Transfusion-related acute lung injury in the critically ill: a translational approach
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Chapter 1

General introduction and outline of this thesis

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Introduction

Blood transfusion is one of the most frequent therapeutic interventions in the Intensive Care Unit (ICU). Up to 50% of ICU patients receive a blood transfusion. Although lifesaving at times, blood transfusion is associated with increased morbidity and mortality. At this moment transfusion–related acute lung injury (TRALI) is the leading cause of transfusion related morbidity and mortality.

TRALI is a subcategory of acute lung injury/acute respiratory distress syndrome (ALI/ARDS). By definition, the occurrence of ALI within 6 hours of transfusion of a blood product should be considered as TRALI (table 1). Characteristics of TRALI are indistinguishable from acute lung injury due to other causes (e.g. pneumonia, trauma or sepsis). Thus, if acute lung injury developed in a patient with an underlying risk factor who also received a blood transfusion, designating symptoms to the blood transfusion or to the alternative cause is a problem. To distinguish such cases, the term ‘possible TRALI’ was proposed, which allows for the presence of another risk factor for ALI. This term has enabled estimates of incidence in the critically ill, in whom other ALI risk factors are often present. Although TRALI is assumed to be rare, it is probably under-reported. Lack of specific disease markers hampers the diagnosis of TRALI, which may contribute to its under-recognition. Several recent studies have reported an increase in the incidence of TRALI, in particular in patients on Intensive Care Units (ICU). At the same time, however, fewer blood transfusions are being given to critically ill patients. The concomitant increase in the incidence of TRALI and the decrease in blood transfusions in ICU patients require an explanation. Either increased awareness has led to an increase in reported TRALI cases, or other disease entities causing hypoxia in the critically ill are being mistaken for TRALI. It has been suggested that TRALI pathogenesis may differ in critically ill patients in comparison to the general hospital population.

<table>
<thead>
<tr>
<th>Table 1. Definition of Acute Lung Injury (ALI) and Transfusion Related Acute Lung Injury (TRALI, reference 3-5)</th>
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<tr>
<td><strong>Definition ALI</strong></td>
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<tr>
<td>- Acute onset of hypoxia: PaO₂/FiO₂&lt;300mm Hg or SpO₂&lt;90% on room air</td>
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<td>- Bilateral pulmonary infiltrates</td>
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<td>- Pulmonary artery wedge pressure ≤18 mm Hg or the absence of left ventricular overload</td>
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<td><strong>Definition TRALI</strong></td>
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<tr>
<td>- No ALI before transfusion</td>
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<tr>
<td>- ALI during or &lt; 6 hours after transfusion</td>
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<tr>
<td>- No other risk factors for ALI</td>
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<tr>
<td><strong>Definition ‘possible’ TRALI</strong></td>
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<tr>
<td>- No ALI before transfusion</td>
</tr>
<tr>
<td>- ALI during transfusion or &lt; 6 hours after transfusion</td>
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<tr>
<td>- One or more risk factors for ALI present</td>
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TRALI incidence

TRALI incidence in the general hospital population.

The lack of specific disease markers and diagnostic tests have resulted in a large variation in estimations of the incidence of TRALI. In general hospital populations, the initial reported incidence ranged from 1 in 2000 to 5000 transfusions. A more recent study reported an incidence of 1 in 1120 transfused blood products in the United States. Compared to previous figures this is a 4-fold increase. The national haemovigilance office of the Netherlands (TRIP, Transfusie Reacties In Patiënten) registers all reported TRALI cases. Since the start of registration, an increase has been observed in the number of reported TRALI cases. A rise from eight cases in 2002 to eighteen cases in 2005 suggests that TRALI is under-reported in the Netherlands (http://www.tripnet.nl). An increase in reporting may have occurred with increased awareness of TRALI. Nevertheless, a look-back study, in which recipients of blood products from a donor linked to a TRALI-fatality were analyzed for symptoms of TRALI, showed that TRALI was frequently not recognized. Therefore, under-diagnosing is not merely a consequence of awareness, but also of a failure to recognize the syndrome.

TRALI incidence on the ICU.

