Chapter 9

Discussion and Future Perspectives
Allogeneic (leukocyte-containing) red blood cell (RBC) transfusions have profound effects on the recipient’s immune system. Besides induction of allo-antibody formation, also immunomodulation occurs impairing the allograft rejection after renal transplantation. This suppression of the renal allograft rejection led to concern about deleterious effects of blood transfusions on cancer recurrence and susceptibility for postoperative infections [1,2]. This hypothesis resulted in several observational studies and a limited number of randomized controlled trials (RCTs), mainly investigating the effect of leukocyte-containing blood transfusions on postoperative infections. Only two RCTs investigated cancer recurrence. One study compared leukocyte-containing RBC with leukocyte-depleted filtered RBC transfusions and did not show a difference in distant metastasis nor local recurrence between the two groups at 2-years and 5-years follow-up [3,4]. The other RCT compared allogeneic versus autologous, both buffy-coat depleted, leukocyte-containing RBC transfusions, also without a difference between the two groups [5]. In contrast, controversial results in different patient populations using different study designs were found for an effect of leukocyte-containing RBC transfusions on postoperative infections [6]. Our group conducted two RCTs on the role of leukocyte-containing RBC transfusions on the occurrence of postoperative infections in colorectal surgery. Two studies, one aforementioned in colorectal cancer surgery and one in miscellaneous gastro-intestinal and vascular surgery, found no evidence for increased susceptibility for postoperative infections after leukocyte-containing RBC transfusions [3,7].

The possible adverse immunomodulatory effects of blood transfusions are referred to as transfusion-related immunomodulation (TRIM). The existence and possible mechanisms of TRIM are hitherto purely descriptive. Extensive animal and laboratory studies investigated the possible mechanisms of immunomodulatory effects of blood transfusions and their relationship with clinical manifestations. Several factors have been suggested to play a role. Most suspected are: allogeneic mononuclear and apoptotic cells, soluble biological response modifiers circulating in plasma and leukocyte-derived cytokines and chemokines. Allogeneic leukocytes or soluble factors released by leukocytes during storage have been most extensively studied in the past years [8].

Clinical Effects of Allogeneic Leukocytes in Cardiac Surgery

Compared with other adverse transfusion effects, the clinical effects of leukocyte-mediated TRIM on mortality after cardiac surgery is more excessive (Figure 1) [10]. In cardiac surgery strong and dose-dependent associations between blood transfusions and postoperative morbidity and mortality are present. However, it is obvious that patients with more
Discussion and Future Perspectives

Preoperative risk factors and undergoing more complex surgery are at higher risk for the development of postoperative complications and these patients receive also more blood transfusions, as compared to patients undergoing to less complex surgery. RCTs, balancing such confounding factors, may help to distinguish between patient, surgery and transfusion factors affecting outcome. Because allogeneic leukocytes are the most important factor held responsible for the clinical effects of TRIM; RCTs investigating their role are indispensable.

Figure 1 | Estimates of risk of death per unit transfused RBCs for several transfusion-related complications in patients undergoing cardiac surgery. (with permission, from Despotis et al. Transfusion 2008; 48:25-30S)

The first RCT in cardiac surgery was performed in the Netherlands [9]. This study comprising 914 patients randomized to receive 3 different RBC products; buffy-coat depleted RBCs were compared with either pre-storage filtered freshly drawn RBCs filtered after storage prior to transfusion, containing during storage accumulated leukocyte-derived soluble factors. An increase in postoperative infections between transfusion of buffy-coat depleted RBCs and the two types of leukocyte-depleted RBCs was only found in patients receiving more than 3 units of RBCs. Moreover a significant higher 60-day mortality rate in patients receiving buffy-coat depleted RBCs compared with both types of leukocyte-depleted RBCs was found. This difference in mortality was mainly due to death with diagnosis of multiple-
organ-dysfunction-syndrome (MODS) in the patients receiving buffy-coat depleted RBCs. In this study MODS was not scored as an endpoint.

