TRANSFUSION-RELATED ACUTE LUNG INJURY

The role of donor antibodies

Daniëlle Mathijssen-van Stein

Transfusion-Related Acute Lung Injury - The role of donor antibodies

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TRANSFUSION-RELATED ACUTE LUNG INJURY – THE ROLE OF DONOR ANTIBODIES

Transfusiegerelateerde acute long schade – De rol van donor antistoffen

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Promotor: Prof.dr. D.J. van Rhenen

Overige leden: Prof.dr. J.L.C.M. van Saase
Prof.dr. A. Brand
Prof.dr. F.W.G. Leebeek

Copromotoren: Dr. E.A.M. Beckers
Dr. A.P.J. Vlaar

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General introduction and outline of this thesis

Daniëlle van Stein and Alexander P. Vlaar

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INTRODUCTION

In 1666 Jean Baptiste Denis for the first time transfused a human being.[1] At that time lamb blood was used for transfusion, which gave a severe transfusion reaction. Since that time much effort has been made to make transfusion as save as it is today. However transfusions still presents a risk to the recipient. Probably in the 1950s the first cases of transfusion-related acute lung injury (TRALI) were reported in the literature, although they were not recognized as a distinct clinical syndrome.[2,3] In 1983, over three hundred years after the first blood transfusion, Popovsky and coworkers coined the name TRALI (Transfusion-related acute lung injury), that implies a serious, sometimes fatal complication of blood transfusion.[4] In 1985 they published the first series of 36 TRALI cases from the Mayo Clinic.[5] Since 2003 it is the leading cause of transfusion-related mortality according to the FDA.[6] The diagnosis of TRALI is based on clinical and radiographic findings, there is no pathognomonic laboratory test.

DEFINITION OF TRALI

The definition of TRALI proposed in 1985 by Popovsky et al. has changed little since then.[5] TRALI is a clinical diagnosis. In 2004 the Canadian Consensus Conference formulated a definition of TRALI based on clinical and radiological findings.[7,8] It defined TRALI as a new episode of acute lung injury (ALI) that occurs during or within 6 hours of a blood transfusion and is not temporally related to another risk factor for ALI. ALI is defined as the acute onset of respiratory distress and hypoxemia (PaO_2-to-FiO_2 ratio less than

Table 1. Criteria for (possible) TRALI according to the Canadian Consensus Conference

TRALI
a. ALI
• Acute onset
• Hypoxemia ($PaO_2/FiO_2 \leq 300$ mmHg or saturation < 90% on room air)
• Bilateral infiltrates on chest radiograph
• No evidence of left atrial hypertension (i.e. circulatory overload)
b. No preexisting ALI before transfusion
c. During or within 6 hours of transfusion
d. No alternative risk factor for ALI present*
possible TRALI
a. ALI
b. No preexisting ALI before transfusion
c. During or within 6 hours of transfusion
d. Alternative risk factor for ALI present*

* Risk factors for ALI include sepsis, aspiration, pneumonia, toxic inhalation, lung contusion, near drowning, cardiopulmonary bypass, drug overdose.

300 mm Hg, saturation less than 90% on room air or other clinical evidence), bilateral infiltrates on chest X-ray and no evidence of circulatory overload. When ALI is temporally related to both transfusion and an alternative risk factor the term "possible TRALI" is used (table 1). Other risk factors include for example sepsis, aspiration, pneumonia and multiple trauma.

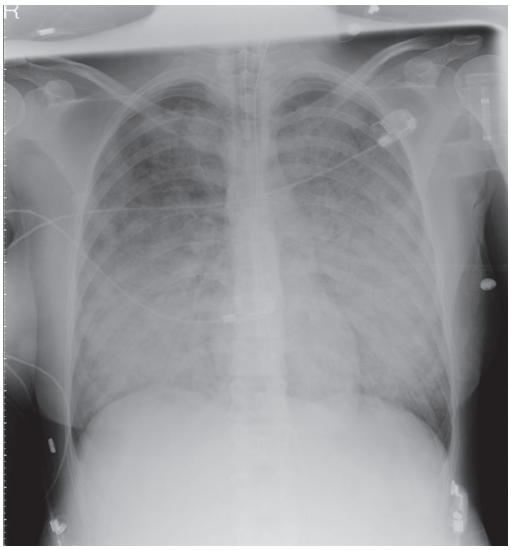

Figure 1. Bilateral infiltrates on chest X-ray in a patient with TRALI

CLINICAL PRESENTATION

TRALI is underdiagnosed and sometimes mistaken for circulatory overload after transfusion or ALI caused by other risk factors. The diagnosis of TRALI is based on clinical findings manifested within 6 hours of transfusion of a blood product. Most often it develops well before the 6 hours after transfusion, sometimes even during the infusion of the blood product. Clinical hallmarks of TRALI include dyspnea, tachypnea, hypoxemia, bilateral pulmonary infiltrates on chest X-ray (figure 1) and frothy edema fluid in the endotracheal tube, in the absence of evidence of circulatory overload and cardiac dysfunction.

Also reported are febrile reactions and hypothermia, both hypotension and (rarely) hypertension, chills and tachycardia.[9-11] In mechanical ventilated patients, the diagnosis should be considered whenever there is an acute unexplained worsening in respiratory status that is temporally related with a transfusion. Clinical signs in these patients may include frothy pink edema in the endotracheal tube, decrease in P/F ratio or increase in airway pressures. There are no specific diagnostic tests for TRALI. The differential

diagnosis includes circulatory overload and this has to be excluded. Echocardiography and measurement of brain natriuretic peptide (BNP) can be useful in these. Transient leukopenia has been temporally associated with the onset of TRALI.[11,12]

PATHOGENESIS

Leukocyte antibodies

The majority of TRALI cases have been related to HLA- or HNA-antibodies in plasma rich transfusion products.[13,14] Red blood cell products (RBCs) and platelet transfusion products (PLTs) are produced with small volumes of plasma but can still cause TRALI.[15-17]A correlation between the HLA and HNA antibody strength and development of TRALI has been indicated which might explain why also small volumes have been related to TRALI.[15,18]

Based on several retrospective studies HLA-class I antibodies account for 14.3-26.7% of TRALI cases, HLA-class II antibodies 0.0-46.7% and HNA 16.7-28.6%.[19-26] These numbers have to be interpreted with caution as many older studies did not test for HNA-antibody prevalence and newer, more sensitive techniques have been developed to detect antibodies.

Threshold model and two hit model

Reported TRALI incidence varies between 0.08-15.1% per patient and 0.01-1.12% per product transfused.[27] The great variety in incidence suggests not all patients or blood products have an equal risk for onset of TRALI. Indeed, studies among critically ill patients showed an increased risk for developing TRALI. On the other hand studies reported that it is possible to develop TRALI in the absence of any underlying condition i.e. (relatively) healthy individuals.[28-30] Furthermore, the majority of antibody containing blood products do not cause TRALI in all patients[20,31-34], even in patients that receive blood products with cognate antibodies.[8] These observations gave rise to the hypothesis that TRALI follows either a "threshold model" or a "two hit model". In the "two hit model" a "first hit" primes neutrophils and attracts them to the pulmonary vasculature. This is followed by a "second hit" that activates these neutrophils with consecutive pulmonary damage. Amongst others, haematogical malignancy, cardiovascular disease, sepsis and emergency cardiac surgery have been identified as risk factors for a "first hit" (figure 2).[16,29,35,36] The "second hit" can be formed by HLA- and HNA-antibodies, biologically active lipids (lysophosphatidylcholines) or other biologic response modifiers (e.g., soluble CD40 ligand). In the "threshold model" the threshold is formed by the level of priming of lung neutrophils, and the ability of the mediators in the transfusion product to activate these primed neutrophils (figure 3).[37] Hence, in the "two hit" it is compulsory

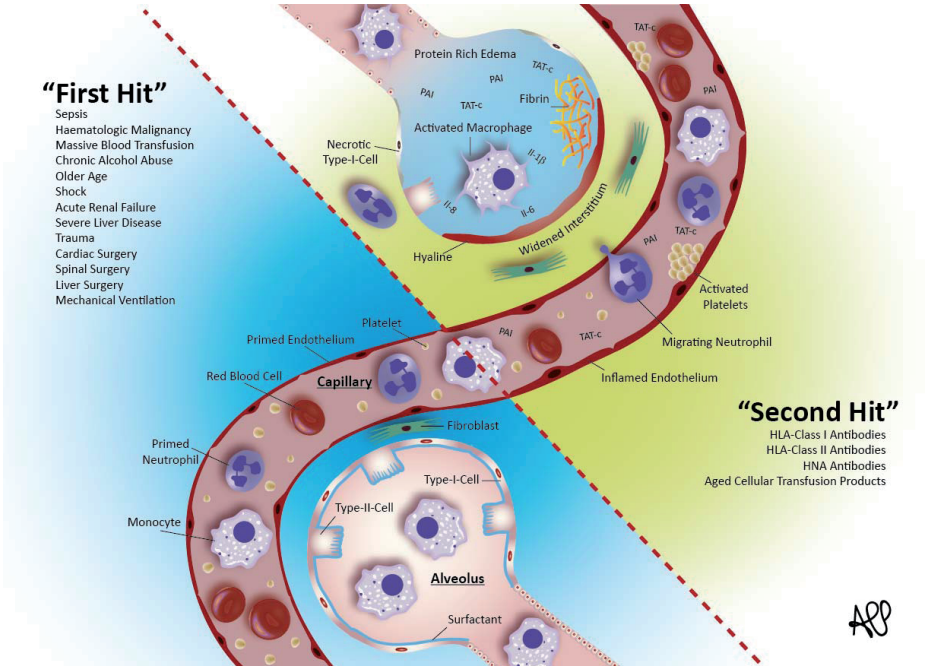

Figure 2. The two hit model of TRALI. The "first hit" consists of patient factors, which primes neutrophils and attracks them to the lung capillary. The "second hit" is the transfusion of a blood product resulting in activation of these primed neutrophils causing pulmonary damage.

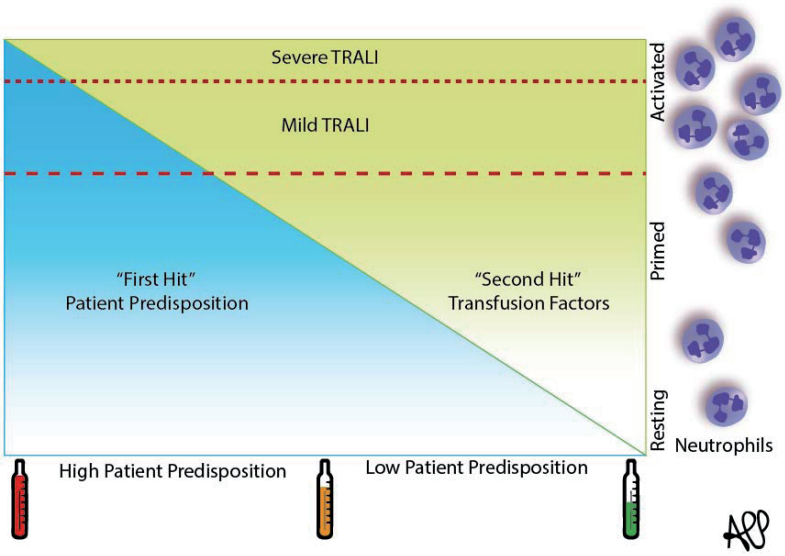

Figure 3. The threshold model of TRALI: In the "threshold model" the threshold is formed by the level of priming of lung neutrophils ("first hit"), and the ability of the mediators in the transfusion product ("second hit") to activate these primed neutrophils

to have a "first hit" to develop TRALI which is mainly the case for non-antibody mediated TRALI. In the threshold model TRALI may develop in the absence of a "first hit" as long as the second hit is strong enough to overcome the threshold. In some TRALI-cases antibodies in the recipient against donor leukocytes have been identified as "second hit" for TRALI.[22] However after implementation of universal leukoreduction for blood products this pathway has become less important.

MANAGEMENT

Whenever a TRALI reaction is suspected in a patient, the attending physician should report this to the blood bank. Involved donors should be identified and tested for leukocyte-reactive antibodies. TRALI is a clinical diagnosis, there is no pathognomonic diagnostic test. Many different specialists are involved in the recognition of, and care for TRALI patients. Reporting of cases to the blood bank differs between the disciplines.[38] Also patient factors were reasons for not reporting suspected cases (e.g. sepsis before transfusion). Reporting is important because involved donors who have proven incompatible antibodies and donors who are associated with a TRALI case two times are excluded from further donations to prevent future TRALI reactions in the Netherlands.

TREATMENT AND PROGNOSIS

There is no specific treatment for TRALI. In most cases, TRALI is self-limiting. Management of TRALI is supportive. Mild cases need supplemental oxygen only, however 70-90% of TRALI cases require mechanical ventilation.[5,39,40] In these patients who need mechanical ventilation a low tidal volume strategy is recommended, as this is beneficial in patients with ALI/acute respiratory distress syndrome and TRALI is considered to be a member of these syndromes.[15] The use of corticosteroids has never been demonstrated to be effective, although some case reports describe the use of these.

Most cases of TRALI are self-limiting and have a good prognosis. The mortality rate of TRALI in the general patient population has been reported as 5% to 10%.[5,41,42]

DIAGNOSTIC WORK-UP OF DONORS

To diagnose antibody-mediated TRALI antibodies directed against recipient's HNA and/or HLA antigens have to be detected in the involved blood donor(s). In the evaluation of TRALI cases, donors are tested for presence of HNA and HLA-antibodies. There are

several techniques to detect these antibodies. These assays are usually only available in specialized centres. They are used to confirm antibody mediated TRALI and identify donors who pose a potential risk for future patients. As it can take several weeks before the results are known, they have no value in diagnosing an acute TRALI-reaction.

HLA-antibodies

In 1964 Terasaki and McClelland developed the complement-dependent cytotoxicity assay (CDC assay) which has been the golden standard for detecting HLA-antibodies for many years.[43] Serum to be tested is incubated with HLA-typed donor lymphocytes and rabbit complement. If antibodies present in the serum bind to the cognate antigen on the lymphocyte, the complement cascade is activated, leading to cell death. By using fluorescent dyes cell death was observed using microscopy and used as an indicator of the presence of HLA Class I antibodies. The CDC-assay is labour-intensive, requires highly skilled staff and fresh preparation of lymphocyte panels of donors with known phenotypes.[43-45]

The CDC was the "gold standard" for years, but most laboratories have replaced it with antigen-based assays. These assays use isolated HLA antigens fixed to wells or micro beads to detect antibodies.[46,47]

HNA-antibodies

The ISBT Working Party on Granulocyte Immunobiology recommends combining the granulocyte immunofluorescence test (GIFT) and the granulocyte agglutination test (GAT), both serological methods, to detect HNA-antibodies.[48] GIFT is considered to be the most sensitive method, but GAT has a better ability to detect HNA-3a antibodies.[49]

In the GIFT a granulocyte suspension is incubated with serum and FITC-labelled rabbit-anti-human IgG serum. The product of this assay, a suspension of labelled cells can be examined under an immunofluorescence microscope.[50] The subjectively observed fluorescence is a measure for antibody activity in the serum. Antibody specificity is assessed by testing a panel of neutrophils from donors of known HNA genotypes.

The GAT is performed according to Jiang and Lalezari.[51] A granulocyte suspension is added to dilutions of test plasma. Cell aggregation and proportion of cells aggregating are examined under an inverted phase microscope after which the reaction is graded from 0 to 4.

The monoclonal antibody-specific immobilization of granulocyte antigens (MAIGA) is another serological method to detect HNA-antibodies.[52] The MAIGA is mostly used for confirmation of GIFT and GAT results.

These classic, serological methods to identify granulocyte antibodies require daily fresh typed granulocytes. The methods are time-consuming and cannot be used for detection of neutrophil antibodies in large populations, as for example blood donors.

Moreover, the International Granulocyte Immunology Workshop demonstrated that there was a wide variation in the proficiency of antibody detection among the different laboratories (16.7 – 100% correct detection of antibodies, mean of 57.5%).[48] More recently analogous to HLA-antibody detection, Luminex bead-based assays have been developed to detect HNA-antibodies.[53]

Cross-matching

Cognate antibodies in donors can also be assessed by performing cross-matches between donor plasma and patient lymphocytes and granulocytes. These cross-matches can be performed in the CDC, LIFT, GIFT and GAT.[43,50,51] Instead of using typed donor lymphocytes or granulocytes in these assays, fresh cells from the patient who experienced a TRALI reaction are used and incubated with plasma from the involved donor(s). When antibodies directed to HLA or HNA antigens in the patient are present, the cross-match will be positive. Cross-matches only identify incompatible antibodies and cross-matches can have false negative results. Antigens may have been upregulated during a TRALI reaction but cross-matching is usually performed weeks or months after the case is reported. Patients have usually recovered by this time and the antigen is downregulated again. This may result in a negative cross-match.

PREVENTIVE MEASURES

Antibody-mediated TRALI caused by the passive infusion of leukocyte-reactive antibodies is considered the most prevalent form of TRALI.[5,55] These antibodies develop most frequently during pregnancy, when women are exposed to the alloantigens from the father. The prevalence of these antibodies increases with each pregnancy, making these antibodies most prevalent in multiparous women.[56] Other allo-exposed individuals are recipients of blood transfusions and organ transplantations.[57] After female donors were recognized as risk factor for developing TRALI, the United Kingdom implemented a preferentially male donor fresh-frozen plasma strategy in 2003. Several countries followed this precautionary measure. Since July 2007 only apheresis plasma from non-transfused male donors is used for single-donor FFP in the Netherlands.

Exclusion of all allo-exposed donors leads to exclusion of a very large group of "safe" donors, who don't have these antibodies. To prevent this unnecessary exclusion, allo-exposed donors could be screened for the presence of HLA and HNA-antibodies.[21,58] To completely minimize the risk of antibody-mediated TRALI, all donors should be screened and all positive donors should be excluded for all blood products containing plasma, regardless of the amount of plasma.

