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

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Chapter 19

Summary and general discussion
Summary

Chapter 1 provides an overview of the current knowledge on transfusion-related acute lung injury (TRALI). TRALI is the leading cause of transfusion-related morbidity and mortality.1-4 The pathogenesis of TRALI is thought to be a “two hit” event.5 The “first hit” is (any) pro-inflammatory pulmonary condition (e.g., pneumonia, sepsis or lung contusion) which results in activation of lung endothelium with sequestration of polymorphonuclear neutrophils. The “second hit” is induced by transfusion of a blood product. Either antibodies against neutrophils are thought to be implicated in the activation of the sequestrated neutrophils, or bioactive lipids (lysophosphatidylcholines (lysoPCs), which accumulate during storage of blood products, induce the “second hit”. However, there are also TRALI cases reported in which a relatively active patient or a healthy research volunteer developed TRALI.6,7 For such cases an additional hypothesis, a threshold model has been suggested, 8 in which 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 pulmonary neutrophils on the one hand and the ability of the mediators in the transfusion to cause activation of primed neutrophils on the other. When the activation status of the “first hit” is too low, it is possible that priming factors in the transfusion are not strong enough to overcome the threshold. This would explain why TRALI does not develop in a transfused patient even when an antibody-antigen match is present. In a critically ill patient with predisposing factors functioning as “first hit”, such as pneumonia, sepsis or trauma, a “second hit” with transfusion of mediators with low neutrophil-priming activity may be sufficient to overcome the threshold to induce a TRALI reaction. Conversely, when the “second hit” is strong enough, e.g. a strong antibody-mediated response, severe TRALI can occur in an otherwise “healthy” recipient. Besides the overview, the general aims and outline of the thesis are described in this chapter.

Chapter 2 reviews the present TRALI animal models and their strengths and weaknesses.9-15 Future animal models are proposed, in which clinically relevant “first hits” can be applied, thereby imitating the complex clinical situation. In this chapter the need for the development of therapeutic approaches for this potentially life-threatening disease are underlined. Several interventions which have proven to be effective in acute lung injury and which may also be beneficial in TRALI are discussed. The application of these interventions requires the development of clinically relevant TRALI animal models, including mechanical ventilation. It has been suggested that ventilator induced lung injury (VILI) may serve as a “first hit” in the onset of TRALI.16

In chapter 3 the development of a clinical relevant model of VILI in mice is described. Mechanically ventilated mice were randomized into 2 ventilation groups, including
mechanical ventilation with low ventilation tidals (protective modus) (~ 7.5 ml/kg) and mechanical ventilation with high ventilation tidals (injurious modus) (~ 15 ml/kg). Ventilation with high tidals resulted in the onset of VILI compared to animals ventilated with low tidals. This was evidenced by an increase in pulmonary levels of cytokines and chemokines, neutrophil influx and protein leakage.