The above-mentioned incidence may not apply to the critically ill. These patients are highly exposed to the risks of transfusion, as up to 50-85% of them receive a blood product during their stay on the ICU. In a randomized clinical trial on transfusion threshold in critically ill patients, a restrictive transfusion policy on red blood cells was associated with a decrease in the incidence of ALI (7.7% vs. 11.4%). This suggests that some of these patients may have had TRALI. Retrospective studies suggest that ALI develops more often in critically ill transfused patients than in those who have not received transfusion. In a cohort of mechanically ventilated patients, transfusion of blood products is related to the occurrence of ALI in up to 33% of cases. A recent prospective study confirms a higher incidence in critically ill patients. In this study, patients consecutively transfused in an ICU were observed for the development of ALI within 6 hours after transfusion. Of 904 transfused patients, 74 developed ALI (8%), which is 50-100 fold higher then in the general hospital population.

Comparison of the incidence rates presented should be done with caution for several reasons. First, the definition used for TRALI differs between studies. Some required the presence of antibodies against human neutrophil antigen (anti-HNA) or against human leukocyte antigen (anti-HLA), whereas others used only clinical
In addition, surveillance systems in some countries, including the United States and the Netherlands, use an alternative definition to the consensus definition (possible or suspected TRALI), in which imputability is scored. A case definition which rules out the possibility of TRALI when another ALI risk factor is present, will lead to lower incidence rates compared to studies that have allowed for an alternative risk, i.e. possible TRALI. In critically ill patients, alternative ALI risk factors are often present. A prospective study in this patient group reported a high incidence of suspected and possible TRALI cases taken together.

TRALI pathogenesis

Presently, there are two hypotheses on the pathogenesis of TRALI. The first hypothesis suggests that TRALI is caused by donor antibodies against human neutrophil antigens (HNA) or human leukocyte antigens (HLA). This antibody-mediated reaction can not explain all TRALI cases however. Many TRALI cases are reported in which no specific anti-neutrophil antibodies are detected. Also, the majority of recipients do not develop TRALI even when their neutrophils express the cognate antigen which the transfused antibody recognizes. The second hypothesis implicates two independent “hits” (figure 1).

Figure 1. Pathophysiology of transfusion-related acute lung injury (TRALI). Neutrophils are attracted to the lung by release of cytokines and chemokines. Firm adhesion to the endothelium of the lung capillaries is realized. Neutrophils are then activated by the "second hit" of bio active lipids or HLA/HNA antibodies. This results in alveolar capillary damage and leakage of fluid into the alveolar space, resulting in the clinical picture of TRALI.
The “first hit” is the clinical condition of the patient at the time of the transfusion. An inflammatory reaction due to any cause attracts neutrophils to the pulmonary compartment. Primed neutrophils, trapped in the microvasculature of the lungs, are functionally hyperactive. The “second hit” is the transfusion. Either anti-neutrophil antibodies or bioactive lipids (lysophosphatidylcholines, lysoPCs) or cytokines that have accumulated during blood storage, further activate the primed neutrophils in the lung vasculature of the recipient. The result is endothelial damage, capillary leak and extravasation of neutrophils. Accordingly, lysoPCs as well as aged blood products were used to cause TRALI in experimental models. Also, observational studies have reported associations between prolonged storage of blood products and ARDS in the critically ill. In conclusion, both leucocyte antibodies and neutrophil priming agents released in stored cellular blood products are considered causative in TRALI. A priming condition may be required for a TRALI reaction to develop.

**Diagnosis of TRALI**

There are no specific tests to diagnose TRALI. Anti-leucocyte (HLA) antibodies class I and II and anti-neutrophil (HNA) antibodies have been implicated in TRALI. Serological workup consists of testing blood from the recipient and the implicated donors for the presence of HLA- and HNA-antibodies. Incompatibility is tested by cross-matching donor plasma against the recipient’s leucocytes. Alternatively, incompatibility can be studied by typing the recipient’s leucocytes for expression of the cognate HLA or HNA antigen. A donor with leucocytreactive antibodies which are incompatible with the patient, is excluded from further donation of blood for transfusion products. Testing for bioactive lipids such as lysoPCs is more difficult. No quantitative tests yet exist. Also, the implicated blood product often is not available. As pointed out above, aetiology and laboratory diagnosis are controversial. Serological tests for TRALI have yet to be validated against an accepted gold standard and serve only to support the clinical diagnosis of TRALI. Also, the serological workup for TRALI takes a few months, rendering this kind of diagnostic procedure unfit for clinical guidance. Therefore, TRALI remains a clinical diagnosis. Serological testing should be regarded as a preventive measure.
TRALI in the critically ill