Some countries use pooled solvent/detergent (S/D) plasma instead of single-donor fresh-frozen plasma. S/D plasma is plasma pooled from 500-1600 donors. Pooling reduces the possible antibody load by dilution. Also the antigens present in the pooled plasma might be able to bind the antibodies, causing inactivity of the antibodies.[59] In 2 studies no leukocyte-reactive antibodies were found in units of S/D plasma.[59,60] Countries that use pooled S/D plasma (e.g. Finland, Luxembourg and Norway) have never reported TRALI-cases after transfusion of S/D plasma.[61]

OUTLINE OF THE THESIS

The clear association between the presence of donor antibodies and the onset of TRALI is the starting point of this thesis. Although the association is acknowledged it is not known which donors are at risk to have antibodies. Furthermore current assays for identification of HLA positive donors have many limitations. Finally strategies aimed at reducing the number of antibody positive donors to prevent the onset of TRALI have been introduced however the overall impact has not yet been evaluated. The thesis focuses on the role of donor leukocyte-reactive antibodies in TRALI, and the translation to preventive strategies. In chapter 2, we analyzed all TRALI-cases in the Netherlands reported to the Sanquin Blood Bank during a 2.5-year period for patient, donor and product characteristics. All patients and involved donors were screened for anti-leukocyte (HLA and HNA) antibodies. In chapter 3 we aimed to examine the relation between female donors and the occurrence of TRALI by studying TRALI patients who had received transfusions either from female donors or male donors alone. Chapter 4 focuses on the contribution of alloexposed donors to the occurrence of TRALI for different blood product types (plasma-rich and plasma-poor products). Reporting and identifying possible causal donors of TRALI cases are keystones in the prevention of future TRALI cases as the implicated donors are excluded from future donations. In chapter 5, we investigated whether the use of bead-based techniques might improve the diagnosis of antibody-mediated TRALI. We compared cellular-based techniques to bead-based techniques for detection of HLA-antibodies in a series of 100 consecutively reported TRALI cases meeting the Canadian consensus conference criteria for TRALI. Chapter 6 is a meta-analysis on the impact of low risk TRALI donor strategies for plasma containing blood products on the onset of TRALI. We performed a meta-analysis of all trials on this topic since 1995. Finally, the results are summarized and discussed in chapter 7 (English) and chapter 8 (Dutch).

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2

Transfusion-related acute lung injury reports in the Netherlands: an observational study

Daniëlle van Stein, Erik A. Beckers, Kees Sintnicolaas, Leendert Porcelijn, Fikreta Danovic, Jacques A. Wollersheim, Anneke Brand, and Dick J. van Rhenen

Transfusion 2010;50:213-220

ABSTRACT

Background

Transfusion-related acute lung injury (TRALI) is a serious, sometimes fatal complication of transfusion, attributed to white blood cell (WBC)-reactive antibodies present in the blood product. This study investigated incidence and etiology in the Netherlands.

Study design and methods

From January 2005 through July 2007, all TRALI cases reported to the Sanquin Blood Banks were evaluated. Only cases meeting the Canadian Consensus Conference criteria for TRALI were included and investigated for patient, donor, and product characteristics. Patients and donors were screened for HLA class I, HLA class II and granulocyte antibodies.

Results

A total of 56 TRALI cases were reported of which 49 were completely evaluated. Seventy-eight percent of the patients needed monitoring or mechanical ventilation on the intensive care unit, 10 patients died. In 61% of cases large-volume plasma products were involved. WBC-reactive antibodies in donors were found in 73% of cases, with proven incompatibility in 21 out of 44 (48%) investigated cases. Possible TRALI cases, as defined by Canadian Consensus Conference, had statistically significant lower incompatibility rates compared to TRALI cases, 18% vs. 58% (p=0.036). In the 21 alloimmune cases, a total of 31 implicated donors were found, of which 26 were female, including 12 fresh-frozen plasma (FFP) products.

Conclusion

TRALI is the most serious transfusion complication in the Netherlands, causing severe morbidity and mortality. Antibodies were found in the majority of the cases, but causality with proven incompatibility could be established in 21 cases (48%). Female FFP products were involved in 57% of proven alloimmune cases and would theoretically be prevented using male FFP only.

INTRODUCTION

Transfusion-related acute lung injury (TRALI) is a serious, possibly fatal complication of blood transfusion. It is now the leading cause of transfusion-associated mortality according to the FDA.[1] TRALI is diagnosed based on clinical and radiographic findings and is defined as a new episode of acute lung injury (ALI) occurring during or within 6 hours of a blood transfusion.[2] The exact pathogenesis of TRALI is not fully elucidated yet. There are two proposed mechanisms: The antibody-mediated mechanism and the two-event model.[3,4] Although it is increasingly recognized in recent years, there still is a lack of clinical recognition of TRALI, which leads to misdiagnosis and underreporting. Although numerous case reports have been documented in the literature, only a few case series have been published. The definition used for TRALI differed between these studies. TRALI incidence reportedly ranges from 1 in 432 to 1 in 5000 blood products.[5,6]

This observational study describes the clinical and laboratory features of all reported TRALI cases in the Netherlands in a 2.5-year period. The occurrence of TRALI cases in the Netherlands, where all red blood cell (RBC) units and half of PLT units have low plasma volumes (less than 30 ml) and where a prestorage leukoreduction policy was implemented in 2002, was not known and might be different from other countries, where PLTs with high plasma volumes (more than 100 ml) or leukoreduction is not in place.

We adhered strictly to the Canadian consensus criteria and used an extensive diagnostic package for screening patients and all involved donors for HLA class I, HLA class II, and granulocyte-specific antibodies. Participating in an active hemovigilance program, a relatively large number of 49 TRALI cases, which represents one of the largest TRALI series, was included and analyzed for patient, donor, and product characteristics.

MATERIALS AND METHODS

Patient study population

All suspected cases of TRALI in the Netherlands reported to the Sanquin Blood Banks during a 2.5-year period (January 2005-July 2007) were evaluated. TRALI was diagnosed by the hospital medical personnel and subsequently reported to one of the transfusion consultants of the blood bank. The Sanquin Blood Bank is divided into four regions, each serving a quarter of the population of the Netherlands. Patients were included from all four blood bank regions. All cases of TRALI were reviewed by one of us (DS, EB, FD, and JW). We followed the consensus criteria for TRALI from the Canadian Consensus conference (Table 1).[2,7] Only cases meeting these criteria were included and investigated for patient, donor, and product characteristics.

Table 1. Criteria for TRALI and possible TRALI from the Canadian Consensus Conference[2]

TRALI	
a.	ALI
	• Acute onset
	• Hypoxemia ($PaO_2/FiO_2 \leq 300$ mmHg or saturation < 90% on room air)
	• Bilateral infiltrates on chest radiograph
	• No evidence of left atrial hypertension (i.e. circulatory overload)
b.	No preexisting ALI before transfusion
c.	During or within 6 hours of transfusion
d.	No alternative risk factor for ALI present*
possible TRALI	
c.	ALI
d.	No preexisting ALI before transfusion
c.	During or within 6 hours of transfusion
d.	Alternative risk factor for ALI present*

* Risk factors for ALI include sepsis, aspiration, pneumonia, toxic inhalation, lung contusion, near drowning, multiple trauma, burn injury, acute pancreatitis, cardiopulmonary bypass, drug overdose.

Patient characteristics collected include age, sex, admitting diagnosis, transfused blood components, and clinical manifestations. All donors of blood products transfused within 6 hours before the development of TRALI were investigated, and donor variables reviewed included sex, age, parity, blood transfusions in past, and results of antibody testing. A donor involved in a TRALI case was designated as implicated when incompatibility was proven between donor antibodies and patient cells. When antibodies were not incompatible with patient antigens or in absence of these antibodies, the donor was flagged as associated with a TRALI case.

Blood products

The patients were transfused with RBCs, PLTs, or fresh frozen plasma (FFP). The RBCs were suspended in saline-adenine-glucose-mannitol additive solution (AS) and prestorage leukoreduced (< 1 x 10^6 white blood cells [WBCs]). The final product contained a mean volume of 20 mL of plasma, the mean total volume was 270 mL, and the mean haematocrite 0.57 L/L. The majority of PLT products transfused were pooled random-donor units (prepared from the buffy coats [BCs] of five donors). Pooled PLTs were prestorage leukoreduced (< 1 x 10^6 WBCs) and suspended in plasma or PLT AS (PAS II). The mean volume was 310 mL. Single-donor apheresis units were only used when HLA-compatible PLTs were needed. When indicated, RBCs and PLTs were washed or irradiated. FFP is derived from single-donor apheresis donations and contains fewer than 1 x 10^6 WBCs. The plasma is quarantined for at least 6 months. The volume was approximately 325 mL. For all patients, type and storage time of the blood products transfused within 6 hours before the development of signs and symptoms were recorded.

Laboratory tests

WBC-reactive antibodies were examined in all patients (in posttransfusion blood samples and, if available, in pre-transfusion samples) and in all donors of blood components transfused to patients within 6 hours preceding the development of TRALI.

All patients and donors were screened for HLA antibodies using a standard complement-dependent cytotoxicity (CDC) assay against a panel of HLA class I-typed donor lymphocytes, to detect complement-fixing antibodies to HLA Class I, and using a two-colored fluorescence test with a panel of HLA class II-typed donor B lymphocytes for the detection of HLA Class II antibodies. In addition, a flow cytometry bead-based screening assay for the presence of HLA Class I and II antibodies (FlowPRA, One Lambda, Inc., Canoga Park, CA) was used.[8]

If samples were positive by CDC and/or flow cytometry screening assay, the specificity of HLA Class I and II antibodies was determined using single antigen beads on the Luminex platform (Labscreen SA, One Lambda, Inc.). A cutoff for a positive Luminex test of 1000 (normalized) mean fluorescence intensity was used. Lymphocyte-reactive antibodies were examined by the lymphocyte immunofluorescence test (LIFT) against two pools of five typed donor-lymphocyte suspensions each, according to Décary et al.[9] Granulocyte-specific antibodies were examined by the granulocyte immunofluorescence test (GIFT; HNA-1a, -1b, -1c, and -2a) based on the method of Verheugt and coworkers[10] and by the granulocyte agglutination technique for HNA-3a, according to Jiang and Lalezari.[11]

The patients and when appropriate (in case of specific patient antibodies) also donors were phenotyped in the GIFT for HNA-1a, 1b, 2a, and 3a and genotyped by using a polymerase chain reaction-based assay with sequence-specific primers (Olerup SSP AB, Saltsjöbaden, Sweden) and/or sequence-specific oligonucleotides primers (One Lambda, Inc.) for HLA Class I and II. WBC incompatibility was also assessed by performing cross-matches between donor serum and patient's lymphocytes and granulocytes in the CDC, LIFT, GIFT and granulocyte agglutination technique. Donors were assigned incompatible if either a positive cross-match or specific antibodies against cognate antigens in the patient were found.

Statistics

Statistical analysis was performed with computer software (SPSS 15.0 for Windows, SPSS, Inc. Chicago, IL). Frequencies were described as mean, median, and range. Statistical differences between the TRALI and possible TRALI group were tested with the Fisher's exact test. p Values less than 0.05 were considered significant.

RESULTS

Recipients

Fifty-six cases of suspected TRALI were reported to the Sanquin Blood Banks in the 2.5-year period. Forty-nine cases were completely evaluated and met all criteria for TRALI. In four cases another diagnosis was more likely to have caused the symptoms (circulatory overload, pulmonary embolism) and from three patients additional information was insufficient for a definitive diagnosis. In 2005, 13 reports; in 2006, 24; and in the first half of 2007, 12 reports met the TRALI criteria. Of these 49 cases 24 (49%) were females. The mean age of the recipients was 52.6 years, with a range of 5 to 81 years (median, 59 years; Table 2).

Table 2. Characteristics of TRALI/possible TRALI patients

Patients	**N=49 (%)**
Age mean	52.6
Median	59
Range	5-81
Male:female	25:24
Diagnosis	
Surgical	26 (53%)
Non-surgical	23 (47%)
Ventilated during transfusion/start of symptoms	11 (22%)
After transfusion: Mechanical ventilation	21 (55%)
Non-invasive ventilation	2 (5%)
Supplemental oxygen	15 (39%)
Fever	14/43 (33%)*
Chills	4/42 (10%)*
Cyanosis	9/36 (25%)*
Hypotension	13/41 (32%)*
Frothy sputum from endotracheal tube*	10/18 (56%)†

* information was not available for all cases

† 17 patients were not intubated during or after the start of the symptoms; in 14 cases no information was available.

The most commonly reported clinical signs and symptoms of TRALI were hypoxemia and pulmonary infiltrates on chest X-ray (both required to meet the criteria for TRALI), shortness of breath, frothy sputum from endotracheal tube, hypotension and fever. Various admitting diagnoses were reported. Of all patients 26 were classified as surgical and 23 as nonsurgical. In the nonsurgical group, 11 patients were diagnosed with hematologic disorders (of which 9 were malignant), and 5 patients had an (active) infection

(e.g., pancreatitis, pneumonia). Eleven patients were already on mechanical ventilation during transfusion or at the start of the signs and symptoms. Of the other 38 patients, 21 (55%) required mechanical ventilation after the onset of the TRALI; the remainder needed supplemental oxygen (Table 2). In 11 patients (22%) an alternative risk factor for ALI was present: 4x sepsis, 3x pneumonia, 1x multiple trauma, 1x cardiopulmonary bypass, 1x acute pancreatitis, and 1x circulatory overload. Four patients were already in the intensive care unit (ICU); 76% (34/45) of the remaining patients were transferred to the ICU. Ten (20%) TRALI patients died; in seven patients the TRALI reaction contributed significantly to the patients' death. In the other three cases it remained obscure whether TRALI was a contributing factor in the cause of death.

Associated blood products

Table 3 lists the characteristics of the involved blood products and donors involved in the 49 cases. The patients received a mean of 4.2 blood products in the 6-hour period before the beginning of the TRALI reaction (median, 3; range 1-17). In 30 of 49 (61%) cases, high-volume plasma products (FFP and/or PLTs) were involved; in 39% of the cases the patient only received RBCs. The mean age of the transfused RBCs and PLTs was 17 (range, 6-34; median, 17) and 4.7 (range, 2-7; median, 5) days, respectively.

Table 3. Involved blood products and donors

Involved blood products	**Cases**
RBCs	19 (39%)
FFP	3 (6%)
PLTs in plasma	2 (4%)
RBCs plus FFP	17 (35%)
RBCs plus PLTs in plasma	4 (8%)
RBCs plus FFP plus PLTs (PAS/plasma)	4 (8%)
Number of blood products	
Mean	4.2
Median	3
Range	1-17
Involved donors	
Total number	259
Mean	5.3
Male : female	156 : 103
RBC number	129 (78 male : 51 female)
BC number	66 (41 male : 25 female)
FFP number	64 (37 male : 27 female)

Donors

The 49 patients received blood products from 259 donors, with a mean number of donors of 5.3 per case (median, 4; range, 1-17; Table 3). The male : female ratio was 157: 102 (61%: 39%) and represented the ratio of blood products stored at the blood bank facilities. The mean age of the donors at time of donation of the involved blood product was 46.9 years (range, 18-69; median, 49 years). Parity information was provided for 94 female donors. Of these 94 donors, 70 donors have been pregnant at least one time (mean, 2.6 pregnancies; median, 2; range, 1-7) and 24 donors have never been pregnant. Seven donors (five women and two men) had a past history of blood transfusion. Two female plasma donors were two times involved in a TRALI case.

Laboratory test results

Patients

Forty-three patients were tested for HLA- and granulocyte-reactive antibodies; 18 (42%) tested positive, of which 11 were female and seven were male. In six cases only patient antibodies were found; in the other 12 cases at least one of the involved donors also tested positive for antibodies.

In nine cases HLA Class I and in two cases a combination of HLA Class I and II antibodies were found. HNA antibodies were found in two patients and five patients had nonspecific WBC-reactive antibodies (positive screening in the LIFT and/or CDC).

Donors

In 36 cases antibodies were detected in at least one of the involved donors (Table 4). In 24 of these cases, the antibodies were found in the donors of the transfused blood

Table 4. Antibody and incompatibility testing in involved donors and products

Donor antibodies	Cases with donor antibodies	Antibodies in donors Number	Male	Female	Involved products from male donors	Involved products from female donors
Incompatible	21	31*	6	25	4 RBCs 2 FFP	10 RBCs 3 BCs 12 FFP
Compatible	10	13*	6	7	5 RBCs 1 FFP	2 RBCs 3 BCs 2 FFP
Compatibility not tested	5	11*	6	5	4 RBCs 2 FFP	2 RBCs 1 BCs 2 FFP
All donors	36	55*	18	37	13 RBCs 5 FFP	14 RBCs 7 BCs 16 FFP

* In some cases antibodies were found in more than one donor

product only; in the other 12 cases antibodies were found in both the donor(s) and the recipient. The specificities of donor antibodies are listed in Table 5.

Table 5. Specificity of donor antibodies (36 cases)

	Incompatible	Compatible	Incompatibility not tested	Total
HLA Class I antibodies	4	5	2	11
HLA Class II	3	1	0	4
HLA Class I and II	9	1	2	12
HLA Class I and HNA	3	0	1	4
HNA	1	0	0	1
IgM	1	3	0	4
Total	21	10	5	36

Incompatibility testing

In 31 cases of the 36 cases with donor antibodies, compatibility testing was performed. In the remaining five cases blood samples for performing cross-matches or DNA typing were not obtained, because either the patient had died or the patient was not traceable anymore. Incompatible donor antibodies were present in 21 (68%) of these cases (Table 4). In two additional cases (with no detectable donor antibodies) patient antibodies were found incompatible with at least one of the involved donors (data not shown).

In 15 cases there was one implicated donor; in, respectively, three, two, and one case(s) there were two, three, and four donors with incompatible antibodies. In all 21 cases with proven incompatible donor antibodies, a total of 31 donors were involved, of which 25 (81%) were female. Fourteen of the 31 products with incompatible donor antibodies appeared to be FFP. Of these, 12 (86%) were from female donors (Table 4). The incompatibility rate was higher in TRALI without risk factors for ALI, compared to TRALI in presence of a risk factor (possible TRALI), and appeared statistically significant (58%% vs. 18%, p= 0,036; Table 6). In cases in which only RBCs were used, the incompat-

Table 6. Antibody and incompatibility testing in cases

Cases	Total	Possible TRALI	TRALI	RBC only	Plus FFP	Plus PLT
All cases	49	11	38	19	24	6
Cases with donor antibodies	36	7	29	10	23	3
Compatibility tested (in presence of donor antibodies)	31	7	24	8	21	2
Proven incompatibility	21	2*	19*	3	18	0
In presence of patient antibodies	10	2	8			

* statistically significant: p=0.036

ibility rate was also significantly lower than in cases with high-volume plasma products (3/17=18% vs. 18/22=82%, p<0.0005; Table 6).

