sensitive to developing disease if exposed, but would not develop disease if unexposed.

The RR minus one gives this number, as a multiple of the number of cases that would occur if there was no exposure at all. The PAR divided by the exposure prevalence also gives this number, but as a multiple of the number of cases that actually occurs at the present exposure prevalence. The exposure prevalence and the PAR (or the exposure prevalence and the RR) can be used to calculate, from the number of cases that would occur if there was no exposure, the number of cases that actually occurs at the present exposure prevalence (and vice versa). Therefore, given a known exposure prevalence, the RR and the PAR contain exactly the same information (which also follows from equation 1 in the Appendix of Chapter 4).

Given that we are interested in the effect of a given risk factor, it does not seem unreasonable to assume we would determine the exposure prevalence, probably even before starting a study into its association with a disease. It would, after all, be impossible to study risk factors with zero prevalence and an incredible waste of effort and resources to study those with near zero prevalence. Furthermore, in case control studies the exposure prevalence has to be estimated to arrive at any effect measure at all. Therefore, the information content of the RR and PAR is identical and can not be an argument for the preference for either. This preference should instead be based on ease of interpretation, which in turn also partly depends on the ease with which related effect measures can be derived.

Ease of interpretation depends heavily on personal preference and prior experience, but the fraction of all disease preventable by removal of exposure seems an extremely intuitive effect measure. The number of times the risk of disease increases upon exposure is, by comparison, a very abstract measure of effect. The only way to increase the comprehensibility of this measure is to supply the baseline risk of the disease as well. The RR therefore, seems more suitable for prediction modeling, where the baseline risk of disease is combined with the effects of many risk factors to give a predicted risk of disease over a given period, given a known exposure.

Estimation of the population attributable risk

A major limitation in the use of the PAR in etiological research has been the difficulty in correcting for the influence of confounders, which is much more straightforward for the RR. Since a confounder, by definition, changes the baseline risk the PAR can not be expected to be constant across strata of a confounder. If the total number of cases changes, while the number caused by exposure remains the same, the PAR also changes. Therefore, the most commonly used method to arrive at a corrected PAR is to correct the RR and

calculate a PAR from the corrected RR.[19] This requires an estimate of the exposure prevalence among cases. However, in the case of confounding the exposure prevalence among cases becomes harder to estimate. Since some strata will contribute more cases due to the causal effects of confounders, those strata will be over-represented among cases, compared to the source population. These strata by definition also have a different exposure prevalence, or there could have been no confounding. Therefore, the actual observed exposure prevalence among cases should not be used, but rather the exposure prevalence in the source population should be ascertained and used in equation 1 in the Appendix of Chapter 4.

Alternatively, the standardization method described in Chapter 4 can be used to arrive at a corrected PAR directly. This method can then also be generalized to use with more than one case per stratum (by weighing each stratum for the number of cases) and can also be used to correct for a known fraction of non-differential misclassification. In the presented case where the method is applied to TRALI, all but one transfusion are non-differentially misclassified (i.e. non-causal). This gives a fraction of misclassification of (n-1)/n, which cancels out against the number of transfusions and gives each stratum an equal weight of one. As shown briefly in Chapter 6, in the application to TRALI, this method is also robust against missing data, as long as it is missing randomly with respect to causal and non-causal transfusions.

Finally, in the before-after comparison in Chapter 7 the PAR was the most easily estimable effect measure. In this case the fraction of TRALI cases that was observed to be prevented by the plasma measure only needed to be corrected for the completeness of observation.

Prevention of TRALI

In conclusion, it seems that many, compounded methodological problems, concerning TRALI research specifically and research of side effect of blood transfusions in general, have distorted previous effect estimates of risk factors for TRALI. The principal conclusion to be drawn from this thesis is that leukocyte antibodies, and thus allo-exposed donors, can only contribute importantly to the occurrence of TRALI caused by plasma rich products. In plasma poor products other risk factor must be more important. Although the risk of TRALI used to be much lower after transfusion of plasma poor products, the prevention of TRALI is currently aimed at TRALI caused by plasma rich products. Therefore, plasma poor products are becoming relatively more important.