The higher incidence of TRALI in patients on the ICU suggests that the aetiology may differ in the critically ill when compared with a general hospital population. The clinical state of a patient plays a significant role in the development of TRALI. At the time of transfusion, considerable neutrophil priming activity can be shown in TRALI patients, which could conceivably be caused by a predisposing inflammatory state. When compared with patients who did not develop TRALI after transfusion, TRALI patients more often had sustained a first event, i.e. a first “hit” before the transfusion. Implicated events include recent surgery, active infection and massive transfusion. Risk factors for the development of TRALI have been identified by a prospective study and epidemiological data from haemovigilance systems. Risk factors include surgery, in particular coronary bypass surgery, and haematological malignancies. Other clinical entities that predispose the lungs to TRALI have not yet been identified.

Possible risk factors for TRALI in the critically ill

As the “two hit” model of TRALI holds that priming of lung neutrophils at the time of transfusion can occur by a pro-inflammatory response of any origin, it is conceivable that clinical conditions causing ALI may also predispose to TRALI. In the critically ill, ALI is a common complication which can result from numerous conditions, ranging from direct pulmonary insults, such as pneumonia and aspiration, to indirect pulmonary insults, such as sepsis. Indeed, in the only prospective study on TRALI in the critically ill, patients developing ALI after transfusion were more likely to have sepsis than were controls. Also, mechanical ventilation may induce or worsen ALI (ventilator-associated lung injury). Of note, one-third of ventilated patients develop ALI after transfusion of a blood product. The risk of acquiring ALI tended to be associated with high tidal volumes, which may suggest that mechanical ventilation is a risk factor for TRALI. If indeed ALI of any origin predisposes to TRALI, the multiple possible “first hits” may explain the increased incidence of TRALI in the critically ill, when compared with the general hospital population.

Threshold model of TRALI

The concept that ICU patients are susceptible to a TRALI reaction due to an inflammatory response resulting in priming of pulmonary neutrophils, has been underlined by the proposal of a threshold model of TRALI. In this model, a threshold must be overcome to induce a TRALI reaction (figure 2). The factors that determine this 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. In this model, one side of the spectrum is a strong antibody-mediated response which can cause overwhelming TRALI in an otherwise “healthy” recipient. Conversely, it is possible that priming factors in the transfusion are not strong enough to overcome the threshold when activation status is too low. This would explain why TRALI does not develop in transfused patient even when an antibody-antigen match is present. At the other side of the spectrum is a patient with predisposing factors, i.e. a critically ill patient with ALI due to another cause. Transfusion of mediators with low neutrophil-priming activity is sufficient to overcome the threshold to induce a TRALI reaction.

In conclusion, ICU patients may run a great risk of acquiring TRALI. Specific conditions that predispose to TRALI are unknown, but may include any pro-inflammatory state.

**Management of TRALI**

Specific treatment for TRALI does not exist. Prevention of TRALI seems the best approach to reduce the incidence. All patients require additional oxygen and in 70-90 %, mechanical ventilation is unavoidable.\(^\text{16,25}\) It has been shown that ventilation with low tidal volume is protective in patients suffering from ALI compared to ventilation with conventional tidal volumes.\(^\text{40}\) In line with these data, it could be speculated that a restrictive tidal volume ventilation should be applied to avoid worsening of lung injury. However no data exist that confirm this.
Transfusion guidelines