In **chapter 4** the influence of mechanical ventilation on the development of TRALI is investigated, combining the mouse VILI model which is presented in **chapter 3**, with a model of antibody-induced TRALI. In animals in which TRALI was induced, mechanical ventilation with low tidal volumes aggravated pulmonary injury, as evidenced by an increase in neutrophil influx and pulmonary and systemic levels of cytokines, as well as worsening of lung histopathological changes compared to unventilated controls. The use of high tidal volume ventilation resulted in a further increase in protein leakage and pulmonary edema. This study showed that mechanical ventilation synergistically augmented lung injury during TRALI, which was even further enhanced by the use of high tidal ventilation. We hypothesized that mechanical ventilation can be a “first hit” in TRALI pathogenesis. In line with the threshold model, the presence of a “first hit” may require a weaker second hit for the induction of TRALI. Therefore, in **chapter 5**, it was investigated whether onset of TRALI is dependent on the titer of major histocompatibility complex (MHC)-I antibodies infused in a combined model of VILI and antibody-induced TRALI. Mice were ventilated for five hours with low or high tidal volume, as described in **chapter 4**. After three hours of mechanical ventilation, TRALI was induced by infusion 0.5 mg/kg, 2.0 mg/kg or 4.5 mg/kg MHC-I antibodies. Controls received saline infusion. After five hours of mechanical ventilation, animals were sacrificed. Mice ventilated with high tidal volumes developed lung injury after infusion of 4.5 mg/kg, but not after infusion of lower concentrations of MHC-I antibodies, or in the presence of low tidal ventilation. Results suggest that decreasing the concentration of MHC-I antibodies in blood products may abrogate or at least decrease the severity of a TRALI reaction in mechanically ventilated patients. Besides immune mediated TRALI, non-immune mediated TRALI is suggested to play an important role in the onset of TRALI in critically ill patients suffering from an underlying condition. It is hypothesized that transfusion of aged cell-containing blood products may cause TRALI in the presence of a “first hit”. In **chapter 6** the effect of transfusion of aged red blood cells (RBCs) on the onset of lung injury is described. Using a syngeneic *in vivo* transfusion model it was investigated whether the transfusion of stored rat RBCs causes lung injury in healthy and in lipopolysaccharide (LPS)-primed rats in a “two hit” event of lung injury. In healthy rats, transfusion of aged RBCs (stored for 14 days) caused mild pulmonary inflammation, but no coagulopathy. In LPS-pretreated rats, transfusion of aged RBCs augmented lung injury by inducing coagulopathy, both in the pulmonary and systemic compartment, when compared
to transfusion with fresh RBCs. We tried to determine which substance caused the lung injury by washing of the aged cells. When transfused separately, supernatant of aged RBCs, but not washed aged erythrocytes, mediated coagulopathy in the “two hit” model. Analysis of the supernatant of aged RBCs showed no bio-active lipid (lysophosphatidylcholine, lysoPC) accumulation. This study showed that not the aged erythrocytes, but factors in the supernatant cause lung injury in a clinically relevant transfusion model, an effect which was modulated by the presence of a “first hit”.

In chapter 7 the effect of aged platelet concentrates (PLTs) on the onset of lung injury was investigated in a newly developed syngeneic in vivo transfusion model using healthy rats as well as in a “two hit” model using LPS-pretreated rats. In addition, the effect of washing of platelets was studied. In healthy rats, transfusion of aged PLTs caused mild lung inflammation. In LPS-pretreated rats, transfusion of aged PLTs, but not fresh PLTs, augmented pulmonary and systemic coagulopathy. When PLTs components were transfused separately, supernatant of aged PLTs, but not washed aged platelets, induced pulmonary injury in the “two hit” model. Supernatants of aged PLTs contained increased concentrations of lysoPCs compared to fresh PLTs, which enhanced neutrophil priming activity in vitro. This study showed that in a “two hit” in vivo transfusion model, aged platelets cause lung injury in which pulmonary coagulopathy is a prominent feature. This effect may be caused by lysoPCs which have accumulated in the aged platelet products. In addition, chapter 6 and 7 suggest that in the preparation of blood products, washing of aged cells may decrease adverse effects of a blood transfusion.

In chapter 8, storage conditions that influence lysoPC accumulation and neutrophil priming capacity were studied in stored RBCs and PLTs products, as produced by Sanquin Blood Bank (SBB). Blood was drawn from healthy volunteers and processed and stored according to SBB protocols. During storage, samples were taken regularly. Neutrophil-priming capacity and accumulation of lysoPCs in RBCs stored in SAGM solution were absent. RBCs with plasma added to the storage solution showed elevated lysoPC levels at day 0, which did not further increase. Accumulation of lysoPCs in cell-depleted plasma occurred at 22 °C, but not at 4 °C. However, both stored plasma’s did not induce neutrophil-priming. These data suggest that lysoPC accumulation is not cell dependent but plasma and temperature dependent. Furthermore, in PLTs, lysoPC accumulation during storage is only a partial explanation of the observed neutrophil-priming effect as removing of the lysoPCs did not decrease the neutrophil-priming capacity.

Chapter 9 reviews the change of perspective of TRALI in the past decades. Two decades ago, TRALI was considered a rare complication of transfusion-medicine. Nowadays, TRALI has emerged as the leading cause of transfusion related mortality, presumably as a consequence of reaching international agreement on defining TRALI
with subsequent increased recognition and reporting of TRALI-cases.\textsuperscript{1-4} Specific patient populations such as critically ill patients have an increased risk to develop TRALI,\textsuperscript{19,20} which may be explained by the two event hypothesis.\textsuperscript{5} Opposed to the traditional view that TRALI has a good prognosis,\textsuperscript{21} TRALI may have a significant impact on morbidity and outcome, at least in specific patient groups.\textsuperscript{20,22} The association of transfusion with adverse outcome calls for blood product and donor management strategies aimed at decreasing the risk of acquiring TRALI. Excluding female donors for plasma donation may reduce onset of TRALI, but does not prevent the occurrence of TRALI.\textsuperscript{23,24} Furthermore, in this chapter it is stressed that studies are needed to identify patients who are at risk for the onset of TRALI. In such studies, the consensus definition should be applied to improve comparability of risk factors and outcome of TRALI across patient populations.