Plasma rich products

From Chapter 8 we can tell that to prevent leukocyte antibodies from entering the blood supply, deferral of all female donors is not strictly necessary. Female donors reporting no previous pregnancies have an identical prevalence of leukocyte antibodies as male donors. Surprisingly, Chapter 8 also shows that deferral of either all allo-exposed donors or all female and transfused male donors will only decrease the fraction of leukocyte antibody positive donors from one in seven to one in eleven. This is in sharp contrast with the high percentages of TRALI cases estimated to be preventable by the same measures (Chapters 5, 6, and 7), suggesting the antibodies in never allo-exposed donors to be less likely to cause TRALI. As also discussed in Chapter 8, this seeming discrepancy could be due to the fact that leukocyte antibodies are largely detected by assays that assess only binding to certain specific epitopes. These antibodies could also include antibodies with little functional or clinical implication. It seems very well possible that these clinically irrelevant, possibly naturally occurring, cross-reactive antibodies form the majority of antibodies detected in never allo-exposed donors and a substantial minority of those detected in allo-exposed donors.

The overall most efficient measure to prevent TRALI would then be the deferral of all self-reported allo-exposed donors from the donation of plasma for transfusion as FFP or the suspension of platelets for transfusion. A similarly effective measure, leading to less donor deferral would obviously be the exclusion of only the plasma from those donors with clinically relevant antibodies. However, with the current assays we can not distinguish clinically relevant from irrelevant antibodies. Deferral of all donors with leukocyte antibodies is therefore the safer option, but screening for antibodies is prohibitively expensive and labor-intensive. Furthermore, given the results from Chapters 5, 6, and 7 it seems unlikely that this would add substantially to the safety of plasma rich blood products beyond the improvement already offered by exclusion of plasma from allo-exposed donors.

Plasma poor products

The prevention of TRALI caused by plasma rich products, by the exclusion of plasma from allo-exposed donors, is thought to be near complete. Therefore, the prevention of TRALI caused by plasma poor products has become the next highest priority. Since leukocyte antibodies seem to contribute little, if anything, to the occurrence of TRALI caused by plasma poor products, other risk factors must be more important. In this context biological response modifiers (BRM) seem the most likely candidate.[20-22] Most of these small-molecule, inflammatory mediators are known to accumulate in cellular blood products during storage.[21,22] However, since the introduction of universal leukoreduction substantial accumulation of specific inflammatory mediators in packed red cells seems unlikely. Non-specific stimulation of inflammatory processes by cell debris, however, could still play an

important role and cell debris is also likely to accumulate during storage. Furthermore, in platelet products, specific inflammatory mediators are still released during storage. Therefore, storage time and conditions are suggested to be the next, most likely important, and most easily investigated risk factors for TRALI caused by plasma poor products.

It should also be remembered, though, that any factor in a blood product capable of either specifically or non-specifically and either directly or indirectly activating recipient neutrophils could cause TRALI. Potential candidates could therefore range from trace amounts of chemicals released by blood bags to inflammatory mediators produced by the donor before donation. The suggestion to investigate storage time as a potential risk factor therefore reflects the expected ease of investigation and high potential for intervention, as much as the perceived likelihood of a causal relation to the occurrence of TRALI.

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Summary

Rutger A. Middelburg

Transfusion-related acute lung injury (TRALI) is currently the most common serious side effect of blood transfusion. As described in Chapter 1 and shown quantitatively in Chapter 2, the TRALI literature until December 2007 largely consisted of case reports and case series. There was a strong suggestion that TRALI could be caused by donor leukocyte antibodies, present primarily in parous female donors and transfused donors, which activate recipient neutrophils. In The Netherlands this suggestion led to the exclusion of female donors and transfused donors from the donation of plasma for transfusion from 1st October 2006. In this thesis we aimed to quantitatively estimate the expected effect of the implementation of this measure.

In Chapters 3 and 4 we describe and solve several common methodological problems in research of side effects of blood transfusions in general and TRALI in particular. Two of the methods proposed in Chapter 4 are then used in Chapters 5 and 6 to study the contribution of female donors and allo-exposed donors to the occurrence of TRALI. Chapter 7 gives an estimate of the actual effect of the plasma measure in The Netherlands on the incidence of TRALI. The results from Chapters 5 through 7, though obtained through completely different approaches and partly in different populations, are in close agreement. Together these chapters suggest nearly all TRALI caused by plasma rich products to be preventable by the deferral of female or allo-exposed donors, while there is no such effect on the incidence of TRALI caused by plasma poor products.

In Chapter 8 we also evaluate the effectiveness of the plasma measure at actually keeping leukocyte antibodies out of the blood supply. This effectiveness is then compared to other potential donor deferral strategies. It is shown that the deferral of only self-reported allo-exposed donors is as effective as deferral of all female and transfused male donors. In terms of donor management deferral of allo-exposed donors only would be more efficient, but in effectiveness to prevent TRALI there is no difference.

Finally, in Chapter 9, we discuss these findings and some more general issues concerning the use of the population attributable risk, as opposed to the relative risk, and correction of the population attributable risk for confounding.