DISCUSSION

The name TRALI was first coined by Popovsky and coworkers in 1983.[12] In 1985 they published the first series of 36 TRALI cases from the Mayo Clinic.[5] The diagnosis of TRALI is based on clinical and radiographic findings, because there is no pathognomonic laboratory test for TRALI. In 2004 the Canadian Consensus Conference defined TRALI[2,7] as a new episode of ALI that occurs during or within 6 hours of a blood transfusion and is not temporally related to another risk factor for ALI. ALI is defined as the acute onset of respiratory distress and hypoxemia (PaO_2-to-FiO_2 ratio of less than 300 mm Hg, saturation less than 90% on room air or other clinical evidence), bilateral infiltrates on chest X-ray, and no evidence of circulatory overload.[6] When ALI is temporally related to both transfusion and an alternative risk factor the term "possible TRALI" is used. Here, we describe all and only TRALI cases fulfilling these consensus criteria that were reported to the Sanquin Blood Banks during a 2.5 year period. Nationwide 49 TRALI reports were thoroughly studied. During the study period, an increase in reports was noticed, from six in the first half of 2005 to 12 reports in the first half of 2007, indicating a greater awareness of TRALI.

The incidence of TRALI reported in the literature ranges from 1 in 1120[6] to 1 in 5000[5] for all blood components and up to 1 in 432 per unit of platelets[6]. In 2006 a total of 700,758 blood products (556,509 RBC, 51,869 PLT and 92,380 FFP units) were issued by the Sanquin Blood Banks to Dutch hospitals. In that year, 24 TRALI reactions were reported, giving an estimated frequency in the Netherlands of approximately 1 in 29,000 (0,003%), which is lower than what was expected from the literature. This difference might be explained by the use of different definitions for TRALI between studies. We have stringently held on to the Canadian Consensus Conference criteria, which require, among other things, a chest X-ray. Milder TRALI cases, in which no chest radiographs were made, might have been missed or excluded in this way.

The types of blood products used in different countries are not always comparable and may not hold the same risk for TRALI. Leukoreduced (RBC, FFP, and PLTs) and plasma-poor (all RBC and 50% of PLTs) blood products are used in the Netherlands. Possibly these products hold a lower risk for TRALI. Also, it should be kept in mind that this observational study was not designed for true incidence measurement. During this study awareness of TRALI increased giving a rise in reported cases, but there probably still is underreporting, especially in circumstances in which an alternative risk factor for ALI was present. Presumably TRALI is also mistakenly taken for fluid overload in some cases.

TRALI is a serious event; in this study 78% of the patients needed monitoring and/or mechanical ventilation in the ICU, and 10 (20%) patients died. These high morbidity and mortality rates indicate that TRALI is the number one severe transfusion reaction in the Netherlands and the leading cause of transfusion-related deaths. The mortality rate of TRALI patients has been reported as 5-10%.[5,13] The strict adherence to the Canadian Consensus Conference criteria, which might apply to only the more severe and life-threatening cases of TRALI, and not to the milder, less fatal cases, might possibly explain the higher mortality in our study.

The pathogenesis of TRALI is not fully elucidated yet. The majority of cases of TRALI are thought to be caused by WBC-reactive antibodies present in the blood product, mainly found in (multiparous) female donors. Antibodies against HLA and neutrophil antigens were shown to bind to their cognate antigens on neutrophils, causing activation of these neutrophils, which then sequestrate in the lungs, causing endothelial damage, capillary leakage, and finally pulmonary edema.[3] The relevance of these antibodies in the pathogenesis of TRALI was shown in an ex vivo rabbit model and an ex vivo rat lung model.[14,15] However, only a few studies attempted to correlate antibodies in the donor with antigens in the patient. They demonstrated incompatibility in 59% to 87.5%[5,16-18]. Two of these studies had only small numbers of TRALI cases. [16,17] Reported incidences of WBC-reactive antibodies in TRALI cases (in donor and/or patient) ranged from 25% to 89%.[5,6,13,19] The prevalence of HLA antibodies in the donor population is 2.3% for males and 17% for females.[20,21] Using these prevalences, we calculated a 37% a priori chance of a random patient in our series to receive at least one blood product from a donor with HLA antibodies, assuming the patient received blood from 5.3 donors with an equal male-to-female ratio to that found in the TRALI cases. In fact, we found WBC-reactive antibodies (mainly HLA antibodies) in at least one of the involved donors in 73% (36/49) of the TRALI cases.

Although we also tested TRALI patients for the presence of WBC-reactive alloantibodies, their significance in the pathophysiology remains to be elucidated. In two cases, in which donor antibodies were not detected, patient alloantibodies were shown to be incompatible with at least one of the involved donors. The first of these patients received 2 RBC units, and the other 1 RBC unit and 1 PLT concentrate. All blood products were leukoreduced. In leukoreduced blood products fewer than $1x10^6$ WBCs are present. The minimum amount of neutrophils required to be activated to cause sufficient oxidative injury to lung endothelium is not known. Whether or not leukoreduction might have failed in one of the involved blood products or a possible causative role for soluble HLA antigens present in the blood products remain speculative.

In 36 cases antibodies were found in one or more of the involved donors. Incompatibility testing was performed in 31 of 36 cases and the presence of incompatible antibodies was proven in 21 cases, either by positive cross-match or by identifying the

cognate antigen by genotyping. In our series a total incompatibility rate of 48% of cases (21/44, five cases not tested) was found. In an additional 10 cases (23%) compatible donor antibodies were found.

In 12 of 21 of the TRALI cases with proven incompatibility (alloimmune TRALI), HLA Class II antibodies, either alone or in combination with HLA Class I antibodies, were detected, suggesting a more prominent role of donor HLA Class II antibodies in the pathogenesis (Table 5). It has been shown that resting neutrophils do not express HLA Class II antigens and that activation of neutrophils is mediated via monocytes, which are rich in HLA Class II antigens.[16] Although the role of antibodies against HLA Class I antigens in neutrophil activation has been established, a dilutional effect is expected, as HLA antigens are also expressed on other cells.

Another pathophysiological hypothesis is the two-event model. The first event is related to the underlying clinical condition of the patient (e.g., infection or surgery) that causes activation of the pulmonary endothelium, leading to the sequestration and priming of neutrophils to the activated endothelium. Transfusion is the second event: transfused WBC-reactive antibodies, biologically active lipids (lysophosphatidylcholines), and/or other biological response modifiers (e.g., soluble CD40 ligand) activate the primed neutrophils, causing endothelial damage and subsequently TRALI.[4,22] These lipids and biological response modifiers accumulate during storage of blood products, suggesting that older blood products are more prone to cause TRALI.[23,24] In 13 of our cases we did not find WBC-reactive antibodies in donors and in 10 other cases compatible donor antibodies were found, together representing 52% of cases (23/44, five not tested). Biologically active lipids or other biological response modifiers might have caused these TRALI reactions. Differences in product storage times between the alloimmune and nonalloimmune TRALI cases were not found (data not shown). However, because most of the patients received more than 1 product (mean, 4.2 products), the causal products could not be identified, which leaves this question unresolved.

Clinically, two distinct groups were identified: the TRALI group with no alternative risk factor for ALI and the possible TRALI group with another risk factor for ALI present. In the TRALI group incompatibility was shown in 19 of 33 tested cases (58%). In the possible TRALI group, incompatible antibodies were found in 2/11 (18%) cases (Table 6). The difference between these two groups was significant (p=0.036). It may be argued that some cases of possible TRALI are actually not transfusion related but are ALI caused by another risk factor, for example, sepsis or pneumonia. It is also possible that indeed a different, non-immune mechanism underlies the pathogenesis of these possible TRALI cases, as was postulated in the two-event hypothesis.

Since July 2007, as a precautionary measure against TRALI, only apheresis plasma from nontransfused male donors is used for single-donor FFP in the Netherlands. In 19 of 49 TRALI cases in this study, at least one of the blood products was a female FFP. In 12 of

these cases incompatibility was proven, indicating that, in retrospect, 12 of 21 (57%) alloimmune TRALI cases might theoretically have been prevented with this precautionary measure.

Of the 10 patients who died, seven patients received only RBCs and three patients were transfused with female FFPs. All three female FFPs contained antibodies: one proven incompatible, one compatible, and one not tested. Therefore, at least one death (and possibly two; the not tested involved FFP contained a wide variety of antibodies against HLA Class I antigens) might theoretically have been avoided, if the preventive measure had already been effective. If the preventive measure would also be applicable to female-derived BCs used for pooled PLTs, a third case of a TRALI -associated death might have been prevented. In this case, two female BCs contained incompatible antibodies, against HLA I /II and HLA I/II and HNA, respectively.

The next few years will prove whether this male-only FFP measure will actually give a decline in TRALI cases. SHOT reports indicated a decrease in TRALI cases after the introduction of the use of predominantly male plasma for transfusion. However, in the SHOT classification of TRALI, nonalloimmune cases are regarded as unlikely and are not taken into account.

In conclusion, TRALI is the most serious transfusion complication in the Netherlands, causing severe morbidity and mortality. In 73% of the 49 cases fulfilling the Canadian Consensus Conference criteria, WBC-reactive donor antibodies were found. Incompatibility was proven in 48% of the cases. The antibodies most frequently found were HLA Class I and/or II antibodies. Female FFP was the most frequently implicated blood product. Fifty-seven percent of the alloimmune TRALI cases might theoretically have been prevented with the male-only FFP measure. We aim to investigate prospectively the effect of this precautionary measure, irrespective of the presence of WBC-reactive antibodies, in the next year after the introduction.

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3

Female donors and transfusion-related acute lung injury

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

Rutger A Middelburg, Daniëlle van Stein, Barbara Zupanska, Małgorzata Uhrynowska, Ognjen Gajic, Eduardo Muñiz-Diaz, Nuria Nogués Galvez, Christopher C Silliman, Tom Krusius, Jonathan Wallis, Jan P Vandenbroucke, Ernest Briët and Johanna G van der Bom

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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 alloexposures 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:

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

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.

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).

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

	Red cells		Plasma		Platelets	
	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.

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).

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

	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]

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 3. Percentage of TRALI cases preventable by the exclusion of female donors

	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

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

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.

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).

4

Alloexposed blood donors and transfusion-related acute lung injury: a case-referent study

Rutger A. Middelburg, Daniëlle van Stein, Femke Atsma, Johanna C. Wiersum-Osselton, Leendert Porcelijn, Erik A.M. Beckers, Ernest Briët, and Johanna G. van der Bom

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ABSTRACT

Background

Donor white blood cell (WBC) antibodies are thought to increase the risk of transfusion-related acute lung injury (TRALI). WBC antibodies can be present in blood products from donors who have been allo exposed. Alloexposed donors are increasingly excluded from donating plasma, but can still donate plasma-poor products. We aimed to quantify the contribution of alloexposed donors to the occurrence of TRALI for different blood product types.

Study design and methods

We performed a case-referent study including all reported TRALI patients and all Dutch blood donors. Data on alloexposure 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

Alloexposure 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 alloexposed 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 [RBCs]), alloexposure 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%).

Conclusions

Alloexposed donors conferred an increased risk of TRALI in recipients of plasma-rich products, but not in recipients of plasma-poor products. Although WBC antibodies are an important risk factor for TRALI, amongst RBC 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 6 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 white blood cell (WBC) antibodies arise from exposure of the immune system to allogeneic cells and tissues (alloexposure).[14,15] This alloexposure 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 WBC antibodies.[14-19] The prevalence of these antibodies increases from below 5% in subjects without known alloexposure to 10-15% after blood transfusions or a single pregnancy to well over 30% after three or more pregnancies.[14-19] Alloexposed 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 [PLT] 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, transfu-

sion history, and presence of WBC 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 WBC antibody containing plasma is necessary to cause TRALI.[28] If the small amount of plasma present in red blood cells (RBCs) is sufficient, this could have the obvious implication of excluding alloexposed donors from all forms of blood donation. On this subject, only anecdotal evidence exist to date and further investigation of differences between product types is 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 alloexposed 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.

METHODS

Study design

Ethical approval was granted by 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 to October 2008. For each included TRALI patient donors of transfused blood components were identified and their alloexposure status was determined (see appendix for details). Donors were considered alloexposed 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.

Alloexposure status of donors associated with TRALI patients was compared to the alloexposure status of a reference group of donors (see appendix 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 alloexposure status of the donors of each TRALI patient was matched to the alloexposure status that would have been expected, based on the alloexposure status of the reference group (as described below).

TRALI patients: definition, reporting, and 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 6 hours after completion of a blood transfusion.[1,2] In accordance with the consensus definition, a distinction was also 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 hemovigilance office in the Netherlands). Reports from hospitals are made by either the hospital's hemovigilance 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 alloexposure 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.

Blood products

Transfused blood products were classified as either plasma-poor or plasma-rich. PLT 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 RBCs, the PLTs from donors supplying only PLTs (i.e. not plasma) for a pooled PLT product (i.e. including four of every five donors for PLTs in plasma and all donors for PLTs in PLT 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 PLTs (and plasma) from the donor supplying both PLTs and plasma for pooled PLTs in plasma. Although apheresis PLTs are a standard product for some indications in The Netherlands, their use is relatively rare and no TRALI cases occurring after the transfusion of apheresis PLTs were therefore included in any analyses.

Plasma measure

Since October 1, 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 1, 2006, was reported. Therefore, all TRALI cases occurring since September 2007 are considered "postplasma measure". For the primary analyses only TRALI cases occurring before September 2007 are included, since after the plasma measure it was not possible for plasma to be donated by an alloexposed donor. However, the subgroup 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 to October 2008.

Statistical analyses

We aimed to estimate the contribution of alloexposed 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 alloexposed 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 alloexposed 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 alloexposed donor. The difference of this number from the number of TRALI patients expected to be caused by alloexposed donors was considered the excess number of TRALI patients caused by alloexposure of donors. The maximum excess number was the total number of TRALI patients minus the number expected to be caused by alloexposed donors. Dividing the excess number by the maximum excess number gives the PAR (the fraction of TRALI cases that could have been prevented by the exclusion of all alloexposed donors).

We first performed these analyses for all TRALI patients, giving an estimate of the effect of exclusion of all alloexposed 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 to 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.

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.

From September 2007 to October 2008, an additional 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 2: PAR of alloexposed donors, according to product types*

		Number in analyses			
		Patients†	Donors	PAR (%) 95% CI	
Overall‡		50	267	51%	(14% to 88%)
Plasma-poor‡	Before measure§	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%)

* A negative PAR value can only be interpreted as indicative of some protective effect, but not of any size of that effect
† Includes all patients until September 2007, except for the third row where, as indicated, patients were included until October 2008.
‡ Overall: all reported TRALI patients. Plasma-poor: patients receiving only products containing less than 40 mL plasma per donor. Plasma-rich: patients receiving only products containing more than 200 mL plasma per donor. Mixed: patients receiving both plasma-rich and plasma-poor products.
§ Before 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.

Alloexposure in reference subjects

A final number of 1040 donors with known alloexposure status was used to determine the expected alloexposure 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 alloexposed donors 354 (98%) were female. Alloexposed donors constituted 68% of all female donors.

The mean number of donations in 2007 was 2.67 for male donors and 1.84 for female donors. Alloexposure status was not associated with the number of donations. It was 1.86 for non alloexposed female donors and 1.84 for alloexposed female donors.

Alloexposed donors and TRALI risk

The expected percentage of alloexposed donors among 267 donors involved in 50 TRALI cases was 27% (standardized for products types donated and year of donation); 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 alloexposed donors (PAR). The overall PAR of receiving a transfusion from an alloexposed donor was 51% (95% confidence interval [CI], 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 alloexposed donor was 94% (34% to 100%; Table 2). In 19 patients who had received only plasma-poor products (mostly RBCs) 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 (Fig. 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%).

DISCUSSION

Among recipients of only plasma-poor blood products alloexposure of donors was not associated with an increased risk of TRALI. However, among recipients of both plasma-rich and plasma-poor blood products alloexposure of the donors was a major risk factor for TRALI. This suggests first that alloexposure of the donor is an important risk factor for TRALI when plasma-rich components are transfused and second that the plasma-rich

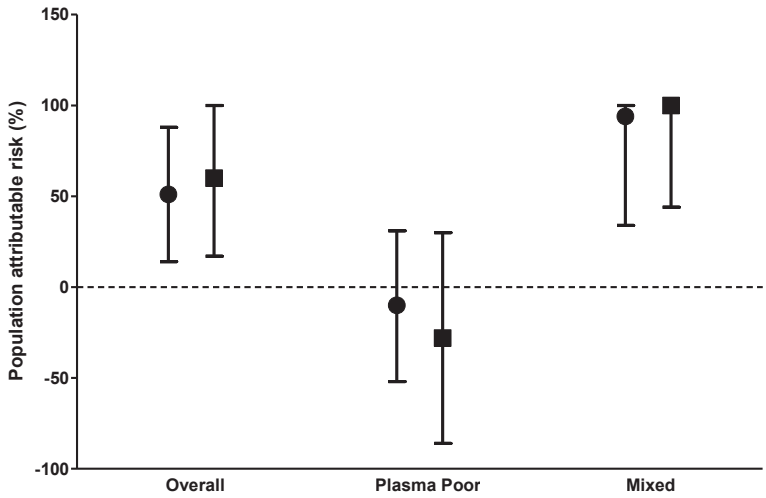

Figure 1: Population attributable risk of allo-exposed donors for all 50 TRALI patients and for 39 TRALI patients, excluding all possible TRALI. (●) The PAR for all 50 TRALI patients; (■) the PAR for 39 TRALI patients after excluding all cases of possible TRALI. "Overall" indicates the estimate for all patients, regardless of product mix. "Plasma-poor" indicates the estimate for patients receiving only plasma-poor products (<40 mL plasma per donor). "Mixed" indicates 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% Cis. 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.

products are more likely to have caused the TRALI in recipients of both plasma-rich and plasma-poor products.

We used alloexposure of the donors as a marker for the increased prevalence of WBC antibodies. Although only a minority of alloexposed donors actually develops WBC antibodies, nearly all WBC antibodies are found in alloexposed donors. We assumed that there is no other reason, besides WBC antibodies, why alloexposed donors can increase the risk of TRALI. It can then be shown that the percentage of TRALI cases preventable by the exclusion of alloexposed donors equals the percentage preventable by the exclusion of all donors with WBC antibodies. This can be understood since, first, exclusion of alloexposed donors also excludes donors with WBC antibodies and therefore prevents the cases caused by those antibodies. Second, we do not expect it to prevent any other cases than those caused by WBC antibodies since alloexposed donors are not known to cause TRALI through any other mechanisms (i.e., other alloexposure-related, but antibody-independent mechanisms). This is one of the major advantages of using the PAR as the effect estimate, since it removes the need to actually determine the presence or specificity of WBC antibodies.