In \textbf{chapter 10} the results of a survey on diagnosing acute lung injury are presented. Incidence reports on acute lung injury (ALI) vary widely. Insight in diagnostic preferences of critical care physicians when diagnosing ALI may improve identification of the ALI patient population. Critical care physicians in the Netherlands were surveyed using vignettes involving hypothetical patients and a questionnaire. The results showed that of the respiratory determinants, PaO$_2$/FiO$_2$ and PEEP level are considered important determinants in diagnosing ALI, whereas pulmonary artery occlusion pressure and results of chest X-ray are considered less important. A history of heart failure, level of compliance or presence of a risk factor for ALI had no impact on diagnosing ALI. Furthermore, the medical background specialty influenced preferences of diagnostic determinants.

In \textbf{chapter 11} the results of a survey on diagnosing TRALI are presented. TRALI is a clinical diagnosis. Although a international consensus definition exists, disciplines involved in TRALI diagnosis may differ in diagnostic preferences. A survey was conducted among critical care physicians, hematologists, hemovigilance workers and transfusion medicine physicians, using case vignettes and a questionnaire. The vignettes varied in patient and blood product related factors that may influence the decision to report a TRALI-case. Preferences in favor of reporting TRALI were ‘classic’ TRALI symptoms: onset within 1 hour, after transfusion of FFP, in the absence of lung injury prior to transfusion. An admission diagnosis of sepsis was a negative preference. Massive transfusion (6 RBC + 4 FFP units) was a negative preference for transfusion medicine physicians, but a positive preference for the other disciplines. This shows that the presence of a “first hit” is considered a reason not to report a suspected TRALI-case. Furthermore, disciplines involved in managing TRALI differ in decision-making on the reporting of suspected TRALI–cases and asking for an immunologic work-up. This may partly explain variances in TRALI diagnosis and estimates of incidence.
In chapter 12 the result of a retrospective case control study on the incidence, risk factors and outcome of TRALI in a cohort of critically ill patients is presented. Screening of 2,024 patients for the onset of ALI yielded 109 suspected TRALI-cases. Compared to transfused controls, risk factors for TRALI included emergency cardiac surgery, hematologic malignancy, massive transfusion, sepsis, mechanical ventilation and high APACHE II score. The amount of plasma was associated with TRALI in the univariate analysis. However, this association disappeared in the multivariate analysis. TRALI contributed to adverse outcome, with longer duration of mechanical ventilation and lower survival. In line with the ‘threshold model’, incidence of TRALI is high in the critically ill, which can be explained by an underlying systemic inflammatory condition of such patients and by transfusion of high volume plasma products and platelet concentrates.

Chapter 13 is a multicenter trial on the effect of a blood transfusion on pulmonary vascular permeability in a cohort of cardiac surgery patients (n=60), using the Pulmonary Leakage Index (PLI). Cardiac surgery resulted in an elevated PLI. Transfused patients had a higher PLI compared to non-transfused patients. The amount of red blood cell products, but not of fresh frozen plasma (FFP) or platelets, was associated with an elevated PLI. The causative factor in the blood products was not found, as the median concentration of bio-active lipids transfused nor the presence of human leukocyte or human neutrophil antibodies (HLA/HNA) antibodies were associated with an elevated PLI. In line with findings in the transfusion model mentioned in chapter 6 and 7, these results emphasize that transfusion can cause mild pulmonary leakage, before meeting the TRALI diagnostic criteria.

