Transfusion-related acute lung injury in the critically ill: a translational approach
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Citation for published version (APA):
Transfusion-related acute lung injury in cardiac surgery patients is characterized by pulmonary inflammation and coagulopathy

A prospective case control study


Submitted
Abstract

Background: Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion-related morbidity and mortality. Clinical data on TRALI pathogenesis are sparse. In the present study we determined markers of systemic and pulmonary inflammation and coagulation in a cohort of cardiac surgery patients developing TRALI.

Methods: Cardiac surgery patients requiring cardiopulmonary bypass were prospectively screened for the onset of TRALI. Transfused and non-transfused controls were randomly assigned to TRALI cases in a 2:1 ratio. Blood samples were taken pre- and post-operatively and at onset of TRALI. A non-directed bronchoalveolar lavage (BALF) was performed at onset of TRALI.

Results: In all patients, cardiac surgery caused systemic inflammation and neutrophil activation, evidenced by an increase of IL-6, IL-8 and EA complexes compared to pre-surgery levels (p<0.001). Prior to onset, systemic IL-8 and IL-6 levels were higher in patients developing TRALI compared to control groups (p<0.01). In the BALF of patients developing TRALI, levels of IL-8, IL-6 and EA complexes were elevated compared to control groups (p<0.05 for all). Both systemic and pulmonary levels of TATc were enhanced in TRALI patients compared to control groups (p<0.01). BALF levels of PAA% were decreased due to an increase in PAI-1 levels in patients developing TRALI compared to control groups (p<0.01), indicating decreased fibrinolysis.

Conclusions: Prior to the onset of TRALI, there is systemic inflammation and activation of neutrophils. TRALI is characterized by both systemic and pulmonary inflammation and activation of neutrophils, as well as enhanced coagulation and suppressed fibrinolysis.
Introduction

Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion-related morbidity and mortality.\textsuperscript{1-4} Although traditionally regarded as a rare syndrome, recent studies show that the incidence is high in critically ill patient populations,\textsuperscript{5-7} and significantly contributes to adverse outcome.\textsuperscript{7,8} A two event hypothesis has been postulated that may explain the high incidence in the critically ill.\textsuperscript{9,10} The first event is an underlying inflammatory condition, causing priming of the pulmonary neutrophils. The second event is the transfusion of a blood product containing either antibodies or factors that accumulate during storage, providing additional signals for neutrophil-mediated endothelial damage and lung injury. Cardiac surgery may be a risk factor for acquiring TRALI. Cardiopulmonary bypass causes systemic neutrophil priming.\textsuperscript{11} Also, deflation of the lungs during surgery may contribute to a pulmonary inflammatory condition. Both conditions may serve as a first event in TRALI pathogenesis. Finally, these patients are frequently exposed to blood products. In accordance, cardiac surgery was found to be a risk factor for TRALI in observational studies.\textsuperscript{7,12}

TRALI is considered to be part of the spectrum of acute lung injury/acute respiratory distress syndrome (ALI/ARDS), but also has distinct entities of its own. ALI/ARDS is characterized by pulmonary inflammation and coagulopathy.\textsuperscript{13} Knowledge on TRALI pathogenesis is largely restricted to animal models, in which it was suggested that neutrophils are critical in the interaction with endothelial cells.\textsuperscript{14,15} In a transfusion model, we have recently shown that transfusion-induced pulmonary injury is characterized by enhanced coagulation and decreased fibrinolysis.\textsuperscript{13,16,17}

Clinical data on priming and activation pathways in TRALI are largely absent. To gain insight in the pathogenesis of TRALI, we performed a prospective case control study in a cohort of cardiac surgery patients requiring cardiopulmonary bypass, in which markers of inflammation, neutrophil activation and coagulation in TRALI patients were compared to matched controls, before and at onset of TRALI.

