Experimental strategies directed at inflammation and coagulation in ARDS and TRALI
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Blood transfusion during cardiac surgery is associated with inflammation and coagulation in the lung: a case-control study

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Abstract

Introduction: Blood transfusion is associated with increased morbidity and mortality in cardiac surgery patients, but cause and effect relation remain unknown. We hypothesized that blood transfusion is associated with changes in pulmonary and systemic inflammation and coagulation, occurring in patients who do not meet the clinical diagnosis of transfusion-related acute lung injury (TRALI).

Methods: We performed a case control study in a mixed medical-surgical intensive care unit of a university hospital in The Netherlands. Cardiac surgery patients (n=45) were grouped as having received no transfusion, restrictive transfusion (1-2 units) or multiple transfusions (≥5 units). Non-directed bronchoalveolar lavage fluid (BALF) and blood were obtained within 3 hours post-operatively. Normal distributed data were analyzed using ANOVA analysis and Dunnett post-hoc test. Non-parametric data were analyzed with Kruskal Wallis and Mann-Whitney-U test.

Results: Restrictive transfusion increased BALF levels of IL-1β and D-dimer compared to non-transfused controls (P < 0.05 for all), and IL-1β levels were further enhanced by multiple transfusion (P < 0.01). BALF levels of IL-8, TNFα and thrombin-antithrombin complex (TATc) were increased after multiple transfusion (P < 0.01, P < 0.001 and P < 0.01 resp.) compared to non-transfused controls, but not after restrictive transfusion. Restrictive transfusion was associated with increased pulmonary levels of plasminogen activator inhibitor-1 (PAI-1) compared to non-transfused controls with a further increase after multiple transfusions (P < 0.001). Concomitantly, levels of plasminogen activator activity (PAA%) were lower (P < 0.001), indicating impaired fibrinolysis. In the systemic compartment, transfusion was associated with a significant increase in levels of TNFα, TATc and a decrease in PAA (P < 0.05).

Conclusion: Transfusion during cardiac surgery is associated with activation of inflammation and coagulation in the pulmonary compartment of patients that do not meet TRALI criteria, an effect that was partly dose-dependent, suggesting transfusion as mediator of acute lung injury. These pulmonary changes were accompanied by systemic derangement of coagulation.
**Introduction**

Blood transfusion can be a life-saving intervention. However, it is increasingly recognized that transfusion itself contributes to morbidity and mortality in specific patient populations, including critically ill, cardiac surgery and trauma patients. Transfusion-related (TR-) acute lung injury (ALI) is the most serious cause of transfusion related morbidity and mortality. It is characterized by acute bilateral pulmonary permeability edema with subsequent hypoxia, classically developing within 6 hours after transfusion.

Observational studies in critically ill patients indicate that transfusion is dose-dependently associated with ALI. In these studies however, the temporal relation between transfusion and adverse outcome has not clearly been determined. In an effort to capture the association between transfusion and ALI, the term ‘delayed TRALI’ was coined, allowing for ALI to develop after a longer time span than 6 hours. In line with this, TRALI criteria are fulfilled only in a minority of patients after cardiac surgery, although hypoxia is a frequent finding following this procedure. Also in a heterogeneous population of critically ill, transfusion of red blood cells units (RBCs) dose-dependently and transiently decreased oxygenation. Together, this may suggest that transfusion can result in lung injury without fulfilling the clinical consensus criteria of TRALI.

In contrast to this view, some authors argue that the association between blood transfusion and adverse outcome does not mean that transfusion actually mediates disease. It may merely be a marker of illness severity. Observational studies on the association of transfusion and adverse outcome have been recognized to share a common limitation: they do not distinguish between residual confounding and actual causation.