Beyond the distinction between TRALI and possible TRALI, we ignored all other data on potential patient risk factors. All diseases can be considered multicausal 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 patient's predisposition for developing TRALI is usually not readily influenced.

An assumption necessary for the used analyses to be valid is that the calculated probability of receiving a transfusion from an alloexposed 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 of a nonselected, random sample of all actively donating donors. We have no reason to assume that in the few years of our study the average alloexposure 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 alloexposed donors, any association seen in the patients receiving both must be due to plasma-rich products. Since the PAR 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 PAR 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 or 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 WBC antibodies or alloexposed donors, this could cause bias in the conclusions that TRALI after plasma-poor products is not associated with alloexposed donors and that TRALI after plasma-rich products is. However, since this information was not available to the reporting physicians, this reporting bias cannot be a problem in our study.

Our results indicate that half of all TRALI cases may be preventable by the exclusion of all alloexposed donors, which is in close agreement with a previous estimate of the PAR of female donors.[26] Furthermore, our findings confirm that alloexposure 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 Serious Hazards Of Transfusion (SHOT) report.[25] Of 17 TRALI cases described in this report, 11 involved the transfusion of RBCs (six received only RBCs). In none of the ten completely investigated cases were concordant WBC antibodies found in donors of the transfused RBCs. For all five cases in which WBC antibodies were identified, the implicated products were either fresh frozen plasma (three cases) or PLTs (two cases).[25] In this study we did not observe a sharp decrease in the total annual number of reported TRALI cases after the plasma measure. However, as previously described by Wiersum-Osselton and colleagues[30] during the same period in The Netherlands we did see the previous trend of strong increase level off. Simultaneously a shift was observed from plasma-rich towards plasma poor-products.[30]

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 mean of effects exerted through plasma-poor and plasma-rich products. Changes therefore reflect the weighted mean 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 alloexposed donors, which are used as a proxy for WBC antibodies. However, this association was only observed for plasma-rich products. Alloexposed donors are almost exclusively female and female donors are increasingly being excluded from donation of plasma-rich products. As shown recently, deferral of all donors reporting a history of alloexposure would effectively decrease the prevalence of WBC antibodies among donors to a similar extend as excluding all female and transfused male donors.[31] In the Dutch situation this would cause a total deferral of 25% of all donors.[31] Irrespective of the exact deferral strategy chosen, most blood banks in developed countries now have a deferral strategy aimed at the exclusion of WBC antibodies in place. Therefore, the contribution of plasma-rich products from alloexposed donors to the occurrence of TRALI is dwindling and the relative importance of RBC transfusions is steadily growing.

Already nearly half of the reported TRALI cases in this study were caused by RBCs alone. At present little is known about risk factors for TRALI related to RBCs. With the growing contribution of RBCs to the occurrence of TRALI these risk factors need to be identified. In this context biological response modifiers seem the most likely candidate.[13,32,33] Most of these small-molecule, inflammatory mediators are known to accumulate in cellular

blood products during storage.[13,33] However, a clear association of TRALI with storage time has not yet been demonstrated.

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APPENDIX

Donors of TRALI patients: identification, alloexposure 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 alloexposure 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 alloexposed if they reported either one or more pregnancies, one or more blood transfusions, or both.

For donors for whom the alloexposure 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 alloexposure of all donors for that patient will be underestimated. In the second case the total alloexposure 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: alloexposure status

Male and female donors have different alloexposure 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 alloexposure 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 alloexposure among male donors. The fraction of donations from female alloexposed donors was calculated

in the same way and these two fractions were added to estimate the total fraction of donations from alloexposed 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). Alloexposure 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

alloexposure status among donors donating products transfused to TRALI patients who only received products from routine donations.

Sanquin records were used to link the alloexposure status of each individual donor to the donation frequency of that donor. Numbers of donations from alloexposed donors were calculated by multiplying the numbers of alloexposed donors by their donation frequency. The fractions of products from alloexposed 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).

5

Underdiagnosing of antibody mediated transfusion-related acute lung injury: evaluation of cellular-based versus bead-based techniques

Daniëlle van Stein, Erik A.M. Beckers, Anna L. Peters, Leendert Porcelijn, Rutger A. Middelburg, Neubury M. Lardy, Dick J. van Rhenen, and Alexander P.J. Vlaar

Submitted

ABSTRACT

Background

Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion-related mortality. To support the diagnosis of antibody mediated TRALI, HLA and HNA antibodies are tested in involved blood donors. Identification of antibody positive donors is important as exclusion of these donors is part of preventative strategies against TRALI. We compared cellular-based versus bead-based techniques for diagnosis of antibody mediated TRALI.

Study design and methods

All reported TRALI-cases in the Netherlands during a 5-year period were evaluated. Donors were screened for the presence of HLA class I and II antibodies using both cellular-based and bead-based techniques.

Results

In total 100 TRALI-cases were reported of which 91 were fully tested. In 113 donors HLA antibodies were detected of which 84 were only detected by bead-based techniques, 12 only by cellular-based tests and 17 by both assays. Antibody mediated TRALI was diagnosed in 44/91 of reported cases. Twenty-one (48%) of these cases would not have been identified using only cellular-based assays.

Conclusion

Bead-based techniques show a higher sensitivity for detecting incompatible donors in TRALI-cases than cellular-based assays. These results suggest that the use of bead-based assays will result in a significant reduction of future TRALI reactions as more antibody positive donors will be excluded from future donations.

INTRODUCTION

Transfusion-related acute lung injury (TRALI) is a serious, sometimes fatal complication of blood transfusion. It is the leading cause of transfusion related morbidity and mortality.[1] TRALI is defined according to the Canadian Consensus Conference (CCC) criteria (Table 1) as the acute onset of hypoxemia ($paO_2/FiO_2 \leq 300$ mmHg or saturation <90% on room air) with new or worsening bilateral infiltrates on the chest radiograph, occurring during or within 6 hours of a blood transfusion in the absence of circulatory overload.[2-5] Although in recent years the awareness of TRALI has been increasing, it is still misdiagnosed and underreported.[6-8]

Table 1. Definition transfusion-related acute lung injury (TRALI) according to the Canadian TRALI Consensus conference and US National Heart, Lung and Blood Institute Definition.[2-5]

Suspected TRALI	Acute onset within 6 hours of blood transfusion PaO2 /FI O2 <300 mm Hg, or worsening of P to F ratio Bilateral infiltrative changes on chest radiograph No sign of hydrostatic pulmonary edema (pulmonary arterial occlusion pressure ≤18 mm Hg or central venous pressure ≤15 mm Hg) No other risk factor for acute lung injury
Possible TRALI	Same as for suspected TRALI, but another risk factor present for acute lung injury

TRALI has been attributed to two mechanisms of onset, the antibody and non-antibody mediated TRALI.[9] Antibody-mediated TRALI is caused by incompatibility between donor white blood cell (WBC) antibodies (human leukocyte antigens (HLA) class I or II or human neutrophil antigens) and patients' white blood cells.[10,11] Binding of these antibodies to their cognate antigens causes endothelial and neutrophil activation. These activated neutrophils sequester in the lungs, causing endothelial damage, capillary leakage and pulmonary edema. Non-antibody mediated TRALI is thought to be caused by pro-inflammatory mediators (e.g. biologically active lipids, soluble CD40 ligand) which accumulate during storage of cell-containing blood products.[12-14] Older blood products would therefore be more prone to cause TRALI.

TRALI diagnosis is based on clinical and radiographic findings; there is no pathognomonic laboratory test to confirm the diagnosis. To identify antibody-mediated TRALI the clinical diagnosis is supported with laboratory investigations. All donors from blood products transfused to the patient within a period of 6 hours before the start of the TRALI symptoms are tested for HLA class I and II antibodies and HNA-antibodies. To establish incompatibility the patient is typed for HNA and HLA antigens and cross-matches between donor serum and patient leukocytes are performed. Donors are assigned incompatible and possibly causal if there is a positive cross-match or if specific antibodies against cognate antigens are found. Reporting and identifying possible causal donors

of TRALI cases are keystones in the prevention of future TRALI cases as the implicated donors are excluded from future donations. However, as it can take several weeks before the results are known, they have no value in diagnosing an acute TRALI-reaction. Until recently the standard methods to detect white blood cell antibodies were cellular-based. These methods are very time-consuming and are dependent on highly skilled staff for obtaining reliable results. The proficiency to detect antibodies varies from 16.7 to 100% between different laboratories[15] and recently it was reported that detection of HNA-antibodies with bead-based assays is more reliable than detection with cellular-based assays.[16,17]

To investigate whether the use of bead-based techniques might improve the diagnosis of antibody mediated TRALI, we compared cellular-based techniques to bead-based techniques for detection of HLA antibodies in a series of 100 consecutively reported TRALI cases.

MATERIALS AND METHODS

Patient study population

All suspected cases of TRALI in the Netherlands reported to the Sanquin Blood Bank (the national blood supply organization) during a 5-year period (January 2005 till January 2010) were evaluated as previously published.[18] TRALI was diagnosed by the hospital medical personnel and subsequently reported to one of the transfusion consultants of the blood bank. All cases were additionally reviewed by physicians familiar with TRALI. Only cases fully meeting the consensus criteria for TRALI from the Canadian Consensus Conference (Table 1) were included and investigated for patient, donor and product characteristics. Data was analyzed by DvS and EB. Patient characteristics collected included age, sex, admitting diagnosis (surgical vs non-surgical), and transfused blood components. All donors of blood products transfused within 6 hours before the development of TRALI were investigated, and donor variables reviewed included sex, age, parity, blood transfusions in past, and results of antibody testing. A donor involved in a TRALI case is designated as implicated when incompatibility was proven between donor antibodies and patient cells. When antibodies are not incompatible with patient antigens or in absence of these antibodies, the donor is flagged as associated with a TRALI case. Donors flagged two times as associated with a TRALI case are excluded from further donation in The Netherlands.

Blood products

The patients were transfused with red blood cells (RBCs), platelets (PLTs), or fresh-frozen plasma (FFP). The RBCs were suspended in saline-adenine-glucose-mannitol (SAGM) ad-

ditive solution (AS) and pre-storage leukoreduced (<1 × 10^6 white blood cells [WBCs]). The final product contained a mean volume of 20 mL of plasma, the mean total volume was 270 mL, and the mean hematocrit level was 0.57. The majority of PLT products transfused were pooled random-donor units (prepared from the buffy coats [BCs] of five donors). Pooled PLTs were pre-storage leukoreduced (<1 × 10^6 WBCs) and suspended in plasma or PLT AS (PAS II). The mean volume was 310 mL. Single-donor apheresis units were only used when HLA-compatible PLTs were needed. When indicated, RBCs and PLTs were washed or irradiated. FFP was derived from single-donor apheresis donations and contained fewer than 1 × 10^6 WBCs. Since July 2007 only apheresis plasma from non-transfused male donors was used for FFP. The plasma was quarantined for at least 6 months. The volume was approximately 325 mL. For all patients type of blood products transfused within 6 hours before the development of signs and symptoms were recorded.

Laboratory tests

WBC-reactive antibodies were examined in all patients (in post-transfusion blood samples and, if available, in pre-transfusion samples) and in all donors of blood components transfused to patients within 6 hours preceding the development of TRALI.

Complement-dependent cytotoxicity (CDC) assay

All patients and donors were screened for HLA Class I antibodies using a standard complement-dependent cytotoxicity (CDC) assay against a panel of HLA Class I-typed donor lymphocytes.[19] Serum to be tested was incubated with the lymphocytes and rabbit complement. If antibodies present in the serum bound to the cognate antigen on the lymphocyte, the complement cascade was activated, leading to cell death. By using fluorescent dyes, cell death was observed using microscopy and used as an indicator of the presence of HLA Class I antibodies. More than 20% cell death was considered as a positive reaction in the CDC technique. For the detection of HLA Class II antibodies a two-colored fluorescence test with a panel of HLA Class II-typed donor B-lymphocytes was used as described by van Rood et al.[20]

Bead-based screening assay

In addition, a flow cytometry bead-based screening assay for the presence of HLA Class I and II antibodies (FlowPRA, One Lambda, Inc., Canoga Park, CA) was used. In this assay 30 individual beads for both HLA Class I and II were combined. Each bead was coated with purified HLA Class I or Class II antigens. Serum was incubated with the beads and stained with a FITC labeled anti-human IgG antibody. Class I and II beads were distinguishable on a flow cytometer by their different fluorescent properties. The percentage of beads showing a positive fluorescence represented the percentage panel reactive antibodies

(PRA). For HLA Class I PRA over 12% was considered positive. For HLA Class II this was 4%. If samples were positive by CDC and/or flow cytometry screening assay, the specificity of HLA Class I and II antibodies was determined using single antigen beads on the Luminex platform (Labscreen SA, One Lambda, Inc.). In this assay all individual beads were coated with one HLA Class I or II antigen. A phycoerythrin (PE)-conjugated anti-human IgG antibody was added. The luminex platform could identify the color-coded beads and PE-fluorescence emission at the same time. A cutoff for a positive Luminex test of 1000 (normalized) mean fluorescence intensity was used. The cut off was based at a reference group of male non-transfused donors.

Lymphocyte immunofluorescence test (LIFT)

Lymphocyte-reactive antibodies were examined by the lymphocyte immunofluorescence test (LIFT) against two pools of five typed donor-lymphocyte suspensions each, according to Décary.[21]

HNA-antibodies

HNA-1a, -1b, -1c and -2a antibodies were examined by the granulocyte immunofluorescence test (GIFT) based on the method of Verheugt et al.[22] Granulocytes from donors of known HNA genotypes were incubated with serum from the involved donors and subsequently with FITC-labelled rabbit-anti-human IgG. The fluorescence observed under a fluorescence microscope was a measure for antibody activity. HNA-3a antibodies were examined by the granulocyte agglutination technique (GAT), according to Jiang and Lalezari.[23] The test serum was placed under oil in a microtiter tray. The typed granulocytes were added and the tray was incubated. The size of the aggregation and the proportion of cells participating were examined under an inverted phase microscope

Incompatibility testing

Incompatibility of antibodies found in the bead-based assays was tested by HLA genotyping the patients using a polymerase chain reaction-based assay with sequence-specific primers (Olerup SSP AB, Saltsjöbaden, Sweden) and/or sequence-specific oligonucleotides primers (One Lambda, Inc.) for HLA Class I and II. WBC incompatibility was also assessed by performing cross-matches between donor serum and patient's lymphocytes in the CDC and LIFT. Donors were assigned incompatible if either a positive cross-match or specific antibodies against cognate antigens in the patient were found.

RESULTS

TRALI-cases

In the 5-years period 100 cases of suspected TRALI reported to the Sanquin Blood Bank met all CCC criteria for TRALI. Of these 100 cases, 54 patients were males. Table 2 displays patient characteristics. Mean age of the patients was 55 years (range 2 to 89 years). Fifty patients had recent surgical treatment, 48 were classified as nonsurgical. In 2 cases the diagnosis at admittance was unknown. In 26 cases an alternative risk factor for ALI was present (possible TRALI according to CCC criteria for TRALI). Ninety-one of these cases were fully tested for donor antibodies and incompatibility (in the other 9 cases, either the donors could not be tested or there was no patient blood to test incompatibility, mainly because the patient had died). In the 91 fully tested cases a total of 451 blood products (RBCs, FFP, buffy coats or PLTs) were involved. The pooled PLTs were count for 5 blood products, because 5 buffy coats were used and subsequently 5 different donors were involved. The mean number of blood products transfused during the 6-hour period before the onset of TRALI was 4.96 (median 3, range 1-17). The overall male:female ratio of the donors was 1.8 (290:161). We found HLA antibodies in 113 donors (Table 3b) implicated in 62 of 91 TRALI cases. In 10 cases HNA-antibodies were detected. In 5 of these cases also HLA-antibodies could be found.

Table 2. Characteristics of patients implicated in TRALI reactions.

	Patients (n=100)
Male sex (%)	54
Age, years (mean and range)	55 (2 – 89)
Diagnosis at admittance (%)	
Medical	48
Surgical	50
Other/unknown	2
Survival (%)	79

CDC/LIFT versus bead-based techniques

Overall, in 62 (68%) of 91 fully tested TRALI cases donor antibodies were found in one or more donors using both CDC and bead-based techniques. The type of products transfused stratified for incompatible and compatible cases are presented in Table 3a. In the vast majority of cases HLA antibodies were detected solely by bead-based assays (Table 5). In 113 donors HLA antibodies were detected, 84 were detected by bead-based techniques; 17 by both cellular and bead-based assays. In 12 donors only cellular-based tests were positive, of which 7 had non-specific IgM (IgM antibodies without any identifiable HLA-specificity) in the CDC, 3 had aspecific LIFT reactivity and 2 had specific HLA

Table 3a. Products involved in antibody positive TRALI cases

	Cases	RBC	BC	FFP
Incompatible cases, (n)	44	- -	- -	- -
-Total products		131	98	76
-Products with antibodies		45	28	22
Compatible cases, (n)	18	- -	- -	- -
-Total products		38	25	15
-Products with antibodies		13	6	4

RBC= red blood cells, BC=buffy coat, FFP=fresh frozen plasma

Table 3b. HLA antibodies in products implicated in TRALI cases.

	Cases	Number of Donors		
		Male	Female	Total
Total donors involved	91	290	161	451
Total cases with antibodies	62	40	73	113
Incompatible cases	44			
Incompatible antibodies	—	16	43	59
Compatible antibodies	—	16	18	34
Compatible cases	18			
Antibodies	—	8	12	20

Table 4. General results of antibody testing in donors and patients

	Number of Cases (%)
Total cases reported	100
Total cases fully tested	91 (100)
Total donor antibodies	62 (68.1)
HLA-class I	58
HLA-class II	24
HLA-class I and HLA-class II	21
Patient and donor antibodies	26 (28.6)
Patient antibodies only	14 (15.4)
Incompatible cases	44 (48.4)
Compatible cases	18 (19.8)

antibodies found in the CDC. Positive results in the CDC or LIFT were mostly not informative of the specificity of the HLA antibodies. In 103 donors (male:female=34:69) specific HLA antibodies were identified, only 2 of these antibodies were found by cellular-based assays only. There were 58 donors with HLA-I antibodies, 24 with HLA-II antibodies and 21 with both HLA-I and II antibodies.

The use of bead-based techniques would have identified an additional 21 antibody-mediated TRALI-cases compared to CDC based techniques only.

Table 5. Results of HLA antibody detection in donor plasma by cellular and bead-based techniques. Cellular techniques include the CDC and LIFT.