In chapter 14 the results of a prospective nested case control study on the incidence and risk factors of TRALI in cardiac surgery patients are presented. Patients were prospectively observed for the onset of TRALI according to the consensus definition. Blood products were screened for bio-active lipid accumulation and storage time. Samples from donors were analyzed for the presence of HLA/HNA antibodies. Of 668 patients included, 16 patients developed TRALI (2.4%). Patient-related risk factors for onset of TRALI were age and time on the cardiopulmonary bypass. Transfusion related risk factors for TRALI were the total amount of blood products transfused, the number of red blood cells stored > 14 days, the volume of plasma transfused, the presence of HLA/HNA antibodies in the donor plasma and the total amount of bio-active lipids transfused. When adjusted for patient risk factors, only the presence of HLA or HNA antibodies in the associated blood products remained a risk factor. In-hospital mortality of TRALI patients was high (13%) compared to transfused and non-transfused control patients (0% and 3% respectively). We show that the incidence of TRALI is high in cardiac surgery patients, contributing to an adverse outcome. Risk factors include duration of cardiopulmonary bypass and transfusion of antibody containing blood products.
In chapter 15 the results of measurements of markers of systemic and pulmonary inflammation and coagulation in the nested case control study of a cohort of cardiac surgery patients mentioned in chapter 14 are shown. In all patients, cardiac surgery caused onset of systemic inflammation and neutrophil activation, evidenced by an increase of IL-6, IL-8 and elastase-alpha(1)-antitrypsin complexes compared to pre-surgery levels. Post-surgery and prior to onset of symptoms, systemic IL-8 levels were higher in patients developing TRALI compared to controls, suggesting that IL-8 may mediate priming of pulmonary neutrophils. In the broncho-alveolar lavage fluid of patients developing TRALI, levels of IL-8, IL-6 and EA complexes were elevated compared to controls. Both systemic and pulmonary levels of thrombin–antithrombin complexes were enhanced in TRALI patients, together with decreased levels of plasminogen activator activity due to an increase in plasminogen activator inhibitor–1 activity levels. Results of this study shed some light on mechanisms of TRALI pathogenesis, which are for the most part unknown. TRALI is preceded by high systemic levels of IL-8 and IL-6, which may add to priming of the pulmonary neutrophils, at least in cardiac surgery patients. A TRALI reaction is mediated by activated neutrophils and characterized by high pulmonary levels of pro-inflammatory cytokines, as well as enhanced coagulation and disturbed fibrinolysis, both in the pulmonary and systemic compartment.

Chapter 16 describes the result of a prospective cohort study in which transfusion triggers were determined in consecutively admitted patients during a 10–week period, to provide insight into determinants of the decision of ICU–physicians to transfuse. Using a questionnaire, the reason of ICU physicians to transfuse RBC was evaluated. Of 310 admissions, 90 patients (29%) received a RBC transfusion. RBC were transfused on a mean Hb–level of 7.4±1.1 g/dl, which accords with the landmark study of Hebert, showing that a Hb-level of 7.0 g/dl is safe in critically ill patients. Residents transfused RBC on a higher Hb–level compared to staff (7.7±1.0 versus 6.9±1.3, p<0.05). The most important reason for physicians to transfuse RBCs was suspicion of bleeding. The average order for RBC transfusion was 4 units. Of each order, 38% of the units was not administered. This study shows that RBC transfusion trigger is restrictive. However, RBC transfusion behavior does not take specific patient characteristics into account.

Chapter 17 describes the results of a survey study on the reasons of ICU-physicians to transfuse fresh frozen plasma (FFP), to provide insight into determinants of the decision of ICU–physicians to transfuse. 67% of the FFPs were transfused in bleeding patients and 33% of the FFPs in non-bleeding patients. FFP was transfused at a pro-thrombin time (PT) of 19 sec [17-22]. On average, 3.2 FFP units were ordered, of which 28% was not transfused. The major reason to transfuse platelets was bleeding. Platelets were transfused at a platelet count of 95 [36-116] x10^9/L in bleeding and 13 [10 - 18] x10^9/L in non-bleeding patients. On average, 1.4 platelet
units were ordered, of which 20% was not transfused. Chapter 16 and 17 show that a significant number of transfusions in the intensive care are given in the absence of bleeding. Whether this transfusion policy is rational or irrational could not be determined in these studies. From a prevention perspective, reduction of the amount of products transfused will decrease the risk of TRALI.