To date, there are no clinical studies unequivocally showing the causal relationship between transfusion and ALI. Therefore, in the present study, we determined pulmonary and systemic effects of a blood transfusion following cardiac surgery. We choose cardiac surgery patients for our study, because cardiac surgery is a known risk factor for the development of TRALI and because this group is a relatively homogenous critically ill patient group, that is frequently transfused. We hypothesized that transfusion activates several pathways of inflammation that also mediate ALI/acute respiratory distress syndrome (ARDS) due to other causes and that such inflammatory processes may occur before meeting TRALI criteria. Pathways of interest include production of pro-inflammatory cytokines and chemotactic glycoproteins, as well as activation of coagulation and attenuation of fibrinolysis, all of which processes found during lung injury. Also, we determined whether the effects of transfusion accumulate with increased amounts of transfused blood, as dose-dependency may be an additional indication of a causal relationship.
Materials and methods

Setting
The study was part of a larger trial performed in the mixed medical-surgical intensive care units (ICUs) of two university hospitals in The Netherlands [22], designed to look for an effect of transfusion on pulmonary permeability in cardiac surgery patients, in which 60 patients were included. Both ICUs are a “closed format” department in which patients are under the direct care of the ICU-team. Patients included in the present analysis were derived from patients in one clinic, since samples for analysis were taken in only one clinic.

Design
The study was approved by the Institutional Review Board (IRB 07/098# 07.17.0539). Prior to valvular and/or coronary artery bypass surgery, patients of 18 years or older were asked informed consent for participation in the study. Exclusion criteria were off-pump surgery, emergency surgery or the use of immunosuppressive drugs. Patients were divided in three groups: patients who received a restrictive transfusion of 1-2 red blood cells (RBCs) (n=18), multiple transfused patients, defined as transfusion of five or more units, consisting of at least 2 RBCs, 2 fresh frozen plasma (FFPs) and 1 unit of platelets of 5 donors (PLTs) (n=10) and a control group receiving no transfusion (n=17). The definition of multiple transfusion included transfusion of different blood products, which is a reflection of current transfusion practice. Transfusion was given in the operation room or within the first three hours post-operatively. During the study, all transfused RBCs were leukoreduced (buffy coat removed and the erythrocyte suspension was filtered to remove the leukocytes (< 1x10⁶), which is the standard of practice in the Netherlands.[23]

Cardiothoracic surgery/Anesthesia procedures
Patients were anesthetized according to local institutional protocol, with lorazepam, etomidate, sufentanil, and rocuronium for induction of anesthesia and sevoflurane plus propofol for maintenance of anesthesia. 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. Cardiopulmonary bypass was performed under mild to moderate hypothermia (28°C-34°C), using a membrane oxygenator and a non-pulsatile blood flow. During the procedure, lungs were deflated. After the procedure, all patients were transferred to the ICU with mechanical ventilation. Patients were ventilated in a pressure controlled mode with tidal volumes targeted at 6 ml/kg.

Non-directed broncho-alveolar lavage technique
Within 3 hours post operatively, a non-directed broncheo-alveolar lavage was performed by instilling 20 ml of sterile 0.9% saline via a 50 cm, 14 gauge tracheal suction catheter.
as described previously.[24,25] In short, the distal end of the catheter was introduced via the endotracheal tube. Immediately after instillation of 20 mL over 10–15 seconds, fluid was aspirated before withdrawal of the catheter.

**Specimen processing and assays**

Bronchoalveolar fluid (BALF) and blood samples were centrifuged at 1500 × g for 15 minutes and supernatant was stored at −80°C until assays were performed. Interleukin (IL)-1β, IL-4, IL-6, IL-8, Tumor Necrosis Factor alpha (TNFα), von Willebrand Factor (VWF), prothrombin fragment 1 and 2 (F1+2), thrombin-antithrombin complexes (TATc), and plasminogen activator inhibitor type 1 (PAI-1) were measured using specific commercially available ELISAs according to the instructions of the manufacturer (IL-1β, IL-4, IL-6, IL-8 and TNFα from PeliKine-compact™, Sanquin, Amsterdam, The Netherlands; PAI-1 from Hyphen BioMed, Andrésy, France; VWF antibodies from Dako, Glostrup, Denmark; F1+2 and TATc from Siemens Healthcare Diagnostics, Marburg, Germany). D-dimer levels were determined with a particle-enhanced immunoturbidimetric assay (Innovance D-Dimer, Siemens Healthcare Diagnostics). Elastase-alpha (1)-antitrypsin complex (EA) [26] were measured by ELISA according to instructions from the manufacturer (Sanquin, Amsterdam, The Netherlands)