Methods

Setting

The study was performed in a 32 bed mixed medical-surgical ICU of a university hospital in The Netherlands. The ICU is a “closed format” department in which patients are under the direct care of the ICU-team. The study was approved by the Ethical Committee. All patients > 18 years of age were asked informed consent prior to undergoing cardiac surgery. Exclusion criteria were pulmonary thrombo-
endarterectomy and emergency surgery. From November 2006 until February 2009, 1000 cardiac surgery patients requiring cardiopulmonary bypass were screened consecutively. Included patients were observed for the onset of TRALI during surgery and up to 30 hours afterwards on the ICU.

Design
Cases were 1:2 randomly matched with controls. Controls were transfused patients not developing ALI and patients not transfused not developing ALI. Suspected TRALI was defined using the consensus definition of TRALI (new onset hypoxemia or deterioration demonstrated by a \( \text{PaO}_2/\text{FiO}_2 < 300 \) mmHg, occurring within 6 hours after transfusion, with bilateral pulmonary changes on chest radiograph and a pulmonary arterial occlusion pressure of \( \leq 18 \) mmHg).\(^1,3,18\) As part of standard peri-operative monitoring, a pulmonary artery catheter was inserted. Chest radiographs were scored for the presence of new onset bilateral interstitial abnormalities by two independent physicians blinded to the predictor variables. When interpretation differed, chest radiograph and the description by the radiologist were reviewed to receive consensus.

Cardiothoracic Surgery/Anesthesia Procedures
The study was performed in a university hospital in The Netherlands. Patients were anesthetized according to local institutional protocol, with lorazepam as premedication followed by etomidate, sufentanil, and rocuronium for induction of anesthesia and facilitation of intubation. During the surgical procedure, sufentanil was used as analgesic and sevoflurane plus propofol were used to maintain anesthesia. Muscle relaxants were not given during the surgical procedure. Small doses of morphine and midazolam could be given at the end of the procedure. Steroids were given at the discretion of the cardio-anesthesiologist. As part of standard care, a pulmonary artery catheter was inserted for peri-operative monitoring.
In all patients, cardiopulmonary bypass was performed under moderate hypothermia (28°C–32°C), using a membrane oxygenator and a non-pulsatile blood flow. During the procedure, lungs are deflated. After the procedure, all patients were transferred to the ICU with mechanical ventilation.

ICU Management
The postoperative ICU protocol involves fluid infusion with normal saline and starch solutions, blood transfusion to maintain hemoglobin concentration (≥5.0 mmol/L), dopamine and norepinephrine in continuous infusion to achieve mean arterial blood pressure ≥ 65 mm Hg, and dobutamine and/or enoximone to achieve a cardiac index
≥2.5 L/min/m² or a mixed venous oxygenation >60%. Propofol was continuously infused until core temperature reached 36.0°C, after which propofol infusion was stopped. Analgesics include acetaminophen and morphine.

**Blood and broncho-alveolar lavage fluid collection**

Pre-operatively, on arrival at the ICU and at onset of TRALI, blood samples were collected from an indwelling arterial catheter and centrifuged at 1,500 x g for 10 minutes at 4°C. The supernatant was collected and stored at −80°C until measurements were performed. At onset of TRALI, a nondirected bronchoalveolar lavage was performed as described previously. Controls were lavaged within 30 hours of ICU admission. A 50 cm, 14 Fr tracheal suction catheter was inserted via the orotracheal tube and advanced until significant resistance was encountered, after which 20 ml 0.9% saline was instilled over 10 seconds and immediately aspirated. The recovered bronchoalveolar lavage fluid (BALF, 4 – 8 ml) was centrifuged at 1,500 x g for 10 minutes at 4°C. The supernatant was collected and stored at −80°C until measurements were performed. The cell pellet was re-suspended in PBS and cell counts were determined using a hemacytometer (Beckman Coulter, Fullerton, CA). Differential counts were done (up to 100 cells per slide) on cytospin preparations stained with a modified Giemsa stain, Diff–Quick (Dade Behring AG, Düdingen, Switzerland).