In chapter 18 the results of a randomized controlled trial on the effect of correction of mild coagulation disorders on bleeding during and after percutaneous dilatational tracheotomy (PDT) is presented. PDT is a simple bedside procedure in critically ill patients. ICU-patients however, frequently have coagulation disorders which could place them at higher risks for bleeding during or after PDT. ICU-patients planned for bedside PDT with a prolonged prothrombin time, thrombopenia or treatment with acetylsalicylic acid, were randomized to receive infusion with FFP and/or platelets ("correction") versus no transfusion ("no correction") before PDT. 35 patients were randomized to the "correction" group and 37 patients to the "no correction" group. There were no patients with clinically significant bleeding in either group. Median blood loss in the "correction" group was 3 [IQR: 1 – 6] grams compared to 3 [IQR: 2 – 6] grams in the "no correction" group (P = 0.96). The duration that blood was visible in tracheal aspirates was short (a median of 1 hour) and not affected by the study intervention. Bleeding during or after bedside PDT in ICU patients with mild coagulation disorders is rare. In this pilot study, correction of subclinical coagulation disorders by transfusion of FFP and/or platelets did not affect bleeding during or after PDT.
General discussion

TRALI in the critically ill patient population

The incidence of TRALI is high on the ICU. In agreement with our studies, incidence was reported to be 8% in a critically ill patient population in the US. The high incidence may be explained by the “two hit” hypothesis, in which inflammatory conditions commonly found in the critically ill, predispose for the occurrence of TRALI. Results from the present studies identifying TRALI risk factors underline this hypothesis. We found that sepsis, mechanical ventilation and cardiac surgery are risk factors for the onset of TRALI and that prior to the onset of TRALI in cardiac surgery patients, there is systemic inflammation and activation of neutrophils. Our results suggest that established risk factors for ALI (e.g. massive transfusion, sepsis) may actually be risk factors for the onset of TRALI. These findings raise the question whether TRALI should be regarded as a diagnosis after exclusion of other ALI risk factors. Rather, we suggest that TRALI may be viewed as an entity of the ALI spectrum. Generally considered to have a good prognosis, we show that TRALI may contribute to adverse outcome. Taken together, we show that TRALI is a significant problem in the critically ill patient population.

Immune-mediated TRALI

Results from experimental studies in the transfusion models suggest that mechanical ventilation may serve as a “first hit” in the onset of TRALI and that it aggravates the course of the disease. These data support observational studies suggesting that mechanical ventilation may be a risk factor for TRALI. Furthermore, we show that immune-mediated TRALI can also be a “two hit” event. It is generally believed that the “two hit” hypothesis applies to TRALI caused by bio-active substances. However, also TRALI mediated by antibodies seems to depend on the patient’s predisposition. Taken together, results of our studies may explain why the incidence of TRALI is high in the critically ill patient population.

Non-immune mediated TRALI

We show that both aged RBCs and PLTs induce mild pulmonary injury in healthy rats. It could be speculated that after multiple transfusions, adverse effects accumulate, resulting in acute lung injury, thereby contributing to the widely observed transfusion-related morbidity. In a “two hit” model, injury induced by aged RBCs or PLTs is characterized by coagulopathy and is abrogated by washing. The results suggest that washing of aged RBCs and PLTs may decrease pulmonary complications in patients with an inflammatory condition who are exposed to a blood transfusion. Washing of RBCs has been shown feasible in the clinical setting.
Clinical trials investigating whether washing of RBCs will reduce onset of pulmonary complications after RBCs transfusion are needed to confirm our results. With respect to the causative agent in the supernatant, lung injury caused by transfusion of aged PLTs was associated with lysoPC accumulation during storage. However, we found that accumulation of lysoPCs during storage only partially mediates neutrophil-priming capacity. Also, in contrast with previous studies, we found no accumulation of lysoPCs during storage of RBCs.\textsuperscript{14,35,36} It should be mentioned that considerable differences in manufacturing and storage protocols exist between blood banks. Our data may have relevance for manufacturing processes, suggesting that storage conditions have significant effect on accumulation of lysoPCs and \textit{in vitro} neutrophil priming capacity.

Our data suggest that the “first hit” plays a key role in the critically ill patient population for both the immune and non-immune mediated TRALI. For this reason, the term ‘possible TRALI’, which delineates TRALI in the presence of another risk factor, is misleading. TRALI should be seen as part of the ALI spectrum and not as a diagnosis per exclusionem. Directions for further research should be focused on identifying and confirming other clinical relevant risk factors (“first hits”) for the onset of TRALI, such as sepsis and pneumonia. Insight in risk factors of TRALI may help to identify specific patient populations which would benefit most from an adapted transfusion policy.