Plasminogen activator activity % (PAA%) was measured by an amidolytic assay.[27] Briefly, 25 μl of plasma was mixed to a final volume of 250 μl with 0.1 M Tris-Cl, pH 7.5, 0.1% (v/v) Tween-80, 0.3 mM S-2251 (Chromogenix, Mölndal, Sweden), 0.13 μM plasminogen, and 0.12 mg/ml CNBr fragments of fibrinogen (Chromogenix, Mölndal, Sweden). The results are expressed as %. Assays were performed batchwise to keep inter-assay variability as low as possible.

**Data collection**

Pre-operative European System for Cardiac Operative Risk Evaluation (EuroSCORE), physical status classification system according to the American Society of Anesthesiologists (ASA-score), predicted Vital Capacity, Forced Expiratory Volume in 1 second (FEV₁) and left ventricular function were determined. Left ventricular function was categorized as good (ejection fraction (EF) > 45%), moderate (EF <45 % but > 30%) or bad (EF ≤ 30%). Data on total operation room (OR) time, clamp time and time on cardiopulmonary bypass were extracted from the electronic patient data system. Duration of mechanical ventilation and PaO₂/FiO₂ ratio at the time of lavage were scored. Data on storage time of RBCs were obtained from the National Blood Bank. Suspected TRALI was scored using the consensus definition of ALI (new onset hypoxemia or deterioration demonstrated by a PaO₂/FiO₂ < 300 mmHg, within 6 hours after transfusion, with bilateral pulmonary changes, in the absence of cardiogenic pulmonary edema) [28-30]. Cardiogenic pulmonary edema was identified when pulmonary arterial occlusion pressure was > 18 mmHg, or by the presence of at least two of the following; central venous pressure > 15 mmHg, pre-operative a history of heart failure or
valve dysfunction ejection fraction < 45% as estimated by echocardiogram and a positive fluid balance. Chest radiographs were scored for the presence of new onset bilateral interstitial abnormalities by two independent physicians who were blinded to the predictor variables. When interpretation differed, chest radiograph and the description by the radiologist were reviewed to receive consensus.

Statistics
Data were checked for distribution. Data are expressed as mean (±SD) or median (IQR) where appropriate and in graphs as boxplots, lower hinge defined as the 25th percentile and upper hinge as the 75th percentile. Normal distributed data were analyzed using ANOVA analysis and Dunnett post-hoc test. Non-parametric data were analyzed with Kruskal Wallis and Mann-Whitney-U test. A P value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS 16.0.

Results
Patient characteristics are shown in Table 1. The multi-transfused group had a higher EuroSCORE compared to the other two groups. There were no differences in cardiac and pulmonary function between the groups, nor in clamp time. We found no difference in storage time of administered RBCs. PaO₂/FiO₂ ratio after 3 hours on the ICU did not differ between multiple-, restrictive- and non-transfused patients (Table 1). There was no difference in peri-operative use of dexamethason between groups. Multi-transfused patients, however, received prolonged mechanical ventilation compared to restrictively and non-transfused patients (Table 1). Of the transfused patients, only 2 met the clinical diagnosis of suspected TRALI.