We performed a second randomized controlled trial in more complicated cardiac surgery associated with a higher probability of multiple RBC transfusions in order to explore the relationship between leukocyte-containing transfusions on MODS and mortality (Chapter 2). In this study mortality at 90 days after surgery was the primary endpoint and hospital mortality rate and the incidences of MODS and postoperative infections were the secondary endpoints. The 90-day mortality showed a non-significant reduction of 40% and the hospital mortality was halved in the group that received pre-storage leukocyte-depleted RBCs, confirming the results of the previous study. The differences between these endpoints were more pronounced in the patient groups who received more than 3 units of RBCs. The main cause of excess mortality was MODS; despite the incidence of MODS was similar in both patient groups. Furthermore the other endpoint (postoperative infections) was reduced significantly in the group that received pre-storage leukocyte-depleted RBCs. Few RCTs followed to investigate the role of leukocyte-depleted RBCs in cardiac surgery [11-14]. The characteristics and results of these studies are presented in Table 1. A meta-analysis of these studies in cardiac surgery revealed an increased short-term mortality after transfusion of leukocyte-containing RBCs [15].

This second RCT confirmed the findings of the previous study with respect to a role of leukocyte-containing RBC increasing postoperative infections and mortality. Surprisingly, the incidence of MODS was not affected by the type of RBCs, while MODS was dose-dependently associated with more transfusions in both groups. Apparently, a larger number of leukocyte-containing RBC units influence the course, although not the incidence of MODS. For further understanding, we analyzed in more detail the causes of death in the two randomized controlled trials in cardiac surgery from the Netherlands (Chapter 3). We found that patients who received standard buffy-coat-poor, leukocyte-containing RBCs, compared with leukocyte-depleted RBCs, excessively died with a combination of MODS and the presence of infection in the postoperative period. Other causes of death (i.e. cardiac reasons and bleeding) were not different between both types of RBCs. These results suggest an important role of allogeneic leukocytes in RBCs aggravating the clinical course of MODS by co-occurrence of infections. These infections could precede or develop when MODS is already manifest. Possibly, transfusion of allogeneic leukocytes results in more postoperative infections, due to long-standing post-surgical immune suppression. However this hypothesis is not confirmed by prospective designed studies.
Table 1 | RCTs in Cardiac Surgery comparing Leukocyte-Containing with Leukocyte-Depleted RBCs.