Samenvatting
(Dutch summary)

Rutger A. Middelburg

Transfusiegerelateerde acute longschade (transfusion-related acute lung injury; TRALI) is op het moment de meest voorkomende ernstige bijwerking van bloedtransfusies. Zoals algemeen beschreven in Hoofdstuk 1 en op kwantitatieve wijze aangetoond in Hoofdstuk 2, bestond tot december 2007 de literatuur over TRALI voornamelijk uit beschrijvingen van één of meer patiënten, zonder controle groep. Er werd wel gesuggereerd dat TRALI voornamelijk veroorzaakt zou worden door leukocyten antistoffen van de bloeddonor die de neutrofiele granulocyten van de ontvanger zouden activeren. Deze antistoffen worden voornamelijk gevonden in vrouwelijke donoren, die zwanger zijn geweest, maar ook in getransfundeerde donoren. In Nederland wordt daarom vanaf 1 oktober 2006 alleen nog plasma voor transfusie gedoneerd door mannelijke, nooit getransfundeerde donoren. In dit project wilden we kwantificeren welk effect we van deze maatregel kunnen verwachten.

In Hoofdstuk 3 en 4 beschrijven we een aantal methodologische problemen die veel voorkomen in het transfusieonderzoek in het algemeen en het TRALI-onderzoek in het bijzonder. In Hoofdstuk 4 stellen we ook verschillende oplossingen voor één van deze problemen voor. Twee van deze methoden worden vervolgens in Hoofdstuk 5 en 6 toegepast om de bijdrage van vrouwelijke donoren en donoren die blootgesteld zijn aan allogene antigenen aan het optreden van TRALI te kwantificeren. In Hoofdstuk 7 geven we een schatting van het werkelijke effect van de invoering van de plasmamaatregel in Nederland. Hoewel Hoofdstukken 5 tot en met 7 heel verschillende methoden en gedeeltelijk verschillende populaties gebruiken komen de resultaten goed overeen. Samen laten deze hoofdstukken zien dat bijna alle TRALI die veroorzaakt wordt door plasmarijke producten voorkomen kan worden door het uitsluiten van vrouwelijke donoren en donoren die blootgesteld zijn aan allogene antigenen. Voor TRALI die veroorzaakt wordt door plasma-arme producten bestaat dit verband echter niet.

In Hoofdstuk 8 laten we zien welk deel van de leukocyten antistoffen door de huidige plasmamaatregel uit de bloedvoorziening gehouden kan worden. Daarnaast tonen we aan dat het uitsluiten van plasma van alleen vrouwelijke donoren die zwanger zijn geweest en getransfundeerde donoren net zo effectief is als het uitsluiten van plasma van alle vrouwelijke en getransfundeerde donoren.

Tot slot bespreken we in Hoofdstuk 9 het verband tussen de resultaten uit alle eerdere hoofdstukken. In Hoofdstuk 9 bespreken we ook wat algemenere onderwerpen zoals het gebruik van een populatie attributief risico in plaats van een relatief risico en correctie van het populatie attributief risico voor confounding.

Dankwoord

Rutger A. Middelburg

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Curriculum Vitae

Rutger A. Middelburg

Rutger Anton Middelburg werd geboren op 25 februari 1978 te Voorschoten. In 1996 behaalde hij zijn VWO diploma aan het Adelbert College te Wassenaar (cum laude). Hierna studeerde hij Biomedische Wetenschappen aan de Universiteit Leiden, waar hij gedurende tweeënhalf jaar verschillende onderzoeksstages deed en in 2001 zijn doctoraal examen haalde. Vervolgens deed hij een jaar lang onderzoek bij Numico Research in Wageningen, waarna hij drie jaar bij de afdeling Klinische Oncologie van het VU medisch centrum in Amsterdam werkte. Sinds mei 2006 deed hij, bij de afdeling Klinische Epidemiologie van het Leids Universitair Medisch Centrum, in samenwerking met de afdeling Onderzoek en Opleiding van de Sanquin Bloedbank regio Zuidwest, een promotieonderzoek naar de oorzaken van transfusion-related acute lung injury. De resultaten van dit onderzoek zijn in dit proefschrift beschreven. Tijdens dit onderzoek volgde hij ook de opleiding tot epidemioloog B. Inmiddels is hij als post-doc werkzaam bij de divisie Research van Sanquin, waar hij onderzoek doet naar predictiemodellen voor bloedingen en effectiviteit van plaatjestransfusies bij patiënten met acute myeloïde leukemie.