Effect of blood transfusion on pulmonary and systemic inflammation
Transfusion was associated with an increase in levels of TNFα, IL-1β and IL-8 in the BALF when compared to non-transfused patients (fig. 1). Multiple transfused patients had higher levels of IL-8 compared to restrictively transfused patients. Transfusion tended to increase pulmonary IL-6 and EA, and to decrease IL-4 levels compared to non-transfused controls (fig. 1).

In the systemic compartment, multiple transfusion was associated with an increase in TNFα compared to restrictive and non-transfused patients (312 (345) vs. 64 (127) vs 182 (190) pg/ml resp., P<0.01). EA levels in plasma were non-significantly elevated after multiple and restrictive transfusion compared to non-transfused controls (287 (441) vs 256 (254) vs 202 (249) ng/ml resp., P=0.50) (data not shown in a graph). Other markers of systemic inflammation, including plasma levels of IL-1β, IL-4, IL-6 and IL-8 were not clearly affected by blood transfusion (data not shown).
Table 1. Demographics, baseline characteristics and peri-operative data of cardiac surgery patients.

<table>
<thead>
<tr>
<th></th>
<th>Non-transfused (n=17)</th>
<th>Transfused</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>64 (±11)</td>
<td>64 (±15)</td>
<td>71 (±6)</td>
</tr>
<tr>
<td>Sex, male</td>
<td>15 (88)</td>
<td>11 (61)</td>
<td>5 (50)</td>
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<tr>
<td>EuroSCORE</td>
<td>3.8 (±1.8)</td>
<td>4.2 (±2.4)</td>
<td>8.5 (±4.4)</td>
</tr>
<tr>
<td>ASA-score</td>
<td>2.8 (±0.6)</td>
<td>3.0 (±0.4)</td>
<td>3.2 (±0.4)</td>
</tr>
<tr>
<td>Left ventricular function^†</td>
<td>0 (0)</td>
<td>0</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Bad</td>
<td>6 (35)</td>
<td>4 (22)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Moderate</td>
<td>11 (65)</td>
<td>14 (78)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Good</td>
<td>0 (0)</td>
<td>0</td>
<td>1 (10)</td>
</tr>
<tr>
<td>FEV1, % predicted*</td>
<td>91 (24)</td>
<td>98 (25)</td>
<td>84 (23)</td>
</tr>
<tr>
<td>Type of surgery</td>
<td>0.763</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CABG</td>
<td>12 (71)</td>
<td>9 (50)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Valve replacement</td>
<td>2 (12)</td>
<td>6 (33)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (18)</td>
<td>3 (18)</td>
<td>4 (40)</td>
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<td>Clamp time, min^*</td>
<td>55 (47)</td>
<td>67 (54)</td>
<td>79 (62)</td>
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<td>Pump time, min^*</td>
<td>99 (60)</td>
<td>90 (68)</td>
<td>107 (90)</td>
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<td>OR-time, min^*</td>
<td>313 (126)</td>
<td>315 (95)</td>
<td>339 (156)</td>
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<td>CVP, mmHg^*</td>
<td>7.7 (5.9)</td>
<td>7.7 (5.6)</td>
<td>7.8 (9.6)</td>
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<td>CO, liters/min^*</td>
<td>4.7 (1.7)</td>
<td>3.8 (2.9)</td>
<td>4.4 (2.2)</td>
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<td>Storage time RBCs, in days^†</td>
<td>-</td>
<td>14.5 (10)</td>
<td>15.0 (8)</td>
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<tr>
<td>PaO2/FiO2 ratio^a</td>
<td>289 (±153)</td>
<td>343 (±94)</td>
<td>305 (±106)</td>
</tr>
<tr>
<td>Hb at ICU, mmol/L^*</td>
<td>5.7 (±0.7)</td>
<td>5.6 (±0.7)</td>
<td>5.0 (±0.4)</td>
</tr>
<tr>
<td>aPTT at ICU, sec^a</td>
<td>26 (4)</td>
<td>27 (3)</td>
<td>34 (7)</td>
</tr>
<tr>
<td>PTT at ICU, sec^a</td>
<td>12 (0.5)</td>
<td>12 (1.3)</td>
<td>18 (2.1)</td>
</tr>
<tr>
<td>MV, total on ICU, hours^*</td>
<td>10 (8)</td>
<td>14 (9)</td>
<td>19 (6)</td>
</tr>
</tbody>
</table>