<table>
<thead>
<tr>
<th>Author; year</th>
<th>No. patients (% transfused)</th>
<th>No. RBCs mean ± SD or median</th>
<th>Main endpoints</th>
<th>Results</th>
<th>p value(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>van de Wetering et al; 1998 [9]</td>
<td>914/ 866 (95)</td>
<td>FF 5.3 ± 4.1</td>
<td>1) Infections</td>
<td>1) 16.9 vs 17.9 vs 23.0%</td>
<td>1) 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SF 5.5 ± 5.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCD 5.4 ± 5.1</td>
<td>2) 60-day mortality</td>
<td>2) 3.6 vs 3.3 vs 7.8%</td>
<td>2) 0.01</td>
</tr>
<tr>
<td>Bracey et al; 2002 [11]</td>
<td>357/ 295 (83)</td>
<td>LD 3</td>
<td>1) Infections</td>
<td>1) ns; data ND</td>
<td>1) ns; data ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCD 3</td>
<td>2) Mortality</td>
<td>2) 5.9 vs 7.5%</td>
<td>2) ns; data ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3) ICU-/Hospital-stay</td>
<td></td>
<td>3) ns; data ND</td>
</tr>
<tr>
<td>Wallis et al; 2002 [12]</td>
<td>597/ 409 (69)</td>
<td>WBF 3.9 ± 3.9</td>
<td>1) Infections</td>
<td>1) 11.3 vs 10.8 vs 17.7%</td>
<td>1) 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCD 3.5 ± 2.6</td>
<td>2) 90-day mortality</td>
<td>2) 0.5 vs 2.9 vs 2.5%</td>
<td>2) 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PR 2.9 ± 1.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilgin et al; 2004 [Chapter 2]</td>
<td>474/ 432 (91)</td>
<td>LD 6.2 ± 7.1</td>
<td>1) Infections</td>
<td>1) 22.6 vs 31.6%</td>
<td>1) 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCD 5.9 ± 6.1</td>
<td>2) MODS</td>
<td>2) 20.4 vs 20.7%</td>
<td>2) 0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3) Hospital mortality</td>
<td>3) 5.5 vs 10.1%</td>
<td>3) 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4) 90-day mortality</td>
<td>4) 8.4 vs 12.7%</td>
<td>4) 0.16</td>
</tr>
<tr>
<td>Connery et al; 2005 [13]</td>
<td>98/ 69 (70)</td>
<td>LD 5.6 ± 13</td>
<td>1) Infections</td>
<td>1) 13.2 vs 25.8%</td>
<td>1) 0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCD 5.6 ± 1.0</td>
<td>2) 30-day mortality</td>
<td>2) 2.6 vs 3.2%</td>
<td>2) 1.0</td>
</tr>
<tr>
<td>Boshkov et al; 2006 [14]</td>
<td>1227/ 562 (46)</td>
<td>ND</td>
<td>1) Serious infections</td>
<td>1) ns; data ND</td>
<td>1) ns; data ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2) 60-day mortality</td>
<td>2) 4.9 vs 9.7%</td>
<td>2) 0.36</td>
</tr>
</tbody>
</table>

*Available only as abstract. †Compared between leukocyte-depleted and leukocyte-containing RBCs. LD=Leukodepleted RBCs; FF=Fresh filtered RBCs; SF=Stored filtered RBCs; BCD=Buffy-coat depleted RBCs; WBF=White blood cell filtered; PR=Plasma-reduced; ND=Not documented; PTI=Pulmonary tract infections.
Analyses on the cost-effectiveness of leukodepletion are scarce and are mainly based on observational data. The cost-effectiveness of leukodepletion in cardiac surgery was analyzed based on data derived from our two studies. The results showed that RBC leukodepletion was cost effective. The benefit of leukodepletion of RBCs was between $220-$310 US per life-year gained in CABG patients [16] and $214 US per cardiac valve surgery patient (Chapter 7).

Because in most well-resourced countries universal leukodepletion (leukoreduction) is implemented, no new randomized controlled trials in this field are expected. We used collected blood samples from the participants of the randomized controlled trial described in Chapter 2, to perform some further analysis of increased postoperative mortality (due to a combination of MODS and infections) after transfusion of allogeneic leukocytes in RBCs.

Laboratory Effects of Allogeneic Leukocytes in Cardiac Surgery

Cardiac surgery results in release of inflammatory mediators, which are presumed to play a role in the development of postoperative complications such as systemic inflammatory response syndrome (SIRS), multiple-organ-dysfunction-syndrome (MODS) and infections. High concentrations of pro-and anti-inflammatory mediators are released during and after trauma or major surgery. Imbalance of the concentration of cytokines can play a pivotal role in a balanced equilibrium after cardiac surgery. Cytokines are low molecular weight polypeptides, which are produced by many cells, such as macrophages, monocytes, neutrophils and platelets. They are divided into two groups with at one end of the spectrum pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-2, IL-8 and IL-12 and at the other end the anti-inflammatory cytokines as IL-4, IL-5 and IL-10. While IL-6 has both pro-and anti-inflammatory properties [17,18]. During and after cardiac surgery several both pro-and anti-inflammatory cytokines are released.