**Assays**

Thrombin–antithrombin complexes (TATc; Behring, Marburg, Germany) were measured using ELISA. Plasminogen activator activity (PAA%), and plasminogen activator inhibitor (PAI)–1 activity were measured by automated amidolytic assays. Interleukin (IL)-6, IL-8, IL-1B and elastase-alpha(1)-antitrypsin complex were measured by ELISA according to instructions from the manufacturer (Sanquin, Amsterdam, The Netherlands). Total protein levels in BALF were determined using a Bradford Protein Assay Kit (OZ Biosciences, Marseille, France) according to manufacturers’ instructions with bovine serum albumin as standard.

**Statistics**

Data were checked for distribution. Normal distributed data were analysed using ANOVA analysis and Bonferroni’s post test. Non-parametric data were analysed with Mann Withney U-test. Repeated measurements were analysed with ANOVA repeated measurements or Wilcoxon signed rank-test dependent on the data distribution. Statistical analysis was performed with SPSS 15.0.
Results

Incidence

A total of 1000 cardiac surgery patients were screened consecutively. Of these, 99 patients did not fulfill the inclusion criteria. Another 233 patients did not give informed consent, leaving 668 patients for inclusion in the study. Of these, 16 patients (2.4%) developed TRALI.

Systemic inflammation and coagulopathy in TRALI

Cardiac surgery caused onset of systemic inflammation, which was shown by an increase in levels of IL-6, IL-8 and EA complexes plasma after surgery compared to pre-surgery levels (figure 1, p<0.001). In patients developing TRALI, plasma IL-8 and IL-6 levels were higher compared to non-transfused controls (p<0.01), but not to transfused controls. Systemic levels of IL-8 and EA complexes decreased at onset of TRALI, while levels of IL-6 remained elevated. Plasma levels of IL-1b were not increased after cardiac surgery nor in TRALI patients.

Figure 1. Systemic levels of IL-8, IL-6, IL-1B and Elastase-alpha(1)-antitrypsin complex (EA) measured in plasma of patients who underwent cardiac surgery and developing transfusion-related acute lung injury (TRALI) compared to control patients (non-transfused and transfused). Data are presented as mean±sem. Mann Withney U-test and Wilcoxon signed rank-test. *p<0.05, **p<0.01 and ***p<0.001. Abbreviations: Pre= pre-surgery measurement, Post= post-surgery measurement, TRALI= onset of TRALI post-surgery. Controls samples were taken within 30 hours of ICU admission.
Markers of coagulation were measured in samples of patients after surgery and at onset of TRALI, but not pre-operatively, as divergent pre-operative use of anti-coagulant therapy pre-operative would have hampered interpretation of results. Systemic TATc levels and of PAA% were within normal levels after surgery (figure 2). At onset of TRALI, there was an increase in TATc levels and a decrease in PAA% levels compared to transfused and non-transfused controls (figure 3, p<0.001, for all).

**Pulmonary inflammation and coagulopathy in TRALI**

Patients developing TRALI had an increase in BALF protein levels compared to transfused and non-transfused controls (table 1, p<0.05 and p<0.01 respectively), indicating pulmonary leakage. This was accompanied by an influx of neutrophils in

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**Table 1.** Cell counts and protein levels in bronchoalveolar lavage fluid

<table>
<thead>
<tr>
<th></th>
<th>Non Transfused</th>
<th>Transfused</th>
<th>TRALI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell count (x10⁴/ml)</td>
<td>45 [13-87]</td>
<td>91 [17-246]</td>
<td>93 [41-240]</td>
</tr>
<tr>
<td>Neutrophil count (x10⁴/ml)</td>
<td>0.6 [0.0-2.5]</td>
<td>3.2 [0.0-9.0]*</td>
<td>4.5 [1.5-75.0]**</td>
</tr>
<tr>
<td>Protein concentration (µg/ml)</td>
<td>47 (15)</td>
<td>85 (26)</td>
<td>239 (85) ††</td>
</tr>
</tbody>
</table>