	HLA-antibodies			
	Cellular techniques	Bead-based techniques	Both	Total
HLA-I	2	49	7	58
HLA-II	—	22	2	24
HLA-I and HLA-II	—	13	8	21
Other*	10	—	—	10
Total	12	84	17	113

*Other antibodies included 7 aspecific IgM CDC cases and 3 aspecific LIFT cases

DISCUSSION

This study is the first to provide evidence that the sole use of cellular-based TRALI diagnostic techniques leads to under identification of incompatible donors in TRALI cases, resulting in under diagnosing of antibody-mediated TRALI. The main findings of this study are; 1) Bead-based techniques results in improved diagnosing of incompatible donors in antibody mediated TRALI reactions compared to cellular-based assays. 2) Use of bead-based assays for detection of leukocyte antibodies in blood donors will likely result in a significant reduction of TRALI as more donors implicated in TRALI will be excluded for future donations.

The aim of the current study was to compare the cellular-based technique to the bead-based technique in identifying donors responsible for TRALI cases. Surprisingly, the bead-based techniques identified 58% (34/59) more incompatible donors in a TRALI reaction and 48% more antibody-mediated TRALI cases. It is tempting to provide an explanation. One explanation may be that the bead-based technique is not depending on the presence of a "first hit". In contrast, the cell-based method uses typed lymphocytes from healthy donors. These healthy donors do not suffer from an underlying condition, which is most often the case in the patient developing TRALI. Previous studies have identified that HLA class antigen up-regulation occurs in the presence of an underlying condition.[24,25] This is in line with the "two hit" hypothesis of TRALI in which the underlying condition of the patient is the "first hit" and results in priming of the pulmonary endothelium and neutrophils.[12] The transfusion is the "second hit" and results in activation of the neutrophils and subsequently to leakage of the pulmonary endothelium resulting in pulmonary edema, i.e. TRALI. In the absence of the "first hit" there is no onset of TRALI. Hence, a blood product may be tested positive in cellular based techniques only when

the immune system is activated which is not the case as cells are obtained from healthy donors. The above mentioned hypothesis of a "two hit" model for antibody mediated TRALI has been confirmed in pre-clinical studies. In a mice model of MHC-I mediated TRALI, TRALI only occurred in the presence of a "first hit" using LPS.[26] This was also confirmed in a rat model of antibody mediated TRALI using MHC-I antibodies.[27] Recently the mechanism has also been tested and confirmed for other antibodies involved in antibody mediated TRALI such as HNA.[28]

Next to improved accuracy, bead-based techniques may also result in cost reduction. Cellular-based techniques are very time consuming. The CDC test requires 16 man-hours. For the CDC test cell panels of 54 typed donor lymphocytes are necessary, which is costly to create and maintain these cell panels. A bead-based antibody screening assay takes only 4 hours, the kits used have lower costs compared to maintaining CDC test cell panels.

Although a recent meta-analysis showed that implementation of male-only donor strategies for high volume plasma products resulted up to a two third reduction of TRALI, accurate testing of involved donors in suspected TRALI cases remains a key stone in the further prevention of TRALI.[29] Male donors still have 1-7% presence of HLA/HNA antibodies.[30-32] Furthermore, RBC products still originate from both female and male donors. As only 10-20ml plasma is sufficient to induce antibody mediated TRALI,[33] it is expected that introducing of bead-based techniques will result in better identification of incompatible donors in antibody mediated TRALI and subsequent prevention of future TRALI cases. Another precautionary measure to prevent TRALI is screening blood donors for HLA and HNA antibodies.[34] Cellular-based tests are very labor-intensive and require highly skilled staff. For these reasons they are hardly appropriate for screening in blood donors. Bead-based assays are more suitable and possibly more effective for screening in large scale populations.

An important issue is that one could argue whether all incompatible antibodies found in the bead-based assay were causal for the TRALI reaction as we did not perform functional test of the incompatibility. Based on retrospective studies, HLA-class I antibodies account for 14.3–26.7% of TRALI cases, HLA-class II antibodies 0.0–46.7% and HNA antibodies for 16.7–28.6%.[35] However, the majority of antibody-containing blood products do not cause TRALI in patients and case reports have shown that the presence of incompatible antibodies does not always result in the onset of TRALI.[36-40] From this perspective, bead-based analysis may result in an overestimation of the causal relation for antibody positive donors in a TRALI case. However, in line with the threshold model it is unclear whether in, for example, critically ill patients, infusion of these incompatible antibodies would have resulted in onset of TRALI. Hence, we do not know what the threshold is for the onset of TRALI for a specific antibody and from this point of view all antibody

positive donors should be excluded. Indeed, drastic TRALI mitigation strategies which excluded all antibody positive donors have resulted in a two third reduction of TRALI.[41-43]

In conclusion, the use of bead-based techniques results in improved identification of incompatible donors in antibody-mediated TRALI compared to cellular-based assays. Our results suggest that the use of bead-based assays will result in a significant reduction of future TRALI reactions as more donors with HLA antibodies will be excluded of future donation.

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6

Low risk TRALI donor strategies and the impact on the onset of transfusion-related acute lung injury; a meta-analysis

*Daniëlle van Stein, *Marcella C. Müller, Jan M. Binnekade, Dick J. van Rhenen and Alexander P.J. Vlaar
*These authors contributed equally

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ABSTRACT

Background

Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion-related mortality. In the past decade blood banks have implemented low-risk TRALI donor strategies, including a male-only donor policy for plasma-containing blood products to prevent onset of TRALI. We performed a meta-analysis to determine whether use of low-risk TRALI donor strategies for plasma indeed reduces onset of TRALI.

Study design and Methods

We searched MEDLINE and Cochrane Central Register of Controlled Trials from January 1995 up to January 2013. Two reviewers independently extracted data on study characteristics, methods, and outcomes. Primary endpoint was onset of TRALI. Subgroup analyses were performed for patient populations prone to develop TRALI and general patient populations.

Results

Ten articles were included. Meta-analysis using a random-effects model taking into account all transfused products showed a significant reduction for the risk of TRALI after implementation of low-risk TRALI donor strategies (odds ratio [OR], 0.61; 95% confidence interval [CI], 0.42-0.88). Data from patient populations prone to develop TRALI showed a significant reduction of TRALI risk (OR, 0.51; 95%CI, 0.29-0.90), while data from general patient populations showed a similar non-significant trend (OR, 0.66; 95%CI, 0.40-1.09). Results were similar when taking only plasma products into account (OR, 0.62; 95% CI, 0.42-0.92).

Conclusion

The introduction of low-risk TRALI donor strategies for plasma-containing products results in a reduction of TRALI.

INTRODUCTION

Transfusion-related acute lung injury (TRALI) is a life-threatening complication of blood transfusion. It causes high morbidity and is the leading cause of transfusion-related mortality for the past 5 years in the United States.[1] TRALI is a clinical diagnosis and defined as a new episode of acute lung injury (ALI) occurring during or within 6 hours after a blood transfusion in the absence of hydrostatic edema.[2;3] TRALI can be the result of a single event (e.g. transfusion), although most TRALI cases are postulated to be a 'two-event' entity. The first event is related to the underlying condition of the patient (e.g., infection or surgery) that causes activation of the pulmonary endothelium, leading to the sequestration and priming of neutrophils in the lung. The second event is the transfusion of a blood product, which activates the primed neutrophils in the lung, causing endothelial damage, and subsequently TRALI.[4]

The transfusion factors can be divided into antibody- and non-antibody-mediated TRALI. Non-antibody-mediated TRALI is caused by transfusion of stored cell-containing blood products. Proinflammatory mediators that have accumulated during storage or the aged red blood cells (RBCs) and platelets (PLTs) themselves have been implicated in non-antibody-mediated TRALI.[4-7] Antibody-mediated TRALI is caused by passive infusion of antibodies, which causes neutrophil activation.[8] The latter form of TRALI is the most prevalent type [9;10] and will be subject of the current meta-analysis. Antibodies are present in plasma-containing blood products. Plasma from female donors has been particularly implicated in the pathogenesis of TRALI.[11-13] Donor leukocyte reactive antibodies (HLA class I and II and anti-granulocyte [HNA] antibodies) are mainly found in (multiparous) female donors. This might be explained by alloimmunization during pregnancies.[14] Therefore, plasma derived from male donors should less likely cause TRALI than plasma derived from women. In 2003, the National Blood Service in the United Kingdom was the first to implement a precautionary measure to reduce TRALI by the preferential use of plasma from male donors for transfusion.[15] Nowadays, more blood collection organizations have taken similar measures (table 1). Most of them use only, or preferentially, plasma from male donors for single-donor plasma. Some blood collection organizations also implemented a (predominantly) male-only donor strategy for other high-volume plasma products such as PLT concentrates.[17] However, the implementation of these low-risk TRALI donor strategies for plasma-containing blood products have serious consequences for the blood supply, in particular for the availability of AB plasma. So far, effects of these high-volume plasma donor policies have only been published in observational studies.[15;18-26]

To quantify the impact of low-risk TRALI donor strategies for plasma on the onset of TRALI, a meta-analysis of all trials on this topic since 1995 was performed.

Table 1 Overview of different low risk TRALI donor strategies[16]

Low risk TRALI donor strategies
Donor deferral based on antibody screening
Screening all donors for HLA and/or HNA antibodies
Screening previously alloexposed donors for HLA and/or HNA antibodies
Donor deferral based on history of pregnancy or history of transfusion
Deferral of all female donors

MATERIALS AND METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations were used for this meta-analysis.[27] We did not register our protocol.

Study Selection Criteria

Selection strategy was to identify and review all studies that met the following criteria: observational controlled trials of transfusion, plasma, TRALI, and ALI.

Search Strategy

To identify literature in electronic databases, MEDLINE from January 1, 1995, through January 1, 2013, was searched, by using the following medical subject heading (MeSH) terms: *blood transfusion, plasma, ARDS and ALI*. The following text words were used: TRALI and transfusion related acute lung injury. To identify observational studies, the MeSH terms *case-control study* and *retrospective study were added*.

The Cochrane Library (2012), which contains the CENTRAL Database of Controlled Trials, the Database of Abstracts of Review Effectiveness, and the Cochrane Database of Systematic Reviews, was also searched.

In addition, the *related articles* feature of PubMed, which identifies related articles by using a hierarchical search engine that is not solely based on MeSH headings, was used. This search was completed with articles selected by two of the authors (AV and MM). Although search was also for non–English language citations, subsequent article review involved only English-language publications.

Study Selection

After all citations based on our search strategy were identified, two of the authors (AV and MM) independently reviewed each abstract to confirm eligibility. Eligible studies evaluated use of low-risk TRALI donor strategies for plasma and onset of lung injury and mortality. Low-risk TRALI donor strategies were defined as predominantly or male-only donor policy for plasma products, plasma from donors screened for absence of HLA and/or HNA antibodies, and plasma from female donors without history of pregnancy. Both

controlled and observational trials were considered eligible. If an abstract was selected as eligible, the same authors independently reviewed the respective article, if available, to confirm that it met inclusion criteria. To resolve discrepancies, the two reviewers either had to reach consensus or use a third reviewer (JB). Interobserver agreement for study selection was determined by κ, with a value above 0.80 indicating good agreement.

Data Extraction

Using a predefined data collection form, data from the studies to describe patient characteristics, study methods, and study findings were extracted. All data were abstracted independently by each of the two primary reviewers and verified for accuracy by the third reviewer, again with discussion used to resolve differences among reviewers. Both primary reviewers were physicians with formal training in clinical epidemiology and biostatistics. Corresponding authors of included studies were requested by e-mail to provide missing data for the meta-analysis.

Data Synthesis and Analysis

Primary endpoint was the effect of the introduction of a low-risk TRALI donor strategy for plasma-containing products on occurrence of TRALI. The secondary endpoint was the effect of the introduction of a low-risk TRALI donor strategy for plasma-containing products on mortality among TRALI patients. Two subsets were analyzed: 1) the group of studies with data from local registries (patients prone to develop TRALI) and 2) the group of studies with data from nationwide registries (general patient population). As patients often receive different types of blood products, we analyzed the effect of TRALI risk reduction strategies for all transfused products (denominator, total number of transfusions; and numerator, all TRALI cases); in addition, we assessed the effects of TRALI risk reduction strategies on plasma-induced TRALI (denominator, total number of plasma transfusions; and numerator, plasma-induced TRALI cases).

The percentage of agreement before discussion among reviewers in study selection, study design, and data abstraction was measured. For data synthesis, evidence tables were constructed, to present data separately for the primary outcome variable, onset of TRALI per transfused blood product, and the secondary outcome variable, 30-day mortality. Furthermore, data were presented based on study population, for example, at-risk patients (intensive care unit and surgery) or general hospital or region population.

The study design was classified as a randomized clinical trial, cohort study (prospective, retrospective, or historical control), case-control study, or outcomes study (cross-sectional).

The methodologic quality of eligible studies was assessed using the modified Newcastle-Ottawa scale, a validated instrument designed to evaluate the quality of

observational studies in systematic reviews and meta-analyses.[28] The quality of cohort studies and case-control studies was assessed separately. Methodology was evaluated in three domains: selection of study population, comparability of study groups, and the quality of outcome assessment. The kappa (κ) statistic was used to assess inter-observer agreement on study quality and a value above 0.80 was defined as good agreement.[29]

Statistical analysis

The meta-analysis was performed with a random effects model.[30] A random-effects model, instead of a fixed-effects model, was chosen to take into account heterogeneity of the studies. The common study characteristics are the incidence of TRALI cases for the total number of blood products transfused. Effect sizes are expressed as odds ratio (OR) and their 95 % confidence intervals (CIs). The odds is the number of TRALI cases by the number of transfused products in a defined context. An OR lower than 1 expresses a protective effect of a low-risk TRALI donor strategy for plasma products to prevent onset of TRALI. An OR higher than 1 shows a protective effect against TRALI of transfused products in the period before the introduction of a male-only donor policy. Heterogeneity was expressed by the chi-square test. A cutoff p value of 0.1 was used to consider heterogeneity. A high p value suggests that the heterogeneity is insignificant. Analysis were performed with computer software (R: A language and environment for statistical computing. R Core Team, 2013, http://www.r-project.org/).[31]

RESULTS

Study selection

Our predefined search strategy identified 118 records after removal of duplicates. After titles were reviewed, 53 citations were excluded and an additional 28 were excluded based on the abstract. Of 37 full-text articles, 10 studies were considered eligible (Table 2) and 27 studies were excluded after detailed review. An overview of the search is presented in Fig. 1. Interobserver agreement for study selection was good ($\kappa = 0.84$).

Study characteristics

No randomized controlled trials were found on the effect of a low-risk TRALI donor strategy for plasma products on the onset of TRALI. Therefore, we only included observational studies. Of these, two studies were prospective.[22,23] The remaining eight studies had a retrospective design. Five studies were carried out within national registries using passive reporting of TRALI.[15,19-21,25] Four studies were carried out in various high-risk TRALI populations (e.g., intensive care and surgery patients) and in these studies TRALI was actively screened for.[22-24,26] One study was carried out in the general hospital

Table 2. Overview of studies.

Reference	Type of study and inclusion	Population	Country	Study year	Endpoint	Reported type of cases*	Plasma or/and PLTs	Percentage male donors after implementation	
								Plasma	PLTs
Wright[26]	retrospective active	surgery	UK	1998-2006	onset TRALI	Mixed cases	Male-only plasma and PLTs	>99%	>93%
Chapman[15]	retrospective passive	national	UK	1999-2006	onset TRALI	Non mixed cases	Predominantly male-only plasma and PLTs	80-90%	80-90% for BC-derived PLT pools
Vlaar[24]	retrospective active	ICU	Netherlands	2004-2007	onset TRALI	Mixed cases	Male-only plasma, PLT strategy not applicable	100%	NA
Eder[19]	retrospective passive	national	US	2008-2011	onset TRALI	Mixed cases	Predominantly male-only plasma, PLT strategy not applicable	>95%	>65% of apheresis PLTs
Wiersum[25]	retrospective passive	national	Netherlands	2002-2009	onset TRALI	Mixed cases	Male-only plasma, PLT strategy not applicable	100%	NA
Nakazawa[22]	prospective active	surgery	Japan	2008-2008	onset TRALI	Mixed cases	Male-only plasma, PLT strategy not applicable	100% †	NA
Lin[21]	retrospective passive	national	Canada	2001-2009	onset TRALI	Mixed cases	Predominantly male-only plasma and PLTs	86-100%	90-99%
Funk[20]	retrospective passive	national	Germany	2006-2010	onset TRALI	Mixed cases	Male-only plasma and plasma from female donors without history of pregnancy or without WBC antibodies. PLT strategy not applicable	NA	NA

Table 2. Overview of studies. (continued)

Reference	Type of study and inclusion	Population	Country	Study year	Endpoint	Reported type of cases*	Plasma or/and PLTs	Percentage male donors after implementation	
								Plasma	PLTs
Arinsburg[18]	retrospective passive	hospitals	US	2006-2008	onset TRALI	Non mixed cases	Predominantly male-only plasma, PLT strategy not applicable	95-100%	NA
Toy[23]	prospective active	hospitals	US	2006-2009	onset TRALI	Mixed cases	One center male-only plasma and predominantly male-only PLTs and one center male or never-pregnant female donors for plasma and PLTs	NA	NA

*for analysis non-mixed cases were converted to mixed cases in order to compare TRALI cases per total number of products transfused/distributed.
† during the study period (October 2007- January 2008)
Non mixed cases = a single product has been identified causing TRALI, mixed cases = all products within the 6 hours prior onset of TRALI have been taken into account.
BC= buffy coat; ICU= intensive care unit; NA= not applicable,

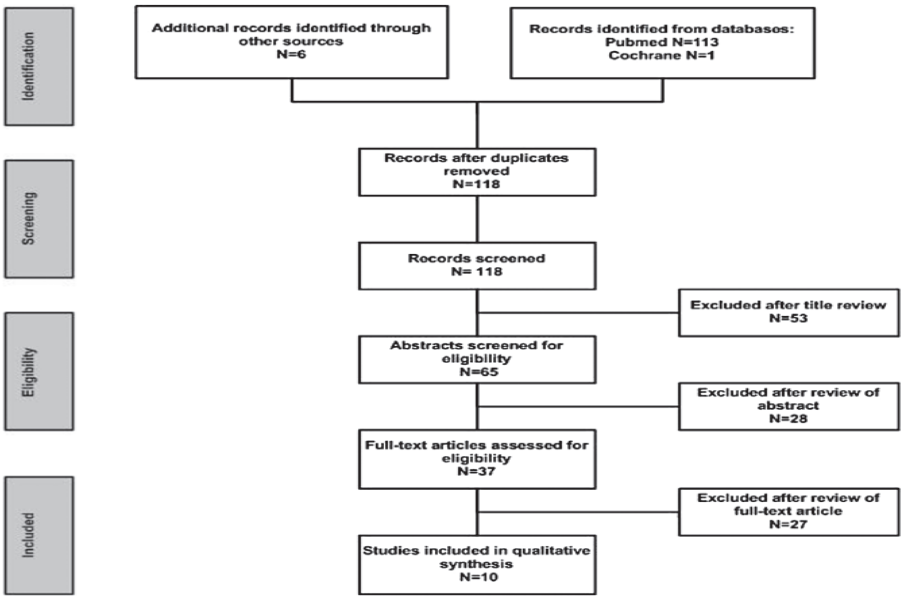

Figure 1: Inclusion flow. Number of articles identified and process for inclusion in the meta-analysis.

patients, but using passive reporting.[18] All authors used Canadian Consensus Criteria to diagnose TRALI.[2,32] In four studies data were also collected before 2004, the year of establishment of the Canadian Consensus Criteria.[15,21,25,26] Of these studies, one used American-European consensus criteria for ALI within 6 hours of transfusion[26] and the second defined TRALI cases as "acute dyspnea with hypoxia and bilateral pulmonary infiltrates occurring during or in the 24 hours after transfusion, with no other apparent cause".[15] The remaining two studies retrospectively applied the Canadian Consensus Criteria.[21,25] The majority of the included studies were able to implement a 95% to 100% male-only plasma donation policy. Two studies used, in addition to male-only plasma, plasma from female donors without history of pregnancy or without HLA and/or HNA antibodies.[20,23] Four studies reported implementation of a male-only donor strategy for PLT products, in addition to a male-only donor strategy for plasma products, as these are high-plasma-volume products as well.[15,19,21,26] Detailed characteristics of included studies are shown in tables 2 and 3.