Few studies investigated the effect of allogeneic (leukocyte-containing) blood products on the cytokine balance. These studies compared cytokine profiles in patients receiving leukocyte-containing RBC transfusions with patients who did not receive any transfusions. In one study in 114 patients an association was found between allogeneic RBC transfusions and postoperative increase of bactericidal permeability increasing protein (BPI), a marker of neutrophil activation, and of the inflammatory mediator IL-6 [19]. However there are no studies that investigated the possible differences between the type of blood products and the concentrations of inflammatory mediators in relation with the outcome of the patients.
We could investigate profiles of some inflammatory mediators in 346 patients participating in our RCT of whom pre- and post-surgical blood samples were available (Chapter 5). We selected four key mediators that represent the inflammatory response after surgery. IL-6 has been shown to be an early predictor for mortality in cardiac surgery. IL-10, an anti-inflammatory cytokine, has been found to be increased after per-operative allogeneic blood transfusions in orthopedic surgery in association with prolonged hospital stay [20]. Pro-inflammatory cytokine IL-12 reflects activation and proliferation of lymphocytes and natural killer cells, which are relevant for the defense against nosocomial infections [21]. The concentration of procalcitonin on the first postoperative day after cardiac surgery has been shown to be an early marker for organ dysfunction with severe outcome [22].

In the analysis of the total patient population we found no differences between the two transfusion arms in the levels of the inflammatory mediators upon arrival at the ICU. However, significantly higher IL-6 levels at arrival at ICU were found in patients after transfusion of 3 or more units BCD-RBCs compared with LD-RBCs. Higher IL-6 and IL-12 concentrations after leukocyte-containing transfusions were present in patients who developed infections and MODS respectively. IL-10 and procalcitonin concentrations were not associated with number and type of transfusions in patients with or without complications, although higher IL-10 concentrations were associated with hospital mortality in both randomisation arms. In a selected patient population staying longer than 2 days at ICU (because of more postoperative complications) we found that the concentration of IL-10 had decreased already on arrival at ICU in both study arms. The increase of IL-6 concentration peaked later and a higher peak level was measured in the group that had received leukocyte-containing RBCs than in the group that had received leukocyte-depleted RBCs (Chapter 5).

Our study is the first showing higher initial pro-inflammatory markers after leukocyte-containing transfusions after cardiac surgery, in particular in multi-transfused subgroups later developing serious clinical complications. These results suggest that leukocyte-containing blood transfusions contribute to an inflammatory response, in addition to an ongoing systemic inflammatory response induced by cardiac surgery. In order to explain increased susceptibility for infections, we presume that this may lead to a more profound counteractive anti-inflammatory response.
Effects of Plasma and Platelet Transfusions in Cardiac Surgery

Cardiac surgery using cardiopulmonary bypass activates the inflammatory, coagulation and the complement system. The complement system can be activated by three pathways: the classical, the alternative and the lectin pathway. While the classical pathway is activated by antibodies and immune complexes, the lectin pathway can be triggered by binding of carbohydrates exposed on a wide range of micro-organisms to mannose-binding lectin (MBL). Based on genetic variation the level of MBL is highly variable. MBL-deficiency in combination with immune-compromising factors is associated with infections and with enhancement of the systemic inflammatory response syndrome and with myocardial injury [23-25]. We investigated in patients participating in our RCT described in Chapter 2, the effects of the type of blood transfusions on post-surgical MBL concentrations. We found that cardiac surgery is associated with considerable MBL consumption, which was independent of leukocyte-containing or leukocyte-depleted RBCs. Furthermore no relation was found between MBL-deficiency and postoperative infections or mortality. In contrast, none of the patients with MBL-deficiency developed MODS, unless they had been transfused with plasma units (Chapter 6). Our findings suggest that plasma transfusions in cardiac surgery can have deleterious clinical effects, at least for a particular patient subpopulation.