Data are presented as median [IQR] or mean (SEM). *p<0.05, Transfused controls vs. non-transfused controls, **p<0.0001 TRALI vs. non-transfused controls, Kruskall Wallis test. ‡p<0.05 TRALI vs. transfused controls, † p<0.01 TRALI vs. non-transfused controls, ANOVA analysis with Bonferonni post test.
Levels of IL-8, IL-6, Elastase-alpha(1)-antitrypsin complex (EA) and IL-1β were elevated in the BALF of patients developing TRALI compared to transfused and non-transfused controls (p<0.05 for all, figure 3). Also, pulmonary levels of TATc and PAI-1 were elevated in TRALI compared to controls (p<0.01 for all, figure 4), whereas levels of PAA% were decreased in patients developing TRALI compared to transfused and non-transfused controls (p<0.01, figure 4), indicating enhanced coagulation and impaired fibrinolysis.

The effect of transfusion on coagulopathy and inflammation

Patients who were transfused but did not meet the clinical criteria of TRALI, displayed systemic and pulmonary changes compared to non-transfused patients. Transfusion resulted in increased plasma levels of TATc and decreased levels of PAA% compared to non-transfused controls (p<0.001 and p<0.01 resp., figure 2).
Transfusion also caused an influx of neutrophils in the pulmonary compartment compared to non-transfused controls (table, p<0.05). However, there was no evidence of pulmonary neutrophil activation as the level of EA complexes were not elevated in the BALF (figure 3, NS). Also, transfusion did not result in increased BALF levels of chemokine IL-8 and pro-inflammatory cytokines. There was a decrease in BALF levels of PAA% in transfused controls compared to non-transfused controls (p<0.01, figure 4), indicating impaired pulmonary fibrinolysis.

**Discussion**

In this relatively large cohort of TRALI patients in which repeated measurements were performed prospectively, the main findings are 1) confirmation that cardiac surgery results in a systemic pro-inflammatory status, 2) TRALI is preceded by high systemic levels of IL-8 and IL-6, 3) TRALI results in influx of neutrophils in the pulmonary compartment.
compartment and is characterized by high pulmonary levels of pro-inflammatory cytokines, as well as enhanced coagulolation and disturbed fibrinolysis, both in the pulmonary and systemic compartment. 4) Systemic coagulopathy and pulmonary neutrophil influx are present in transfused patients not meeting TRALI criteria.

An inflammatory condition is a general finding after cardiac surgery, as confirmed in this study.\textsuperscript{11} We found high levels of IL-6, IL-8 and EA complexes in the systemic compartment, suggesting that cardiac surgery causes a pro-inflammatory response with activated neutrophils, resulting in lysosomal degranulation. Thereby, systemic activation of neutrophils may reflect the “first hit” in TRALI. The key role of neutrophils in the onset of TRALI was shown in a TRALI mouse model. Injection of anti-granulocyte antibody protected mice from the onset of TRALI in an immune-mediated model.\textsuperscript{14} In a 2-event rat model of TRALI using endotoxin as the first event and the infusion of plasma from packed red blood cells as the second hit, depletion of the neutrophils also prevented onset of TRALI.\textsuperscript{22} The role of a systemic “first hit” in inducing TRALI is also suggested by observational clinical studies showing that sepsis is a risk factor for the onset of TRALI.\textsuperscript{5,7} In our patient population, surgery, connection to the heart lung machine and deflation of the lungs during surgery may have served as the “first hit”.\textsuperscript{11,23}

Prior to onset of symptoms, patients that developed TRALI had higher plasma levels of IL-8, but not of EA complexes, compared to post-surgical transfused controls. Previously, IL-8 was found to have a neutrophil-priming effect.\textsuperscript{24} Of interest, infusion of an IL-8 antibody in a “two hit” animal model prevented onset of ALI.\textsuperscript{25} We hypothesize that in response to surgery or cardiopulmonary bypass, endothelial cells produce IL-8, contributing to attraction of neutrophils to the pulmonary compartment and priming, by increasing the surface expression of cellular adhesion molecules.\textsuperscript{26} The conformational change in the β2-integrins on neutrophils induced by IL-8,\textsuperscript{27} may result in adherence of neutrophils to endothelial cells via interaction with adhesion molecules, thereby contributing to a first event in the TRALI pathogenesis.\textsuperscript{26,27}