Methodological quality

The methodological quality of cohort studies is summarized in table 4 and of case-control studies in table 5. Quality assessment revealed that risk of bias of patient selection was low. However, included studies were heterogeneous regarding TRALI surveillance, using passive or active reporting. In addition, in studies carried out within

Table 3a. Transfusion data of cohort studies reporting transfused units

Reference	TRALI cases		Total units transfused		PLASMA transfused		Platelets transfused		Red blood cells transfused	
	Before	After	Before	After	Before	After	Before	After	Before	After
Wright[26]	37	14	2917	1750	1026	629	131	91	1760	1030
Vlaar[24]	17	6	1350	485	174	135	88	25	1088	325
Nakazawa[22]	3	2	1596	1480	467	424	545	556	584	500
Arinsburg[18]	9	1	227913	233685	47756	52230	50041	50143	130116	131312
Toy[23]	23	10	89321	123731	NA	NA	NA	NA	NA	NA

NA=not available

Table 3b. Transfusion data of cohort studies reporting distributed units

Reference	TRALI cases		Total units distributed		PLASMA distributed		Platelets distributed		Red blood cells distributed	
	Before	After	Before	After	Before	After	Before	After	Before	After
Chapman[15]	58	12	16550000	5897000	1874000	634000	1265000	518000	13411000	4745000
Eder[19]	58	127	8320437	33810762	1664598	6695037	637751	3172097	6018088	23943628
Wiersum[25]	68	31	4067000	1377000	545000	183000	279000	98000	3243000	1096000
Lin[21]	105	31	7633560	2364400	1648400	479050	638020	218330	5347140	1667020
Funk[20]	44	4	12170000	6200000	2360000	1080000	880000	490000	8930000	4630000

national registries possibly not all transfused patients were observed for the minimum of 6 hours, which may have resulted in underreporting of TRALI. However, this possible disadvantage was applicable for both periods, before and after implementation of TRALI risk reduction strategies. None of the nationwide studies corrected for confounding that may have been a possible source of bias. Blinded endpoint assessment was carried out in seven studies, leaving the possibility of interpretation bias in the other 3 studies.[15,19,20] Interobserver agreement for the quality assessment was good (median $\kappa = 0.89$ [range 0.40 to 1.0]). Heterogeneity was assessed using I^2 test. Heterogeneity was high ($p<0.01$) for all studies combined and low for predefined subgroup analysis ($p>0.1$).

Meta-analysis on the risk of TRALI

All TRALI cases

The effect of a low-risk TRALI donor strategy for plasma-containing products on the occurrence of TRALI for all transfused products in all studies is summarized in Fig. 2. Figures 3 and 4 show the effect of a low-risk TRALI donor strategy for plasma-containing products for high-risk populations and from nationwide registries, respectively. Four studies showed an association between the introduction of a low-risk TRALI donor strategy for plasma and a reduction of TRALI risk.[18-20,23] In five studies there was a

Table 4. Quality of included cohort studies based on modified Newcastle-Ottowa Scale[27]

Reference	Selection				Comparability	Outcome		
	Cohort representative?	Non-exposed cohort adequate?	Ascertainment of exposure clear?	Outcome not present at start of study?	Adjustment for confounding/bias	Assessment of outcome blinded	Length of follow-up appropriate?	Adequate of follow-up of cohort?
Toy[23]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Chapman[15]	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Eder[19]	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Wiersum[25]	Yes	Yes	Yes	Yes	No	Yes	Yes	No
Lin[21]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Funk[20]	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Arinsburg[18]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes

Table 5. Quality of included case control studies based on modified Newcastle-Ottowa Scale[27]

Reference	Selection				Comparability	Outcome		
	Cohort representative?	Non-exposed cohort adequate?	Ascertainment of exposure clear?	Outcome not present at start of study?	Adjustment for confounding/bias	Assessment of outcome blinded	Length of follow-up appropriate?	Adequate of follow-up of cohort?
Wright[26]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Vlaar[24]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Nakazawa[22]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

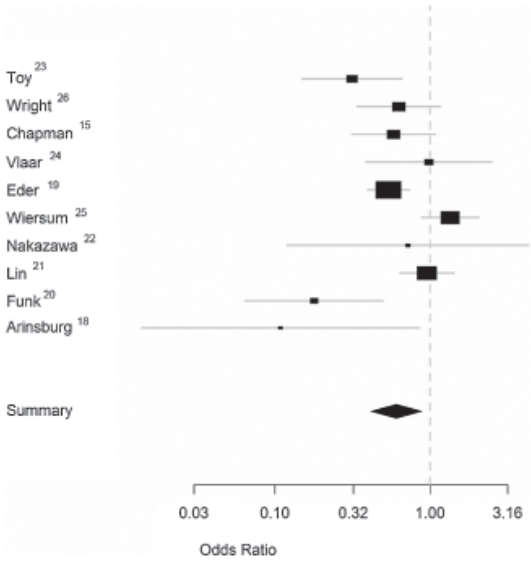

Figure 2. Meta-analysis for the onset of TRALI expressed over all products transfused before and after introduction of a low risk TRALI donor strategy for plasma containing products. Effect sizes are expressed as Odds ratio and their 95 % confidence intervals. The Odds is the number of TRALI cases by the number of transfused products. An Odds ratio lower than one expresses a protective effect against TRALI of products transfused in the period after the introduction of a low risk TRALI donor strategy for plasma.

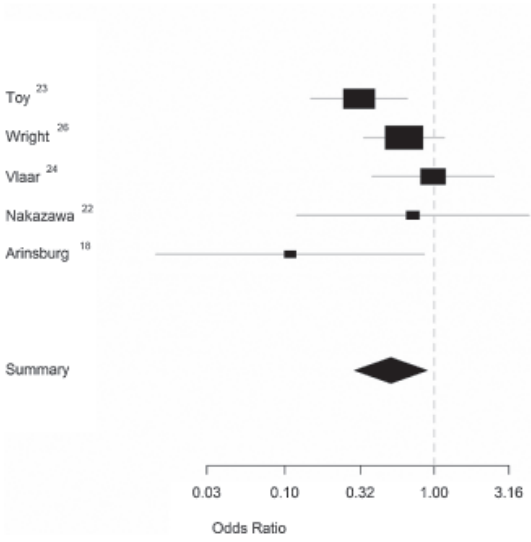

Figure 3. Meta-analysis for the onset of TRALI in at risk patient populations expressed over all products transfused before and after introduction of a low risk TRALI donor strategy for plasma containing products. Effect sizes are expressed as Odds ratio and their 95 % confidence intervals. The odds is the number of TRALI cases by the number of transfused products. An Odds ratio lower than one expresses a protective effect against TRALI of products transfused in the period after the introduction of a low risk TRALI donor strategy for plasma.

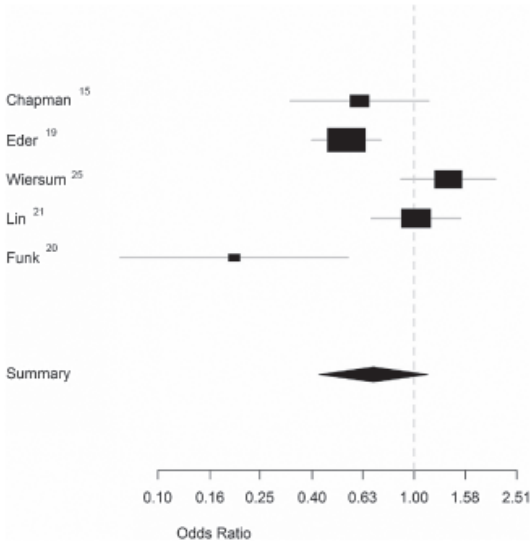

Figure 4. Meta-analysis for the onset of TRALI in general patient populations expressed over all products transfused before and after introduction of a low risk TRALI donor strategy for plasma containing products. Effect sizes are expressed as Odds ratio and their 95 % confidence intervals. The odds is the number of TRALI cases by the number of transfused products. An Odds ratio lower than one expresses a protective effect against TRALI of products transfused in the period after the introduction of a low risk TRALI donor strategy for plasma.

trend to TRALI risk reduction after introduction of a low-risk TRALI donor strategy for plasma.[15,21,22,24,26] TRALI risk was unaffected in one study.[25]

Pooled data of all studies showed a significant reduction for the risk of TRALI after implementation of a low-risk TRALI donor strategy for plasma-containing products (OR, 0.61; 95%CI, 0.42-0.86).

Subgroup analysis revealed that data from local registries showed a significant reduction of TRALI risk (OR, 0.51; 95%CI, 0.29-0.90). Patients in local registries consisted of critically ill patients[24] and patients undergoing major surgery[22,26]. As confirmed by one of the included studies[23] these patients are more prone to develop a TRALI reaction compared to a general patient population that also includes outpatients. Data from nationwide registries (general patient population) showed only a tendency toward protection of a low-risk TRALI donor strategy for plasma containing products against TRALI (OR, 0.66; 95%CI, 0.40-1.09).

Plasma-induced TRALI

Included studies implemented different TRALI risk reduction strategies, which also affected TRALI risk associated with other products than plasma. Therefore, we performed an additional subgroup analysis to assess the effect of risk reduction strategies on

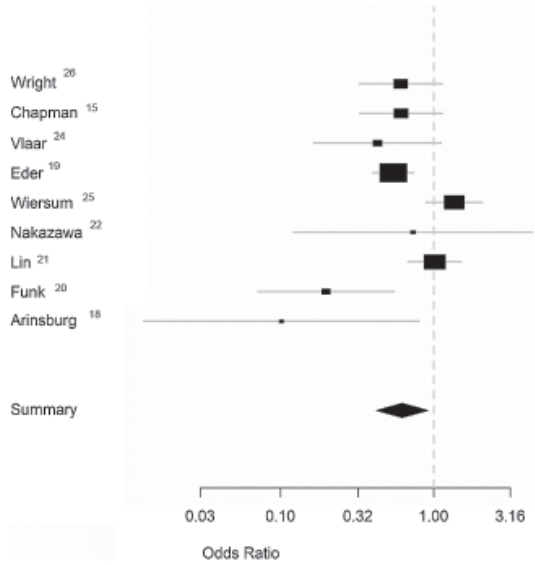

Figure 5. Meta-analysis for the onset of plasma-induced TRALI expressed over all units of plasma transfused before and after introduction of a low risk TRALI donor strategy for plasma products. Effect sizes are expressed as Odds ratio and their 95 % confidence intervals. The odds is the number of plasma-induced TRALI cases by the number of transfused units plasma. An Odds ratio lower than one expresses a protective effect against TRALI of plasma transfused in the period after the introduction of a low risk TRALI donor strategy for plasma containing products.

plasma-induced TRALI. Nine of 10 included studies provided data on plasma-induced TRALI[15,18-22,24-26]. The pooled effect of a low-risk TRALI donor strategy on occurrence of TRALI per transfused unit of plasma is shown in Fig. 5. Figures 6 and 7 summarize the effect of low-risk TRALI donor strategy on plasma-induced TRALI in high-risk populations and from nationwide registries, respectively. Data for plasma-induced TRALI showed a similar pattern as the data for all transfused products. Plasma-induced TRALI was significantly reduced after introduction of a low-risk donor strategy (OR, 0.62; 95% CI, 0.42-0.92). Patients from local registries (prone to develop TRALI) experienced a significant risk reduction (OR, 0.51; 95% CI, 0.31-0.83), while data from nationwide registries (general patient population) showed a tendency to reduced risk of plasma-induced TRALI (OR, 0.69; 95% CI, 0.42-1.13).

Meta-analysis on mortality

Data on 30-day mortality of the total cohort were only available from two studies.[24,26] Both studies showed a non-significant reduction of mortality of the cohort of transfused patients after implementation of a low-risk TRALI donor strategy for plasma products (43% before vs. 35% after and 43% before vs. 23%, NS, respectively). As there were

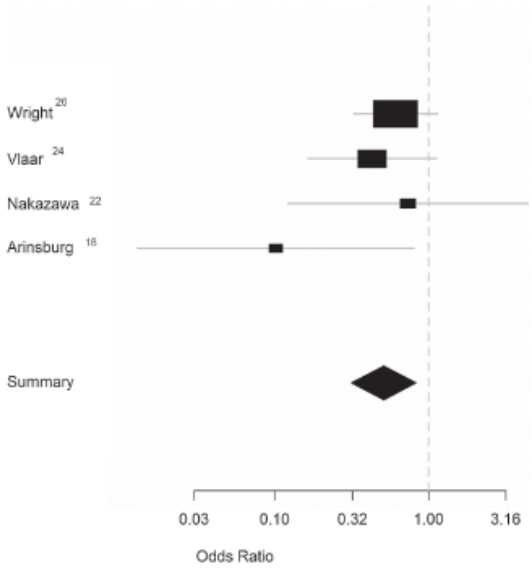

Figure 6. Meta-analysis for the onset of plasma-induced TRALI in at risk populations expressed over units of plasma transfused before and after introduction of a low risk TRALI donor strategy for plasma products. Effect sizes are expressed as Odds ratio and their 95 % confidence intervals. The odds is the number of plasma-induced TRALI cases by the number of transfused plasma. An Odds ratio lower than one expresses a protective effect against TRALI of plasma transfused in the period after the introduction of a low risk TRALI donor strategy for plasma containing products.

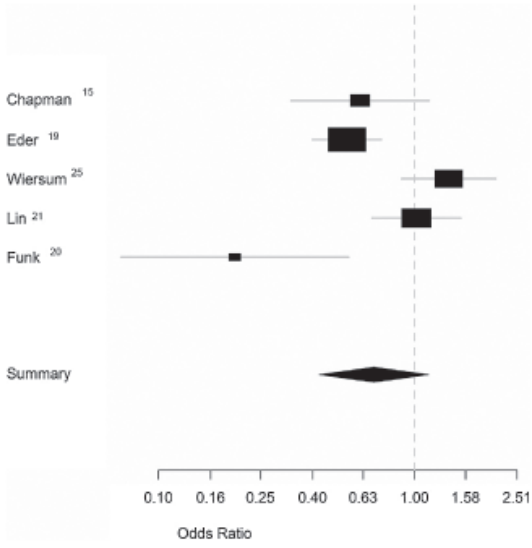

Figure 7. Meta-analysis for the onset of plasma-TRALI in general patient populations expressed over units of plasma transfused before and after introduction of a low risk TRALI donor strategy for plasma. Effect sizes are expressed as Odds ratio and their 95 % confidence intervals. The odds is the number of plasma-induced TRALI cases by the number of transfused plasma. An Odds ratio lower than one expresses a protective effect against TRALI of plasma transfused in the period after the introduction of a low risk TRALI donor strategy for plasma containing products.

only two studies no meta-analysis was performed on these data points. As antibody-mediated TRALI is suggested to be the most severe form of TRALI it can be hypothesized that reducing antibody-mediated TRALI would also result in a reduction of mortality among TRALI patients. Data on 30-day mortality among TRALI patients were available from four studies.[23-26] None of the studies showed a significant reduction of 30-day mortality among TRALI patients after the introduction of a low-risk TRALI donor strategy for plasma-containing products. Pooling of data from these four studies showed a trend to a reduction of mortality among TRALI patients after introduction of a low-risk TRALI donor strategy for plasma containing products (OR, 0.69; 95%CI, 0.27-1.75). An overview of the effect of introduction of a low-risk TRALI donor strategy for plasma-containing products on mortality among TRALI patients is given in Fig. 8.

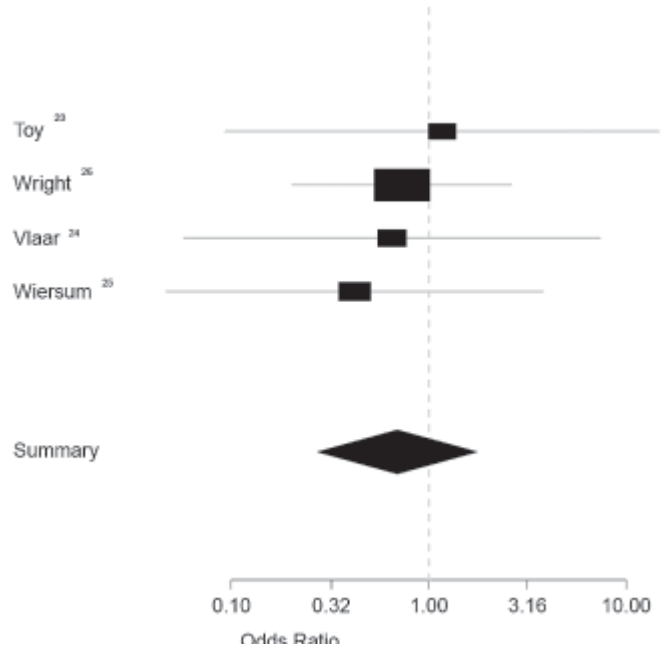

Figure 8. Meta-analysis for mortality among patients developing TRALI before and after introduction of a low risk TRALI donor strategy for plasma containing products. Effect sizes are expressed as Odds ratio and their 95 % confidence intervals

DISCUSSION

This study is the first meta-analysis on the impact of low-risk TRALI donor strategies for plasma on the onset of TRALI. The main findings of this study are; 1) Implementation of a low-risk TRALI donor strategy for plasma-containing products, mainly male-only donor

policies, results in a significant reduction of onset of TRALI (OR, 0.61; 95%CI, 0.42-0.88). 2) Introduction of a low-risk TRALI donor strategy for plasma-containing products results in a trend toward a reduction of 30-day mortality among TRALI-patients (OR, 0.69; 95%CI, 0.27-1.75). 3) At risk patient populations seem to benefit most from the introduction of a low-risk TRALI donor strategy for plasma products.