A substantial proportion of patients undergoing cardiac surgery receive plasma and platelet transfusions. Plasma-containing blood transfusions can contribute to adverse outcome by causing transfusion-related acute lung injury (TRALI), a serious life-threatening condition and an underreported complication of allogeneic blood transfusions. Whether allogeneic leukocytes in blood transfusions play a role in the development of TRALI is unclear [26]. Some observational studies suggested that plasma and platelet transfusions in cardiac surgery are associated with postoperative complications and influence the postoperative outcome [27-33]. However, plasma and platelet transfusions are predominantly transfused to patients who also receive large numbers of RBC transfusions. Therefore it is difficult to determine whether plasma and platelet transfusions are independent risk factors or are only confounders.

Retrospective, multivariate analysis of our two RCTs revealed that plasma transfusions were independently associated with higher all cause mortality (independent of the existence of postoperative infections), while platelet transfusions were associated with mortality in combination with infections present in the postoperative period (Chapter 8). This suggests that, in addition to immunomodulatory effects of RBC transfusions, also plasma and platelet transfusions could play an important role in the outcome after cardiac surgery. Our findings underscore the need for further studies to investigate the aggregate effects of all
the various blood components transfused in cardiac surgery, as well as differentiate between adverse effects possibly associated with a specific blood component(s). How plasma and platelet transfusions indeed contribute by distinct pathways to postoperative morbidity and mortality after cardiac surgery should be evaluated in further studies.

**Allogeneic Leukocytes and the Enhancement of MODS after Cardiac Surgery**

During cardiac surgery blood is exposed to the extracorporeal circuit, hypothermia, ischemia/reperfusion injury and many inflammatory responses are activated. These responses lead to post-perfusion systemic inflammatory response syndrome (SIRS). SIRS is defined by a body temperature less than 36°C or more than 38°C, heart rate more than 90/min, tachypnea with breaths more than 20/min or pCO$_2$ less than 4.4 kPa (32 mm Hg) and leukocyte count less than 4 x 10$^9$/l or above 12 x 10$^9$/l. SIRS can be diagnosed when two or more criteria are present [34]. SIRS is a subset of cytokine storm with an abnormal regulation of cytokines and is immediately counteracted by a compensatory anti-inflammatory response syndrome (CARS) [35]. An overwhelming SIRS causes a dormant state of cell metabolism, referred to as MODS; SIRS usually resolves with adequate supportive therapy and most of the patients recover. However overwhelming SIRS can dominate CARS and progress to MODS, which may lead to mortality. We presume that leukocyte-containing RBC transfusions to patients with an activated inflammatory response act as a second-hit and imbalances the postoperative SIRS-CARS equilibrium further enhancing of prolonging SIRS (Chapter 4).

Both the inflammatory response and the release of pro-inflammatory cytokines lead to activation of the coagulation system and down-regulate the anticoagulant system [36]. Activation of the coagulation factors can in turn activate inflammation. This may enhance the development of infections and microvascular thrombi [37]. Both thrombi and infection play a central role in the development and worse outcome of MODS [38]. This could occur by increasing the circulating RBC mass and vascular rheologic deformations by RBC transfusions. Activated platelets (during storage) may contribute to thrombosis in patients at risk. It has recently been shown that leukocyte-containing RBCs and platelets contain prothrombotic soluble mediators, which interact with leukocytes preceding the apoptosis and death of leukocytes, subsequently producing microparticles with procoagulant activity [39]. Leukocyte-containing RBCs contain prothrombotic soluble mediators, such as CD40L, which induce the synthesis of proinflammatory mediators that can further activate the coagulation system [40]. Recently one study found in the bronchoalveolar lavage fluid besides an increase in proinflammatory mediators IL-8 and TNF-alpha also an increase in trombine-antithrombin complex (TATc), indicating activation of the coagulation system...
in the lung [41]. Some observational studies showed an association between allogeneic blood transfusions and the development of venous thromboembolism [42-44]. The possible association between allogeneic blood transfusions and the formation of thrombosis, as a factor aggravating MODS and having a role in increased mortality due to MODS, is a new subject and should be investigated further.