EuroSCORE: European System for Cardiac Operative Risk Evaluation; ASA-score: physical status classification system according to the American Society of Anesthesiologists; FEV1: forced expiratory volume in 1 second, given in % of predicted; CVP: central venous pressure; CO: cardiac output; ICU: intensive care unit; OR= operation room time; RBCs: red blood cells; Hb: Hemoglobine. aPTT: activated partial thromboplastin time; PTT: partial thromboplastin time. MV: mechanical ventilation. Data are presented in † counts (percents), Φ mean (±SD) or in # median (IQR).

The BALF/plasma ratio of transfused patients for IL-1β, IL-4 and IL-8 was evidently greater than 1 (141; 10 and 375 resp.), indicating that inflammation is more pronounced in the pulmonary compartment. For the other cytokine levels, the mean ratio had a value of around 1, indicating that the level of the cytokines in the pulmonary compartment equalled the level in the systemic compartment after transfusion. In the non-transfused group, the BALF/plasma ratio for IL-1β, IL-4 and IL-8 was greater than 1 (6, 12 and 12 resp.), whereas the ratio for IL-6 and TNFα was clearly below 1 (0.22 and 0.15 resp.).
**Figure 1.** Cytokine levels in the bronchoalveolar fluid of cardiac surgery patients.

Non: non-transfused (n=17); Restrictive: 1-2 units of blood transfused (n=18); Multiple: ≥ 5 units of blood transfused (n=10). ***P< 0.001, **P< 0.01, *P<0.05; ns: not significant. EA: Elastase-alpha(1)-antitrypsin complex. BALF: bronchoalveolar fluid. Non-parametric test were used for analysis.

**Effect of blood transfusion on pulmonary and systemic coagulation and fibrinolysis**

Multiple blood transfusions were associated with activation of pulmonary coagulation, exemplified by an increase in BALF levels of TATc compared to restrictive- and non-transfused controls (fig. 2). For D-dimer, we found higher levels after both restrictive and multiple transfusions compared to non-transfused controls (fig. 2). BALF levels of PAA% were
lower in multiple transfused patients compared to restrictive transfused patients and non-transfused controls, indicative of impaired fibrinolysis. The decrease in PAA% may have been due to an increase in BALF levels of PAI-1 in transfused patients compared to non-transfused patients (fig. 2). Levels of VWF and F1+2 were not significantly different between groups (data not shown).

Figure 2. Markers of coagulation and fibrinolysis in the bronchoalveolar fluid of cardiac surgery patients.

Transfusion had a clear effect on markers of coagulation in the systemic compartment. In plasma, we found a significant higher level of TATc in transfused patients compared to non-transfused controls (fig. 3). Also, fibrinolysis was attenuated, as indicated by a decrease in the level of PAA% in transfused patients compared to non-transfused controls (fig. 3). Levels of D-dimer, VWF and F1+2 were not significantly different between groups (data not shown).

The response to transfusion was clearly dose-dependent for TATc in BALF and plasma, as shown in fig. 4 (Pearson correlation $\rho$ 0.694, $p < 0.001$ and $\rho$ 0.730, $p < 0.001$, resp.), but
was also apparent for TNFα and PAA in plasma and for IL-1β, PAA and PAI-1 in BALF (data not shown).

**Figure 3.** Plasma levels of TATc and PAA(%) in cardiac surgery patients.

**Figure 4.** Thrombin-antithrombin complexes (TATc) in BALF (4.A) and plasma (4.B) according to the total amount of blood products given per patient.