Our study is able to confirm that the use of a low-risk TRALI donor strategy such as a male-only donor policy for plasma indeed results in a reduction of TRALI. The majority of TRALI cases, up to 89%, are thought to be antibody-mediated TRALI, caused by the passive infusion of donor leukocyte-reactive antibodies, present in plasma-containing blood products.[10] These antibodies are mainly induced by pregnancies and blood transfusions.[14] This was the basis for the hypothesis that the use of plasma from non-transfused male donors would minimize the antibody-mediated TRALI. Although this precautionary measure is effective it has serious consequences for the blood supply as the number of (potential) plasma donors is decreased by half, leading to a shortage of donors. In particular, a shortage in the availability of group AB plasma for transfusion is a serious concern[19,21]. From this point of view some countries introduced a preferentially male plasma donor strategy, as it was not feasible to use male-only plasma.[15] It should be noted that using a male-only donor plasma policy does not totally prevent antibody-mediated TRALI. First of all approximately 1% of the male donor population have antibodies.[33] Second, PLT products are also high-plasma-volume products. The plasma added to the pooled plasma does not originate from male-only plasma in all countries. Third, RBCs are suspended in an additive solution. Although the final product contains only a mean volume of 10 to 20 mL of plasma, RBCs are also known to be implicated in TRALI.[13,34] Fourth, not all countries exclude male donors who have a previous history of blood transfusion themselves from donating blood.[35] Of note, an association between previous transfusions and the development of alloreactive antibodies has not unequivocally been demonstrated.[33] The above-mentioned reasons also partly explain residual TRALI cases in included studies.[15,18-22] In addition, residual TRALI cases were reported to be caused by the use of group AB plasma from female donors.[19,21]

Theoretically, to completely minimize the risk of antibody-mediated TRALI, all donors should be screened for HLA and/or HNA antibodies and positive donors should be excluded for all blood products containing plasma, regardless the amount of plasma. Indeed, some blood collection organizations have implemented antibody screening for all donors or all female donors.[36] This results in less donor loss, but accounts for higher costs and is very labour intensive. In addition, the occurrence of HNA antibodies may not be linked to pregnancy or sex. Therefore, further research is warranted before donor antibody-screening can be advocated.

There are alternatives that might be equally effective. Other countries (e.g. Norway, Finland, France) use pooled solvent/detergent plasma (S/D plasma) instead of single-

donor plasma. Pooling has the advantage that it reduces the possible antibody load by dilution and by neutralizing antigens in the plasma pool, which minimizes the risk of TRALI.[37] The countries that use pooled S/D plasma claim that they do not see TRALI reactions associated with this product. Another approach could be to ask the donors for a history of pregnancies and blood transfusions and subsequently test these donors for HLA and HNA antibodies. This method is a reliable predictor of HLA allo-immunisation.[38] It has been suggested, however, that this method may neglect potential HLA- and/or HNA-positive donors as many pregnancies end in the first weeks after conception, while women were not even aware of a pregnancy. Of note, no association was found between early miscarriages and occurrence of alloimmunisation of donors.[33] Also, donors are not always aware of receiving blood transfusions as they are sometimes given during an operation without notification afterward.

The introduction of a low-risk TRALI donor strategy for plasma-containing products seemed to reduce 30-day mortality among patients developing TRALI. However, the observed reduction in mortality was only a trend that can possibly be explained by small number of studies and patients included. An explanation for mortality reduction among TRALI patients is tempting. Antibody-mediated TRALI is reported to induce a more severe form of TRALI compared to non-antibody-mediated TRALI.[39,40] We hypothesize that as a result of a low-risk TRALI donor strategy for plasma-containing products, the frequency of antibody-induced TRALI is reduced, hereby contributing to a reduction in mortality among TRALI patients.

Data from our meta-analysis showed that the implementation of a low-risk TRALI donor strategy for plasma had the highest impact among at-risk patient populations such as critically ill and surgery patients. This may be well explained by the threshold model of TRALI.[41] In this model a threshold must be overcome to induce a TRALI reaction. Factors that determine the threshold are the predisposition of the patient that determines priming of the lung neutrophils and the ability of the mediators in the transfusion to cause activation of primed neutrophils. A strong antibody-mediated response can cause severe TRALI in an otherwise "healthy" recipient. When activation status is too low, it is possible that priming factors in the transfusion are not strong enough to overcome the threshold. This concept has been proven in animal models.[42-44] This model explains why a low-risk TRALI donor strategy for plasma has limited effect in the general patient population as they have no severe underlying condition to overcome the threshold. In this population a high volume of antibodies or a strong antibody-antigen match is needed to overcome the threshold for onset of TRALI.[23]

Our study has several limitations. First of all there are no randomized controlled trials on the effect of a low-risk TRALI donor strategy for plasma on the onset of TRALI. It is also not expected that after the implementation of a low-risk TRALI donor strategy for plasma in many countries a randomized controlled trial will follow. The observational

nature of the studies and reliance on spontaneous reporting to the blood collection organizations or the nationwide registries (which may be far from complete) may have introduced bias. However, this bias would apply equally to the before and after period.

Second, implemented donor policies were not fully equal among all studies. Not all studies were able to achieve a 100% male-only donor policy for plasma due to scarcity of products with a certain blood group. Some studies reported the implementation of a male-only PLT policy in addition to a male-only donor plasma policy. Others also report the introduction, next to male-only plasma, of plasma from female donors without a history of pregnancy or without HLA and/or HNA antibodies. However, all implemented policies assume the same underlying mechanism of excluding (potential) HLA- or HNA-positive plasma donors to prevent antibody-mediated TRALI.

Third, some of the studies included TRALI patients before the consensus criteria for TRALI from the Canadian Consensus Conference in 2004 were established.[2,32] Before that year there were no universal criteria for TRALI, and TRALI was most likely underreported. However, this will rather give an underestimation of the effect of the low-risk TRALI donor strategy for plasma, as the period before 2004 is included in the before group. Fourth, heterogeneity was high for all studies combined and low for subgroup analysis. This can be explained by differences in population and observation period of included studies. The random-effects model used in our study provides an average effect and in this way controls for variation in studies included. From this perspective we believe that the higher heterogeneity in the total group analysis has no or limited impact on our results and conclusions.

In conclusion, this is the first meta-analysis on the effect of implementation of a low-risk TRALI donor strategy mainly based on a male-only donor policy for plasma-containing products to prevent TRALI. This study shows that introduction of a low-risk TRALI donor strategy for plasma-containing products reduces the onset of TRALI, especially in high-risk patient populations. Furthermore, there is a tendency that reduction of antibody-mediated TRALI results in a lower mortality rate among TRALI patients.

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7

Summary and general discussion

SUMMARY

Transfusion-related acute lung injury (TRALI) is a serious complication of blood transfusion, which causes serious morbidity and is the leading cause of transfusion-associated mortality according to the FDA. The majority of TRALI cases (up to 89%) are thought to be antibody-mediated TRALI, caused by the passive infusion of white blood cell (WBC)-reactive antibodies, present in plasma-containing blood products. This thesis focused on the role of donor WBC-reactive antibodies in TRALI, and the translation to preventive strategies

Chapter 2 describes an observational study of all reported TRALI cases meeting the Canadian Consensus Conference criteria for TRALI in the Netherlands in a 2.5-year period (January 2005 through July 2007). A total of 49 patients met all criteria for TRALI. Clinical and laboratory features of these cases were described. All patients and donors were screened for HLA Class I and II, and granulocyte antibodies. There was a high morbidity and mortality. Seventy-eight percent of the patients were admitted to the intensive care unit or needed mechanical ventilation, and 10 patients died. High-volume plasma products (fresh-frozen plasma [FFP] and/or platelets in plasma) were involved in 61% of the cases. The remaining patients received red blood cells only. White blood cell reactive antibodies in one or more involved donors were found in 73% of cases, with proven incompatibility in 21 of 44 (48%) investigated cases. Possible TRALI cases (with an alternative risk factor for acute lung injury) had significantly lower incompatibility rates compared to TRALI cases, 18% versus 58% ($p = 0.036$). In the 21 alloimmune cases, a total of 31 implicated donors were found. Female FFP products were involved in 57% of proven alloimmune cases and would theoretically be prevented using male-only FFP.

It is generally believed that the majority of TRALI cases are caused by female donors. In chapter 3 we aimed to examine the relation between female donors and the occurrence of TRALI by performing an international, multicenter case-referent study. TRALI patients who received blood products only from donors of a single sex were compared to a reference population. The observed sex distribution among the donors of these TRALI patients was compared to the expected sex distribution. Eighty-three TRALI patients who received transfusions from unisex donors were included. The relative risk (RR) of TRALI after a transfusion from female donors among red blood cell (RBC) recipients was 1.2 (95% confidence interval [CI],0.69-2.1). Among plasma-rich blood product recipients the RR was 19 (95% CI, 1.9-191). This indicates that the role of female donors in TRALI cases caused by RBCs is negligible.

WBC-reactive antibodies are mainly found in alloexposed donors. Alloexposure is the exposure of the immune system to allogeneic cells or tissues (occurring through pregnancy, transfusion of blood components or transplantation). In chapter 4 we aimed to quantify the contribution of alloexposed donors to the occurrence of TRALI caused by

plasma-poor and plasma-rich products. A case-referent study was performed, data on alloexposure status of all 223 donors involved in TRALI cases was compared to information on alloexposure status in the total donor population (the expected status). Fifty-one percent of all TRALI cases could have been prevented by deferral of alloexposed donors (population-attributable risk was 51% [95% CI, 14-88%]). In the 19 TRALI patients who received exclusively plasma-poor blood products, alloexposure of the donors was not associated with TRALI. In conclusion, alloexposed donors conferred an increased risk of TRALI in recipients of plasma-rich blood products, but not in recipients of plasma-poor products

To support the diagnosis of antibody mediated TRALI, WBC-reactive antibodies are tested in involved blood donors. Identification of antibody positive donors is important as exclusion of these donors is part of preventative strategies against TRALI. In chapter 5 we investigated whether the use of bead-based techniques might improve the diagnosis of antibody-mediated TRALI. We compared cellular-based techniques to bead-based techniques for detection of HLA-antibodies in a series of 91 consecutively reported TRALI cases meeting the Canadian Consensus Conference criteria for TRALI. In 113 donors HLA antibodies were detected of which 84 were only detected by bead-based techniques, 12 only by cellular-based tests and 17 by both assays. Antibody mediated TRALI was diagnosed in 44/91 (48%) of reported cases. Twenty-one (48%) of these cases would not have been identified using only cellular-based assays. In conclusion bead-based techniques show a higher sensitivity for detecting incompatible donors in TRALI-cases than cellular-based assays. These results suggest that the use of bead-based assays will result in a significant reduction of future TRALI reactions as more antibody positive donors will be excluded for donation.

Antibody-mediated TRALI is the most prevalent type of TRALI. WBC-reactive antibodies are mainly found in (multiparous) female donors. Therefore plasma derived from male donors should less likely cause TRALI than plasma from female donors. In the past decade blood banks have implemented low-risk TRALI donor strategies to prevent onset of TRALI. Most of these strategies are based on using only, or preferentially, plasma from male donors for single-donor plasma or deferral of donors based on history of pregnancy or transfusion. In chapter 6 a systematic review and meta-analysis was performed to further strengthen evidence for these low-risk TRALI donor strategies. Ten articles were included in the meta-analysis. The meta-analysis showed that the implementation of low-risk TRALI donor strategies reduced the onset of TRALI (OR, 0.61; 95%CI, 0.42-0.88). Subgroup analysis showed that the effect was most clear in patient populations more prone to develop TRALI (e.g., critically ill patients and patients undergoing major surgery [OR, 0.51; 95%CI, 0.29-0.90]). The effects in general patient populations showed a similar non-significant trend (OR, 0.66; 95%CI, 0.40-1.09).

GENERAL DISCUSSION

The role of donor antibodies in antibody mediated TRALI

The research presented in this thesis focused on the role of donor white blood cell (WBC)-reactive antibodies in TRALI. The majority of TRALI cases are thought to be antibody-mediated TRALI, caused by the passive infusion of WBC-reactive antibodies, present in plasma-containing blood products.

We found WBC-reactive antibodies in a series of 49 TRALI patients in 73% of the cases, with proven incompatibility in 21 of 44 (48%) investigated cases. In our larger study of 91 fully tested TRALI cases this percentage was confirmed. Reported incidences of WBC-reactive antibodies in TRALI cases ranged from 25% to 89%.[1-4] Only a few studies have attempted to correlate antibodies in the donor with antigens in the patient.[1,5-7] Incompatibility was demonstrated in 59% to 87.5% of these cases. We found a significant difference between the TRALI group and the possible TRALI group (with an alternative risk factor for ALI). In the TRALI group incompatibility was demonstrated in 58% of the cases, while in the possible TRALI group this was only 18%. It is possible that a different, non-antibody mechanism causes TRALI in the possible TRALI cases. Another explanation could be that in possible TRALI cases onset of TRALI follows a threshold model. When the "first hit", the underlying condition of the patient, is strong enough only a small amount of antibodies (below the cut off) may already result in TRALI. Furthermore incompatibility is often tested weeks after the onset of TRALI when the underlying condition of the patient has resolved and the immune system has come to rest. Incompatibility testing may subsequently be false negative.

The previously reported incompatibility rates are higher than the overall 48% incompatibility we found.[1,5-7] Two of these published studies had only small numbers of TRALI cases, these might include only TRALI cases without alternative risk factor.[5,6] Three of the 4 series were published before the establishment of the Canadian Consensus Conference criteria for TRALI. Another explanation may be that there is a publication bias in favor of series with high incompatibility rates.

WBC-reactive antibodies are induced by previous exposure to alloantigens. These alloexposures occur either through pregnancies or through blood transfusions and transplantations. As a consequence WBC-reactive antibodies are mainly found in (multiparous) female donors.[8-11] In our international case-referent study we found that the risk of TRALI was increased among recipients of plasma-rich products (relative risk [RR] 19), but not among recipients of red blood cells (RR 1.2). The percentage of cases preventable by the exclusion of female donors was 7.0% for RBC recipients and 86% for recipients of plasma-rich blood products. These findings suggest that the majority of TRALI cases caused by plasma-rich products are preventable by the exclusion of female donors, whereas in TRALI caused by RBC transfusions the role of female donors is marginal. The

minimal amount of residual plasma, which might trigger TRALI, is not known. Possibly the volume of residual plasma in RBC's is in general insufficient to cause TRALI. However, it is known that WBC-reactive antibodies present in RBC transfusions can cause TRALI.[12] We performed a systematic review of the literature to summarize the direct effect of the relation between female donors and TRALI. We found six studies on this topic.[13-18] None of these six publications investigated the difference between plasma-rich and plasma-poor products.

Diagnosing TRALI

The diagnosis of TRALI is based on clinical and radiographic findings, there is no pathognomonic laboratory test for TRALI. Antibody testing is only supportive in diagnosing TRALI. It takes weeks to months before the results of antibody testing are known. However the detection of WBC-reactive antibodies in involved donors is the keystone in diagnosing antibody-mediated TRALI. We found that bead-based techniques are more proficient in detecting these antibodies in donors involved in TRALI reactions than cellular-based assays. This leads to deferral of more donors and probably in a reduction of future TRALI reactions. An important issue is whether all incompatible antibodies found in the bead-based assay were causal for the TRALI reaction as we did not perform functional test of the incompatibility. Case reports have shown that the presence of incompatible antibodies does not always result in the onset of TRALI.[19-22] These observations gave rise to the hypothesis that TRALI might follow an "threshold model".[23] The threshold is formed by the level of priming of lung neutrophils and the ability of the mediators in the blood product (e.g. WBC-reactive antibodies) to activate these primed neutrophils. TRALI may develop in an (relatively) healthy person as long as the antibody titer is strong enough to overcome the threshold. Contrary, low antibody titers might be able to induce TRALI in patients with an underlying condition (e.g. sepsis, haematological malignancy). Therefore, it is important to trace and exclude all donors with WBC-reactive antibodies irrespective of titer concentrations, as the critical level is not known. This threshold model was supported by *in vivo* TRALI mouse models. Looney et al showed that exposure to pathogens might lower the threshold in mice.[24] Vlaar and co-workers showed that mechanical ventilation lowered the threshold in a HLA-class I antibody mouse model.[25]

International guidelines and standardization of antibody screening assays is necessary to further elucidate the contribution of WBC-reactive antibodies in the pathogenesis of (antibody-mediated) TRALI.

Prevention strategies of antibody-mediated TRALI

In 2003 the United Kingdom was the first to implement a transfusion policy to use predominantly plasma from male donors for the production of high plasma volume

blood products. Nowadays several countries have taken similar measures, mostly based on the use of only, or preferentially, plasma from male donors for single-donor plasma and platelet concentrates. These policies resulted in up to a two-third reduction in TRALI cases. In our meta-analysis of all trials on low-risk TRALI donor strategies we found that that these precautionary measures indeed reduced the risk of TRALI (OR 0.61, 95%CI 0.42-0.86). The effect was most clear in at-risk patient populations (OR 0.51, 95%CI, 0.29-0.90). These results were confirmed in another recent meta-analysis.[26] Schmickl et al found that a male-predominant plasma transfusion policy reduces plasma-related TRALI incidence by 73% (95% CI, 62-80%). These precautionary measures are effective in minimizing the risk of antibody-mediated TRALI, but it can have serious consequences for the blood supply by deferral of half of the potential plasma donors. To overcome this problem of shortage of donors some blood collection organizations screen all donors (or all female donors) for WBC-reactive antibodies. Donors who test positive for these antibodies are excluded for donations. Some countries use pooled solvent/detergent (S/D) plasma instead of single donor plasma, claiming that TRALI reactions are not associated with S/D plasma. In 2014 the national blood supply organization in the Netherlands has introduced S/D plasma instead of fresh frozen plasma. In the next years we will learn whether this will lead to a further decrease in the incidence of TRALI caused by plasma.