\textit{Diagnosing TRALI}

We found that the presence of a pre-transfusion inflammatory condition is a reason to withhold from reporting a suspected TRALI-case. Thereby, the current practice of physicians of reporting suspected TRALI-cases does not reflect belief or knowledge of a “two hit” mechanism. Our data indicate that patients suffering from an underlying condition or receiving massive transfusion will probably be withheld from immunologic diagnosing of TRALI, while implicated donors my cause a future TRALI reaction again. Besides implications concerning patient care, consistent reporting of TRALI cases will provide reliable data on incidence and may increase insight in risk factors for acquiring TRALI. We consider it important that all patients suspected for TRALI are dealt with in a same fashion, using the international consensus case definition instead of local imputability scores.

\textit{Reducing TRALI}

At this moment no treatment is available. Reduction of TRALI incidence is predominantly achieved by prevention. A decade ago, it was shown that a restrictive RBC–transfusion hemoglobin (Hb) level of 7 g/dl is well tolerated in ICU–patients.
and may even reduce mortality in specific patient groups.\textsuperscript{37} However, transfusion practice has changed little during the past decade.\textsuperscript{38} ICU–physicians have been reluctant in adopting conservative RBC–transfusion thresholds\textsuperscript{39-41} and there is wide variation in transfusion practice of RBC.\textsuperscript{42,43} Our study shows that RBC transfusion decisions are predominantly based on Hb levels rather than on patient characteristics. The survey on FFP and platelets transfusion revealed that one-third of FFP transfusions was given to non-bleeding patients and that FFP transfusion failed to normalize prolonged coagulation test results in the majority of the patients. For this reason, we performed a randomized trial investigating whether correction of subclinical coagulation disorders by transfusion of FFP and/or platelets during or after PDT would reduce bleeding. Our results suggest that correction of mild coagulation disorders prior to an intervention is not necessary. Education on indications of transfusion of RBCs, FFP and platelets and improved identification of bleeding are needed and may facilitate a reduction in transfusion rates, thereby reducing both adverse effects as well as health care costs. However, a larger study is needed to confirm this result for a larger patient population and different types of interventions. Finally, these studies should result in proper evidence based transfusion guidelines in the critically ill patient population. An overall reduction of any type of blood transfusion will finally reduce the onset of TRALI.

In the end, transfusion can not be avoided altogether. Another approach is to exclude blood donors which are at risk of inducing TRALI. Female donors, more specific multi-parous donors, have a higher incidence of HLA/HNA antibodies due to sensitization during labour.\textsuperscript{44} Our results suggest that exclusion of HLA or HNA antibody-positive blood products may reduce TRALI incidence, at least in cardiac surgery patients. In accordance, two clinical studies have appeared, indicating that excluding female donor plasma may prove to be effective.\textsuperscript{23,24} Of note, the Dutch National Blood Bank started with the use of male only plasma for the preparation of PLTs products after our study was finished. Whether the use of male-only products will prevent onset of TRALI remains to be established.

Another intervention may be the use of fresh blood only. Our pre-clinical and clinical studies support this hypothesis. However, not all clinical observational studies confirm a beneficial effect of a transfusion policy of fresh blood only.\textsuperscript{45-48} Any recommendation of age of blood products should await results of prospective trials, which are currently underway.\textsuperscript{49} Also, the feasibility of a transfusion policy of ‘fresh blood only’ needs to be investigated, as such a policy may importantly hamper a steady blood supply.

Lastly, we would like to stress that when results of trials investigating the effects of fresh blood only will be available, conclusions should be drawn with caution.
We found considerable influence of storage conditions on lysoPC accumulation and neutrophil priming capacity. Differences in blood processing and storage conditions of blood products worldwide, will hamper generalization of conclusions to countries which were not involved in the trial. Besides trials investigating whether transfusion of fresh blood compared to aged blood reduces mortality, studies are needed that investigate pathways through which detrimental effects of transfusion of aged blood is mediated. Insight in these pathways may help to improve storage conditions, without impeding a continuous reliable blood supply.
Reference List