The Final

Allogeneic blood transfusions are given at different times during and after cardiac surgery. Any intervention by allogeneic RBC transfusions during an already existing inflammatory cascade can be inappropriately timed and can induce a second-hit response. The presence of leukocytes in blood products induces the production and release of proinflammatory cytokines in the recipient, which can aggravate SIRS by both activation of the coagulation system and the inflammatory response. This second-hit response induced by allogeneic leukocytes (and possibly by platelet transfusions as well) may be in combination with infections the cause of a more severe course of MODS (Figure 2).

![Diagram](image-url)

**Figure 2** | Relation between allogeneic blood transfusions, inflammation and coagulation.
To understand the differences between leukocyte-containing and leukocyte-depleted RBC transfusions we investigated several possible causal mechanisms. Soluble mediators derived from deteriorating leukocytes during storage of RBC are unlikely to play a role. This is demonstrated in the first RCT, which observed equal benefit of post- and prestorage filtered RBCs [9]. The complement activation by lectin pathway may be relevant to explore as a causal deleterious effect of plasma transfusions, although does not explain excess death by MODS in association with allogeneic leukocytes. An acute phase reaction represented by procalcitonin could be excluded as a mediator induced by allogeneic leukocytes. A difference in cytokine responses in the recipient was the only significant factor that could be identified as playing a possible causal role (Table 2). Effects of allogeneic leukocytes and also activated platelet transfusions could influence this difference by enhancing interaction between both inflammatory and coagulation systems.

**Table 2 | Factors Related with Outcome after Cardiac Surgery in Relation with Allogeneic Leukocytes**

<table>
<thead>
<tr>
<th>Possible factors</th>
<th>Relation with allogeneic leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of RBC storage lesions</td>
<td>Not related</td>
</tr>
<tr>
<td>Investigated by the storage time of RBCs (Chapter 5)</td>
<td></td>
</tr>
<tr>
<td>Effects of stored plasma-derived factors</td>
<td>Not related</td>
</tr>
<tr>
<td>Investigated by fresh-filtered and stored-filtered RBCs [9]</td>
<td></td>
</tr>
<tr>
<td>Activation of the complement system by lectin pathway</td>
<td>Not related</td>
</tr>
<tr>
<td>Investigated substrate: MBL (Chapter 6)</td>
<td></td>
</tr>
<tr>
<td>Activation of the inflammatory systems</td>
<td>Higher pro-inflammatory and lower anti-inflammatory cytokines</td>
</tr>
<tr>
<td>Investigated substrates: IL-6, IL-10 and IL-12 (Chapter 5)</td>
<td></td>
</tr>
<tr>
<td>Acute phase reaction</td>
<td>Not related</td>
</tr>
<tr>
<td>Investigated substrate: procalcitonin (Chapter 6)</td>
<td></td>
</tr>
<tr>
<td>Activation of the coagulation system</td>
<td>???</td>
</tr>
</tbody>
</table>

The abundant presence of allogeneic leukocytes in blood products is history in the Netherlands and in many well-resourced countries. It seems that cardiac surgery patients are one of the minorities that benefit from this change intended to reduce transmission of vCJD. Donor lymphocytes and granulocyte transfusions are currently only used in special cases in the treatment of hematologic malignancies. Therefore this thesis may be considered
as the "Swan Song" of allogeneic leukocytes in blood products. However the debate about transfusion of (non)-leukoreduced blood products is still continuing.[45]. Furthermore other factors, such as plasma and platelet transfusions (due to activation or storage lesions) and the (possible) activation of the coagulation system by the allogeneic blood transfusions, may remain to play important roles in the development of transfusion-associated complications and are issues for further research in cardiac surgery. Thus many residual questions have still to be answered in the future.
REFERENCES

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