Fewer transfusions will lead to fewer cases of TRALI. Restrictions in transfusion of erythrocytes are well tolerated in ICU patients. However, guidelines for erythrocyte transfusions are not always followed. An alternative approach which is increasingly receiving attention is the transfusion of fresh red blood cells. Stored red blood cells undergo functional and morphologic changes over time, referred to as storage lesions. Studies on the impact of aged blood on respiratory complications have yielded conflicting results. In cardiothoracic surgery patients, respiratory insufficiency and mortality was lower in patients that had received blood stored for less then 14 days compared to patients that had received blood stored for more then 14 days (7.4% vs. 11.0%, P<0.001). However, similar studies did not confirm these findings. The age of platelets has also been associated with ALI in a clinical observational study. Well-designed prospective studies are needed to determine whether patients ‘at risk’ for TRALI (i.e. critically ill or injured patients) would benefit from a differential transfusion policy using fresh products only. Also, fresh frozen plasma and platelets are the blood products most often implicated in TRALI reactions. Guidelines on transfusion of plasma and platelets in the critically ill are less clear than guidelines on transfusion of red cells. Whether a restrictive transfusion policy in patients with a coagulopathy outweighs the risk of bleeding remains to be determined.

Exclusion of women and persons who have previously received a blood transfusion from donorship

After multiple pregnancies, leukocyte antibodies are more often present due to sensitization during labour. This sensitization is also seen in recipients of blood transfusion. In the United Kingdom, women and transfused males have been excluded from donation since 2004. First results after implementation of this policy showed an absence of TRALI cases reported by British blood banks. Based on these results, Sanquin decided to adopt this preventive measure in the Netherlands. Since October 2006, only plasma from non-transfused males is used for preparation of fresh frozen plasma.

In conclusion, present data suggest a relation on one hand between transfusion of plasma from multi-parous donors and on the other hand between age of cell containing products and the onset of TRALI, however the evidence is not strong enough to support a world-wide adoption in transfusion policy. Finally, it is unclear whether other transfusion factors beside the immune and storage related factors are responsible for the onset of TRALI.
Outcome of TRALI in the critically ill

TRALI is generally considered to have a good outcome. However, mortality rates of 45% from TRALI in the critically ill have been reported,\textsuperscript{25,45} as compared to the 5-15% reported mortality in other settings.\textsuperscript{47}

It should be stressed that the “two hit” TRALI model is conceptual. It merges the existing hypothesis about TRALI pathogenesis, but it does not provide guidance on how the relative contributions of host and transfusion factors, i.e. the “first” and “second hit”, should be weighed. Data are sparse, contributing to opposing opinions on the relevance of host factors by experts in the field.\textsuperscript{48} However data are now emerging that point to an important role for host factors in TRALI pathogenesis. In a cohort of mechanically ventilated critically ill patients 33% develops ALI after transfusion.\textsuperscript{23} Mechanically ventilation is known to cause mild pulmonary injury even in a protective modus, which may serve as a “first hit”. A recent mouse experiment showed that MHC-I antibodies induced TRALI reaction in the presence, but not in the absence of LPS as a priming factor, suggesting that host factors are important also in immune-mediated TRALI.

Regardless the role of host or transfusion factors in TRALI pathogenesis, it is clear that transfusion adversely affects clinical outcome in the critically ill. Length of ICU and hospital stay are increased, and a relationship between mortality and transfusion is reported.\textsuperscript{1,21} This clear association is the starting point from this thesis.

Outline of the thesis

The clear association between transfusion and adverse outcome in the critically ill is the starting point of this thesis. Although this association is acknowledged, it is not known whether specific patients are susceptible for the detrimental effects of transfusion. Nor is it understood which transfusion and patient factors contribute to adverse effects. Given the fact that transfusion is a treatment that is applied every day in ICU practice, knowledge on the association is crucial.