Non: non-transfused (n=17); Restrictive: 1-2 units of blood transfused (n=18); Multiple: ≥ 5 units of blood transfused (n=10). ***P< 0.001, **P< 0.01, *P<0.05. TATc: thrombin-antithrombin complexes; PAA(%): plasminogen activator activity %. Non-parametric test were used for analysis for TATc and a parametric test was used for PAA.

The BALF/plasma ratio of transfused patients for D-dimer, TATC and PAA% was evidently smaller than 1 (0.16; 0.28 and 0.36 resp.), and in non-transfused controls the BALF/plasma ratio was also smaller than 1 (0.01; 0.42 and 0.40 resp.), indicating that activation of coagulation and impaired fibrinolysis were more pronounced in the systemic compartment.

Multiple transfused patients had a higher risk of complications following surgery compared to restrictive and non-transfused patients, exemplified by a higher EuroSCORE. The EuroSCORE is calculated using age as well as pulmonary and myocardial function. To account for
confounding patient-related effects, we stratified patients according to their EuroSCORE in low (0-2, n=8), moderate (3-5, n=19) or high (≥ 6, n=16) risk [31] and re-analyzed the data according to these groups. We found no difference in the BALF levels of markers of inflammation and coagulopathy between groups, nor in plasma levels (data not shown). Also, duration of mechanical ventilation (14(8) vs 12(12) vs 14(15) hours resp., P 0.368) was not different between patients when stratified according to the EuroSCORE

Discussion

In this study, blood transfusion during cardiac surgery is associated with a marked pulmonary inflammatory reaction, partly in a dose-dependent manner, characterized by enhanced levels of pro-inflammatory cytokines, and bronchoalveolar activation of coagulation and inhibition of fibrinolysis. Transfusion also is associated with systemic activation of coagulation, impaired fibrinolysis and to a lesser extent in systemic inflammation. Furthermore, we confirm that the amount of transfusion is associated with longer mechanical ventilation in the ICU.

The finding that blood transfusion is associated with inflammation and activation of coagulation and impaired fibrinolysis in the lungs may indicate a mechanism of the observed association between transfusion and post-operative morbidity in cardiac surgery patients. [8] Transfusion has been shown before to up-regulate inflammatory genes and cytokine production.[32-34] To our knowledge, data on pulmonary effects are limited. In this study, the pulmonary inflammatory response after transfusion was characterized by an elevation of IL-1β, IL-8 and TNFα. In accordance, packed red blood cells were found to stimulate leukocyte IL-8 gene expression in vitro and to activate neutrophils to release IL-8.[32,35] Also, donor plasma was shown to activate peripheral mononuclear cells to produce a wide array of inflammatory mediators, including IL-1β, IL-6, TNFα and IL-8 in vitro.[34] Furthermore, there is a trend towards higher levels of IL-6 and EA, and lower levels of the anti-inflammatory cytokine IL-4 after transfusion. These same cytokines are known to be involved in ALI/ARDS. [16] Concurrently, BALF levels of IL-6 and IL-8 are correlated with development of ARDS[36] and high BALF levels of TNF, IL-1, IL-6 and IL-8 were associated with increased mortality.[37] Inflammation and coagulation have tight interaction, i.e. they stimulate each other in both pro-inflammatory and pro-coagulant directions.[36,38]

We found that blood transfusion is associated with activation of pulmonary coagulation and impairment of fibrinolysis. Coagulopathy is a distinct feature of ALI/ARDS due to other causes [16,20,21], contributing to morbidity and mortality.[39] In animals, massive transfusion resulted in extensive numbers of microemboli in the pulmonary vasculature. [40] As the endothelium initiates and regulates coagulation [41], it can be hypothesized that coagulopathy may also play a role in ALI following the systemic ‘hit’ of a blood transfusion. In accordance, we recently showed that lung injury following transfusion was characterized
by profound pulmonary and systemic coagulopathy in a two-hit murine transfusion model. [42,43] Also in this study, transfusion was associated with clear systemic activation of coagulation, whereas systemic inflammation was only mild. A possible mechanism of the observed coagulation dereangements may be activation of coagulation factor IX by the membrane of erythrocytes, which in turn is capable of activating factor X, leading to thrombin generation.[44] Of interest is the finding that transfusion was dose-dependently associated with an increase in the levels of PAI-1, since an increase in PAI-1 levels is of prognostic significance in patients with ALI/ARDS[39], sepsis[45] and pneumonia.[46] Therefore, it may be a marker of pulmonary complications.