Most countries implemented the male-only donor strategies for fresh frozen plasma. Some blood collection organizations (e.g in the Netherlands) also implemented a male-only donor strategy for other high-volume plasma products such as platelet concentrates.[24] In our case-referent study we found that the risk of TRALI was not increased among recipients of plasma-poor products (e.g. RBCs) from female donors. Although it is known that WBC-reactive antibodies present in RBC transfusions can cause TRALI[12,28] our study suggests that a male only policy for plasma-poor products is not rational

Future directions

The implementation of male-only plasma donor strategies has reduced the incidence of antibody-mediated TRALI, but did not totally prevent this serious transfusion reaction. First of all not all countries changed to a male only plasma policy for all high volume plasma products (e.g. pooled platelets). Second approximately 1% of the currently used male donor population for high volume plasma products have WBC-reactive antibodies.[10] Third, not in all countries male donors who had a blood transfusion themselves are excluded from donating blood. And fourth, as mentioned before, plasma poor products such as RBC's contain a mean volume of 10-20 mL plasma and can cause TRALI.[12,25] Also some countries use a preferentially male-only donor policy for FFP, meaning that some FFPs still are derived from female donors, mainly AB plasma. To completely minimize the risk of antibody-mediated TRALI, all donors should be screened for WBC-reactive

antibodies and positive donors should be excluded for all plasma containing blood products, regardless of the amount of plasma.

Occurrence of non-antibody mediated TRALI is not influenced by these measures. Accumulation of bioactive substances are thought to contribute to the occurrence of TRALI.[29-30] Storage time and storage conditions may contribute to the development of these bioactive substances with neutrophil priming ability. However these studies are based on pre-clinical data and observational clinical studies. Pre-clinical (animal model) studies show a clear relation between TRALI and increased storage time of cellular blood products.[30-32] Observational studies however report conflicting results.[33] This may be explained by the heterogeneity of the studies, patients differed in the severity of the first hit and the number of blood products received. Receiving a high number of blood products, raises the chance of receiving "old" blood products. Also patients who receive many transfusions generally are critically ill. Prospective randomized studies are needed to elucidate the factors in non-antibody mediated TRALI.

There is no specific treatment for TRALI, management of TRALI is supportive. Once TRALI occurs it causes high morbidity and mortality. In patients who require mechanical ventilation a low tidal volume strategy is recommended.[34] A restrictive transfusion policy reduces adverse transfusion reactions (e.g. TRALI) and has been proven to be save in the majority of patient populations.[35] Clinicians should be made more aware of the risks of blood transfusions and the importance to apply to restrictive transfusion policies.

In conclusion, most TRALI cases are thought to be caused by WBC-reactive antibodies. The implementation of low-risk TRALI donor strategies for high volume plasma blood products, has significantly reduced TRALI risk. Futures research should be focused on the pathophysiology of non-antibody mediated TRALI, the standardization of antibody screening assays for antibody mediated TRALI and therapeutic strategies for TRALI in general. Also clinicians should be made more aware of the risk of blood transfusions.

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8

Samenvatting

Bloedtransfusie is niet meer weg te denken uit de moderne geneeskunde. Het wordt gezien als een levensreddende handeling. De afgelopen jaren is het echter duidelijk geworden dat het ook een potentieel levensbedreigende interventie is. Transfusiegerelateerde acute longschade (TRALI) is daarbij de meest voorkomende oorzaak van transfusie geassocieerde mortaliteit. TRALI werd eerder gezien als een zeldzame bijwerking van transfusie geneeskunde, recente studies laten zien dat tot wel 1 op 10 hoog risico patiënten die een transfusie ontvangt TRALI ontwikkelt. De meerderheid van de TRALI gevallen, tot wel 89%, wordt gedacht te worden veroorzaakt door de passieve infusie van antistoffen die aanwezig zijn in plasma-bevattende bloedproducten. Deze donorantistoffen zijn gericht tegen de antigenen van de ontvanger en zorgen voor activatie van de witte bloedcellen. Deze activatie leidt tot schade aan de longcapillairen met als gevolg lekkage van eiwitten en vocht vanuit de capillairen naar de longblaasjes (longoedeem). Dit resulteert uiteindelijk in acute benauwdheid bij de patiënt oftewel TRALI. Dit proefschrift richt zich op de rol van donorantistoffen bij het optreden van TRALI. Daarnaast wordt er ook gekeken naar preventieve maatregelen om het optreden van TRALI te verminderen.

Hoofdstuk 2 beschrijft een observationele studie van alle gerapporteerde TRALI gevallen die voldeden aan de Canadese consensus conferentie criteria voor TRALI in Nederland gedurende een periode van 2.5 jaar (januari 2005 tot juli 2007). In totaal voldeden 49 patiënten aan alle criteria voor TRALI. Klinische en laboratorium kenmerken van deze gevallen werden beschreven. Alle patiënten en betrokken donors werden onderzocht op antistoffen gericht tegen witte bloedcellen (HLA klasse I en II en granulocyten antistoffen). Er was een hoge morbiditeit en mortaliteit onder de patienten die TRALI ontwikkelden. Achtenzeventig procent van de patiënten werd opgenomen op de intensieve zorg afdeling en een gedeelte had daarbij mechanische beademing nodig. In totaal overleden 10 patiënten. Hoog-volume plasma producten (vers ingevroren plasma [FFP] en/of bloedplaatjes in plasma) waren betrokken bij 61% van de TRALI gevallen. De overige gevallen van TRALI kregen alleen rode bloedcellen toegediend. In 73% van de TRALI gevallen werd er bij één of meer betrokken donors antistoffen gericht tegen witte bloedcellen gevonden. Hierbij was er sprake van bewezen incompatibiliteit bij 21/44 (48%) van de onderzochte gevallen. TRALI gevallen waarbij bewezen onverenigbaarheid van antistof en antigeen tussen patiënt en donorbloed is aangetoond noemen we antistof-gemedieerde TRALI. Mogelijke TRALI gevallen (aanwezigheid van een alternatieve risicofactor voor acute longschade) hadden een significant lager incompatibiliteits percentage vergeleken met TRALI gevallen, 18% versus 58%. Bij de 21 antistof gemedieerde TRALI gevallen werden in totaal 31 donors met antistoffen gevonden. FFP producten afkomstig van vrouwelijke donoren waren betrokken bij 57% van de bewezen antistof gemedieerde TRALI gevallen. Deze TRALI gevallen hadden theoretisch voorkomen kunnen worden door het gebruik van plasma afkomstig van mannelijke donoren.

In het algemeen wordt aangenomen dat de meerderheid van de TRALI gevallen wordt veroorzaakt door vrouwelijke donoren. In hoofdstuk 3 hebben we de relatie tussen vrouwelijke donoren en het optreden van TRALI onderzocht in een internationale, multicenter case-referent studie. TRALI patiënten die bloedproducten ontvingen afkomstig van donoren van 1 sekse werden vergeleken met een referentie populatie. De geobserveerde sekse verdeling onder donoren van deze TRALI patiënten werd vergeleken met de verwachte sekse verdeling. Er werden drieëntachtig TRALI patiënten geïncludeerd die transfusies ontvingen van uniseks donoren. Het relatieve risico (RR) op TRALI na een transfusie van vrouwelijke rode bloedcel producten was 1.2 (95% betrouwbaarheidsinterval [CI] 0.69-2.1). Onder ontvangers van plasma-rijke bloedproducten was het RR 19 (95% CI 1.9-191). Dit duidt erop dat de rol van vrouwelijks donoren in TRALI gevallen veroorzaakt door rode bloedcel producten verwaarloosbaar is.

Antistoffen gericht tegen witte bloedcellen worden voornamelijk gevonden in donoren die zijn blootgesteld aan lichaamsvreemde antigenen. Deze antistoffen kunnen gevormd worden door blootstelling van het immuunsysteem aan lichaamsvreemde cellen of weefsels (bijvoorbeeld door zwangerschap, bloedtransfusie of transplantatie). In hoofdstuk 4 onderzochten we de bijdrage van aan lichaamsvreemde antigenen blootgestelde donoren aan het optreden van TRALI veroorzaakt door plasma-arme en plasma-rijke bloedproducten. We hebben een case-referent studie uitgevoerd waarbij gegevens over de blootstelling aan lichaamsvreemde antigenen van alle 223 donors betrokken bij TRALI gevallen werden vergeleken met informatie over deze blootstelling in de totale donorpopulatie (de verwachte blootstelling). Eenenvijftig procent van alle TRALI gevallen had mogelijk voorkomen kunnen worden door uitsluiten van aan lichaamsvreemde antigenen geëxposeerde donoren. Bij de 19 patiënten die alleen plasma-arme bloedproducten ontvingen was blootstelling aan allogene antigenen niet geassocieerd met TRALI. Concluderend, donoren die blootgesteld zijn aan lichaamsvreemde antigenen geven een verhoogd risico op TRALI bij ontvangers van plasma-rijke bloedproducten, maar niet bij ontvangers van plasma-arme producten.

Om de diagnose antistof-gemedieerde TRALI te ondersteunen, worden betrokken donors onderzocht op antistoffen gericht tegen witte bloedcellen. Identificatie van antistof-positieve donoren is belangrijk, aangezien exclusie van deze donoren onderdeel is van strategieën ter preventie van TRALI. In hoofdstuk 5 hebben we onderzocht of het gebruik van bead-based technieken bij het opsporen van antistoffen het stellen van de diagnose antistof-gemedieerde TRALI zou verbeteren. In een serie van 91 achtereenvolgend gemelde TRALI gevallen die allen voldeden aan de Canadese consensus conferentie criteria werden cellular-based testen vergeleken met bead-based technieken. Bij bead-based technieken wordt gebruik gemaakt van bolletjes (“beads”) bedekt met antigenen, terwijl bij cellular-based technieken gebruik wordt gemaakt van verse witte bloedcellen. Bij 113 donoren werden HLA antistoffen gevonden, waarvan er 84 alleen

werden gevonden met bead-based technieken, 12 alleen met cellular-based testen en 17 in beide technieken. Antistof-gemedieerde TRALI werd gediagnostiseerd bij 44/91 (48%) van de gerapporteerde gevallen. Eenentwintig van deze antistof-gemedieerde gevallen zouden niet zijn aangetoond bij het gebruik van alleen cellular-based testen. De resultaten van deze studie laten zien dat bead-based technieken een hogere gevoeligheid hebben voor het ontdekken van onverenigbare donoren bij TRALI gevallen ten opzichte van cellular-based testen. Deze resultaten suggeren dat het gebruik van bead-based technieken mogelijk zal resulteren in een afname van toekomstige TRALI gevallen, aangezien meer donoren met antistoffen tegen witte bloedcellen zullen worden uitgesloten van donaties.

Antistof-gemedieerde TRALI is het meest voorkomende type van TRALI. Antistoffen gericht tegen witte bloedcellen worden voornamelijk gevonden bij vrouwelijk donoren die ooit zwanger zijn geweest. Plasma afkomstig van mannelijke donoren zou dus een lagere risico op TRALI moeten geven dan plasma van vrouwelijke donoren. Het laatste decennium hebben bloedbanken laag-risico donor strategieën toegepast om het optreden van TRALI te voorkomen. De meeste strategieën zijn gebaseerd op het gebruik van alleen, of bij voorkeur, plasma afkomstig van mannelijke donoren. In hoofdstuk 6 werd een systematisch review en meta-analyse verricht om het bewijs voor deze preventieve strategieën te bekrachtigen. Tien artikels werden gebruikt voor de meta-analyse. Het invoeren van laag-risico donor strategieën verminderde de kans op TRALI. Analyse van subgroepen toonde dat dit effect het meest duidelijk was in patiëntenpopulaties die meer vatbaar zijn voor het ontwikkelen van TRALI, bijvoorbeeld kritiek zieke patiënten op de intensieve zorg afdeling en patiënten na grote operaties.

Dit proefschrift laat zien dat donorantistoffen een centrale rol spelen in het optreden van TRALI. Producten afkomstig van vrouwelijk donoren die één of meerdere keren zwanger zijn geweest vormen een verhoogd risico op het ontstaan van TRALI, met name hoog-volume plasma producten (vers ingevroren plasma en/of bloedplaatjes in plasma). Het onderbouwen van antistof-gemedieerde TRALI middels laboratorium diagnostiek kan het best gebeuren middels bead-based technieken. Een belangrijke stap in het voorkomen van TRALI is het invoeren van laag-risico donor strategieën, waarbij hoog-volume plasma producten alleen, of bij voorkeur, afkomstig zijn van mannelijke donoren.

Dankwoord
Curriculum Vitae
Publicaties
PhD Portfolio

Dankwoord

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Curriculum Vitae

Daniëlle van Stein werd geboren op 7 februari 1977 in Gouda. In 1995 behaalde zij haar VWO diploma aan het IJsselcollege in Capelle aan den IJssel. In hetzelfde jaar startte zij haar studie geneeskunde aan de Erasmus Universiteit te Rotterdam. In 1999 behaalde zij haar doctoraal examen en in 2001 het artsexamen.

Aansluitend werkte zij als arts niet in opleiding tot specialist op de afdeling Interne Geneeskunde van het Medisch Centrum Rijnmond-Zuid (nu het Maasstad Ziekenhuis). In 2005 startte zij met haar promotie-onderzoek bij de Sanquin Bloedbank in Dordrecht onder supervisie van dr. E.A.M. Beckers en prof. dr. D.J. van Rhenen. In latere instantie werd ook dr. A.P.J. Vlaar als co-promoter betrokken bij het onderzoek. Tijdens dit onderzoek volgde zij het postinitieel masteronderwijs epidemiologie aan het EMGO onderwijsinstituut van het VUmc.

In juni 2008 begon Daniëlle aan de opleiding tot internist in het Ikazia Ziekenhuis te Rotterdam (opleider dr. A. Dees). In mei 2011 vervolgde zij haar opleiding in het Erasmus MC te Rotterdam (opleider prof. dr. J.L.C.M. van Saase). In september 2013 is zij gestart met haar differentiatie Medische Oncologie in het LUMC te Leiden (opleider prof. dr.A.J. Gelderblom). Per 1 augustus 2015 is zij geregistreerd als internist.

Daniëlle is getrouwd met Ron en samen hebben zij twee zoons, Tim en Sander.

Publicaties

Van Stein D, Beckers EA, Peters A-L, Porcelijn L, Middelburg RA, Lardy NM, van Rhenen DJ, Vlaar AP. Underdiagnosing of antibody mediated transfusion-related acute lung injury: evaluation of cellular-based versus bead-based techniques. *Submitted.*

Peters A-L, **van Stein D**, Vlaar AP. Antibody-mediated transfusion-related acute lung injury; from discovery to prevention. Br J Haemat. 2015 Apr 28. doi: 10.1111/bjh.13459. [Epub ahead of print]

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**Both authors contributed equally to the manuscript*

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Middelburg RA, **van Stein D**, Zupanska B, Uhrynowska M, Gajic O, Múniz-Diaz E, Galvez NN, Silliman CC, Krusius T, Wallis JP, Vandenbroucke JP, Briët E, van der Bom JG. Female donors and transfusion-related acute lung injury: A case-referent study from the International TRALI Unisex Research Group. Transfusion. 2010;50:2447-54.

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Middelburg RA, **van Stein D**, Briët E, van der Bom JG. The role of donor antibodies in the pathogenesis of transfusion-related acute lung injury: a systematic review. Transfusion. 2008;48:2167-76.

Leebeek FW, Stadhouders NA, **van Stein D**, Gómez-García EB, Kappers-Klunne MC. Hypercoagulability states in upper-extremity deep venous thrombosis. Am J Hematol. 2001;67:15-9.

PhD Portfolio

Summary of PhD training and teaching

	Year	Workload
General courses		
- Epidemiologisch onderzoek: opzet en interpretatie, EMGO	2005	4 ECTS
- Inleiding SPSS, EMGO	2006	0.5 ECTS
- Principes van epidemiologische data-analyse, EMGO	2006	3 ECTS
- Lineaire regressie en variantie-analyse, EMGO	2006	3 ECTS
- Logistische regressie en analyse van overlevingsduren, EMGO	2006	3 ECTS
- Doelmatigheidsonderzoek: methoden en principes, EMGO	2007	2 ECTS
- Toepassingsgebieden van de epidemiologie, EMGO	2007	3 ECTS
- Clinimetrics: assessing measurement properties of health measurement instruments, EMGO	2007	3 ECTS
Presentations		
- Symposium Regionaal Hemovigilantie Platform Zuidwest, oral presentation	2006	0.3 ECTS
- Sanquin Bloedbankavond, oral presentation	2006	0.2 ECTS
- ISBT Annual Meeting 2006, poster presentation	2006	1 ECTS
- TRIP symposium 2007, poster presentation	2007	0.2 ECTS
- Internistendagen 2008, oral presentation	2008	0.5 ECTS
- ISBT Annual Meeting 2008, oral presentation	2008	1 ECTS
- ISBT Annual Meeting 2010, poster presentation	2010	1 ECTS
(Inter)national conferences		
- European Symposium on Platelet and Granulocyte Immunobiology, 2006, Tromso, Norway	2006	1 ECTS
- ISBT Annual Meeting 2006, Cape Town, South Africa	2006	1 ECTS
- TRIP hemovigilantie symposium 2006, Rotterdam	2006	0.3 ECTS
- Sanquin Spring Seminars 2007, Amsterdam	2007	0.5 ECTS
- ISBT Annual Meeting 2007, Madrid, Spain	2007	1 ECTS
- TRIP hemo- en weefselvigilantie symposium 2007, Utrecht	2007	0.3 ECTS
- Internistendagen 2008, Maastricht	2008	0.5 ECTS
- ISBT Annual Meeting 2008, Macao, China	2008	1 ECTS
- Internistendagen 2009, Maastricht	2009	0.5 ECTS
- Internistendagen 2011, Maastricht	2011	0.5 ECTS
- Internistendagen 2013, Maastricht	2013	0.5 ECTS
- ESMO Annual Meeting 2014, Madrid, Spain	2014	1 ECTS
Other		
- Research Bespreking Sanquin Bloedbank, Leiden	2005-2008	1 ECTS
- Journal Club Sanquin Bloedbank, Leiden	2005-2008	1 ECTS