This dissertation focuses on risk factors, pathogenesis and prevention of transfusion-related acute lung injury. Research subjects included patients from the intensive care departments of the Academic Medical Center and the VU-Medical Center, Amsterdam, The Netherlands. Pre-clinical studies were performed at the laboratory of experimental intensive care and anaesthesiology of the Academic Medical Center, and at the laboratory of the Blood foundation Sanquin, Region Northwest, Amsterdam the Netherlands.
Part 2 of this thesis describes pre-clinical studies of TRALI. In chapter 2, the present status and future directions of animal models investigating TRALI are discussed, calling for clinical relevant in vivo models. In chapter 3 the development of a ventilator induced lung injury (VILI) model using healthy mice is described. VILI serves as a priming factor for the onset of TRALI in an antibody mediated TRALI model, which is shown in chapter 4. As the onset of TRALI is thought to be threshold dependent, we investigated whether onset of TRALI is dependent on the titer of MHC-I antibodies infused in a combined model of ventilator-induced lung injury and antibody-induced TRALI. The study shows that in the presence of injurious mechanical ventilation, onset of TRALI depends on the titer of MHC-I antibodies infused. These results are discussed in chapter 5. Thereby, these 2 chapters give proof of the concept that mechanical ventilation may serve as a “first hit” and that immune-mediated TRALI may be the result of a “two hit” event. Chapter 6 describes a novel syngeinic “two hit” in vivo transfusion model using rats. In this model we investigated the role of storage time of red blood cells (RBCs) in the onset of lung injury in healthy and in neutrophil primed rats. It was found that aged RBCs cause lung injury in the presence of a “first hit”. In this model, infusion of the supernatant of aged RBCs again causes injury, whereas washing of aged erythrocytes prevents onset of lung injury, suggesting that soluble factors mediate TRALI. In chapter 7, the role of storage time of platelets (PLTs) was investigated in a ‘two hit’ murine transfusion model. We show that aged PLTs cause lung injury in the presence of a “first hit”. Again, we found that washing of aged platelets prevents onset of lung injury, suggesting that supernatant of aged PLTs but not the aged cells immediate a TRALI reaction. In the supernatant we found accumulation of bio-active lipids during storage which showed neutrophil priming capacity in vitro. Chapter 8 investigates the contribution of different storage conditions on the accumulation of bio-active lipids during storage of cell containing.

Part 3 describes clinical studies of TRALI. In chapter 9 an overview is given of the change of perspective on TRALI. Generally stated to be a rare event with a good prognosis, this chapter provides data indicating that specific patient groups such as critically patients are at risk of developing TRALI and that TRALI contributes to adverse outcome. In chapter 10 and 11 the practice of diagnosing ALI and TRALI is investigated. Both syndromes are defined using clinical criteria, which need subjective interpretation by the physician. These chapters describe the results of a survey investigation among Dutch clinical and pre-clinical disciplines on the practice of diagnosing ALI and TRALI. The data show that the practice of diagnosing ALI and TRALI is divergent between involved disciplines and that criteria are used which are not part of the international consensus definition. Chapter 12 describes the result of a large retrospective cohort study of critically ill patients on risk factors and
outcome of TRALI. In this study we show that underlying conditions of the patient such as sepsis are a risk factor for the onset of TRALI. During the inclusion period, the National Blood Bank started with excluding female donors for fresh frozen plasma donation. We show that this measure reduces the relative risk for onset of TRALI. In chapter 13 it is shown that peri-operative transfusion of blood products results in an increase of pulmonary leakage measured with the pulmonary leakage index in a cohort of cardiac surgery patients. The transfusion of RBCs is associated with the increase of pulmonary leakage. Chapter 14 describes the incidence, risk factors and pathogenesis of TRALI in a large prospective single center study in a cohort of cardiac surgery patients. The study identifies patient risk factors and shows that antibodies in the associated blood transfusion are a risk factor for the onset of TRALI. Chapter 15 describes the results of the measurement of markers of systemic and pulmonary inflammation and coagulation in the cohort of cardiac surgery patients developing TRALI mentioned in chapter 14. In cardiac surgery patients developing TRALI, there is systemic activation of inflammation prior to the onset of TRALI. TRALI is characterized by both systemic and pulmonary activation of inflammation, as well as enhanced coagulation and decreased fibrinolysis. The next two chapters show the result of a prospective survey study on the transfusion practice of intensive care physicians, fellows and residents in our intensive care unit. Chapter 16 describes the determinants physicians use to order and transfuse red blood cells. Chapter 17 describes the reasons to transfuse plasma or platelets. Both chapters show that a majority of the blood products is transfused in the absence of bleeding. In chapter 18 the result of a randomized controlled trial on the effect of correction of mild coagulopathy before performing tracheostomy in the intensive care is discussed. These data suggest that correction of mild coagulopathy does not reduce the risk of bleeding.
Reference List