The observed effects of transfusion were dose-dependent, at least partially. In agreement, observational studies show that the number of erythrocytes transfused is associated with the onset of TRALI as well as with adverse outcome.[7,47] However, these observational data can not distinguish between confounding and causation.[13,15] The finding of a dose-dependent relationship for the observed inflammatory reaction may contribute to the suggestion that transfusion is a mediator of lung injury and not merely a marker. Of note, not all parameters were dose-dependently affected. However, given that markers showed the same trend, we propose that this may be due to small sample size.

The finding that a single transfusion already elicits pulmonary inflammation, and that these alterations are dose-dependent, support a restrictive transfusion strategy. However, blood transfusion can not be avoided altogether, in particular not in cardiac surgery patients, calling for other strategies to limit pulmonary complications following transfusion. In cardiac surgery patients, an association between non-leukoreduced blood transfusion and mortality was found.[48] Although leukoreduction reduces levels of cytokines in stored blood, adverse transfusion-related outcome continues to occur.[49] In line with these data, we show that leukoreduced blood enhances inflammation and coagulation in the lung in cardiac surgery patients. Thus, leukoreduction may not protect against the occurrence of ALI. Storage time has been implicated in increased risk of postoperative complications as well as reduced short-term and long-term survival in patients undergoing cardiac surgery.[3] Since we found no difference in RBC storage time between restrictive and multiple transfused patients, storage time did not account for the observed differences between the groups.

This study has several limitations. Multiple transfused patients had a higher EuroSCORE than restrictive- and non-transfused patients and displayed a trend for a longer time on the cardiopulmonary bypass machine. Thereby, EuroSCORE and duration of cardiopulmonary bypass may have contributed to the pro-inflammatory response and derangement of coagulation. Therefore, we performed a separate analysis stratifying groups according to low, moderate and high EuroSCORE. We found no difference in levels of inflammatory cytokines
and markers of coagulopathy between groups. These results suggest that the observed effects were attributable to blood transfusion. In line with this, some effects of transfusion were apparent already after restrictive transfusion and this patient group did not differ in EuroSCORE and time on bypass compared to non-transfused controls. In accordance, in a previous study showing increased cytokine levels in transfused cardiac surgery patients, it was shown that transfusion, and not cardiopulmonary bypass, was the most important source for the inflammatory response.[33] In addition, in a prospective study on the mechanisms of TRALI in cardiac surgery patients, we recently found that cardiopulmonary bypass results in transient inflammation, which has subsided at the time of onset of TRALI.[50] Taken together, results may be compatible with the suggestion that blood transfusion mediates pulmonary inflammation. However, we can not exclude that other confounding factors unaccounted for, such as pumptime, may have played a role. Furthermore, our data can not be applied to a general ICU population, since we only studied cardiac surgery patients. A final limitation of this study is the use of multiple comparisons, which can yield a significant difference that actually relies on chance. However, for the majority of differences, P-value was below 0.01.

Conclusions

We show that transfusion is associated with pulmonary and systemic inflammation and activation of coagulation and impaired fibrinolysis, an effect that was in part dose-dependent. These data may indicate that transfusion is a mediator of lung inflammation in patients after cardiac surgery and not merely a marker of disease. Insight in the effects of a blood transfusion may contribute to the risk-benefit assessment of the decision to transfuse cardiac surgery patients.
Reference List