Patients that developed TRALI showed an influx of neutrophils in the lungs and increased pulmonary levels of EA, suggesting that neutrophils have migrated to the pulmonary compartment and are activated. In line with this observation, pre-clinical TRALI models show that a “first hit” of endotoxin results in neutrophil sequestration and priming in the pulmonary compartment. However, activation of these neutrophils only occurs after a “second hit” of infusion of antibodies or supernatant of stored plasma from red blood cells or platelet concentrates.\textsuperscript{28,29} Our study is the first to confirm above mentioned pre-clinical findings in the clinical setting.
As activation of coagulation is a characteristic of ALI/ARDS, we determined markers of coagulation in our cardiac surgery patients. Patients developing TRALI showed activation of coagulation and decrease in fibrinolytic activity both in the pulmonary and systemic compartment. Enhanced coagulation was not yet present after surgery. In TRALI, we hypothesize that coagulopathy is induced by pro-inflammatory cytokines, levels of which were enhanced in our TRALI patients. Pro-inflammatory cytokines are important mediators of activation of coagulation. Infusion of TNF-α into healthy human volunteers induces activation of coagulation. Subsequent, blocking of IL-6 attenuated activation of coagulation both in the systemic and pulmonary compartment. Furthermore, inflammation may partly induce coagulopathy by a relative insufficiency of the natural anticoagulant systems, with a simultaneous suppression of the fibrinolytic system. Our results suggest that inflammation is an early event in TRALI, followed by systemic and pulmonary coagulopathy. This suggestion is in line with our previous findings in transfusion models, in which aged red blood cells and platelet concentrates induced systemic and pulmonary activation of coagulation and decrease in fibrinolytic activity. The implication of these findings is that there may be a place for interventions that target the coagulation system in TRALI. Indeed, activated protein C and antithrombin were found to ameliorate lung injury in several experimental designs.

Our results do not confirm previous findings indicating systemic IL-6 level as a possible marker for the onset of TRALI. Here, systemic IL-6 levels were elevated in all groups post-surgery and remained elevated. An explanation for this finding is tempting. In the present study, the inflammatory response following cardiac surgery may have caused the systemic IL-6 increase. Alternatively, it is unclear whether the time relation between onset of TRALI and sampling was equal in both studies. The results from our study rather point out IL-8 as a possible marker for TRALI. However, we would like to stress out that our study was not designed to establish markers for diagnosing TRALI.

Of interest, in patients not meeting TRALI criteria, transfusion resulted in distinct abnormalities. Here, we show that transfusion resulted in an influx of neutrophils in the BALF in patients not meeting the clinical TRALI definition. However, neutrophils were not activated in the pulmonary compartment as shown by the absence of EA complexes in the BALF, possibly preventing full onset of ALI. Furthermore, some systemic coagulopathy was observed after transfusion. In line with these results, we found previously that transfusion causes mild lung inflammation, including the influx of neutrophils, in animal models of TRALI. A significant number of studies indicate an association between transfusion and pulmonary injury in surgery, trauma and critically ill patients, the nature of which remains largely unknown. Although
the clinical significance remains to be determined, we hypothesize that transfusion-induced mild neutrophil-mediated lung injury may contribute to the widely observed adverse reactions associated with transfusion.41

Conclusion

In a cohort of cardiac surgery patients, TRALI is preceded by high systemic levels of IL-8 and IL-6. A TRALI reaction is mediated by activated neutrophils and characterized by high pulmonary levels of pro-inflammatory cytokines, as well as enhanced coagulolation and disturbed fibrinbolysis, both in the pulmonary and systemic compartment. Furthermore, in transfused patients not meeting TRALI criteria, transfusion resulted in systemic coagulopathy and pulmonary neutrophil influx.
Reference List


