Transfusion associated complications in cardiac surgery: the swan song of the allogeneic leukocytes?
Bilgin, M.Y.

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Chapter 4

Transfusion-Related Immunomodulation (TRIM): A Second Hit in an Inflammatory Cascade?

YM Bilgin
A Brand

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ABSTRACT

Allogeneic blood transfusions are dose-dependently associated with postoperative complications. Leukocytes present in blood components may play a role in these effects, referred to as transfusion-related immunomodulation (TRIM). Of 19 randomised controlled trials of the effect of allogeneic leukocytes in transfusions, 13 looked into the effect of leukocyte-containing red blood cells (RBCs) in the surgical setting on the occurrence of postoperative infections and/or mortality. In contrast to conflicting outcomes of the trials in other settings, in cardiac surgery there is evidence that leukocyte-containing RBC increase postoperative complications associated with mortality. The studies performed in cardiac surgery show less heterogeneity than studies in other surgical interventions and had been conducted either in one or a few participating centres. In this review we discuss possible explanations for these results in cardiac surgery (as opposed to other settings), which may relate to clinical as well as transfusional factors. We suggest that leukocyte-containing transfusions during and after cardiac surgery add a second insult to the cardiopulmonary bypass procedure induced systemic inflammatory response.
INTRODUCTION

Transfusion-related immunomodulation (TRIM) refers to immune suppression after blood transfusion, currently presumed to be mediated by allogeneic leukocytes. In the 1970s, observational studies revealed better survival of kidney grafts in previously transfused patients compared with non-transfused patients [1]. Concern for wider consequences of such a transfusion effect on cancer growth and immunity against infections resulted in hundreds of publications [2]. However, the underlying reasons for transfusion likely confounded the patient outcomes. Only randomised controlled trials (RCTs) circumvent this. Recently two meta-analyses of available RCTs on the role of peri-operative leukocyte-containing transfusions were published [3,4]. As little new information emerged since the publication of these two overviews, there is no need for an update of a systematic review [5]. In this review we discuss these RCTs and focus on the particular clinical circumstances of cardiac surgery that may enhance susceptibility to TRIM.

Selection Criteria

For this review we used MedLine (1980-May 2008), searching the terms "leukocyte-reduced, leukocyte-depleted, filtered (red blood cell or erythrocyte) blood products", "haemoglobin trigger, transfusion dose", "transfusion-related immunomodulation" or "pre-transplant blood transfusion." Only randomised controlled trials, (systematic) reviews and meta-analyses with clinical endpoints were selected. We excluded studies restricted to leukocyte-depletion of platelet components, or comparing in-vitro parameters. Publications of RCTs, re-analyses and follow-up publications of the original cohort were evaluated for information on transfusion products, transfusion dosage, and proportion of transfused patients. We also searched the references of the selected publications to find relevant abstracts or commonly referenced key publications. We found one meta-analysis of 7 studies comparing a liberal versus restrictive transfusion policy based on the hemoglobin (Hb) trigger in adults [6] and 4 similar studies in children [7-10]. Sixteen RCTs compared leukocyte-depleted red blood cells (RBC) with those containing leukocytes of which 14 RCTs (two still in abstract form) investigated transfusions administered for surgery or trauma [11-24]. One RCT evaluated all hospital patients [25] and one was conducted in human immunodeficiency virus (HIV) positive patients [26]. In prospective kidney transplant recipients, we found three RCTs investigating the effect of a blood transfusion on graft outcome [27-29].
RCTs Comparing Restricted with Liberal Transfusion Policy

Seven hemoglobin (Hb)-trigger studies comprising 1.703 patients used non-leukocyte-depleted RBCs. The meta-analysis by Carson et al shows that a restrictive transfusion trigger entails no more mortality than a liberal transfusion trigger [6], rather the opposite was found (OR: 0.80; 95% CI: 0.63-1.02). This outcome was influenced by one large RCT in 838 patients, staying at an intensive care unit (ICU), in which patients were either transfused to maintain the Hb value between 7 and 9 g/dl (restrictive) or above 10 g/dl (liberal). Patients assigned to a restrictive trigger received an average of 2.6 units of RBCs compared with 5.6 units in the liberal group. Mortality at 30 days, the primary outcome measure, was not significantly different between the groups: 18.7% versus 23.3% (OR: 0.80; 95% CI: 0.61-1.04) in favour of the restrictive trigger. In subgroups of patients younger than 55 years of age and those with a lower APACHE (Acute Physiology And Chronic Health Evaluation) risk score, mortality was significantly lower in the restrictive group than in the liberal group: 5.7% versus 13% (p=0.02) and 8.7% versus 16.1% (p=0.03), respectively [30]. A similar study, using leukocyte-depleted RBC products in 637 paediatric ICU patients, confirmed that a lower transfusion trigger substantially reduces the number of transfused patients and the number of units transfused without a negative clinical effect [7]. Multi-organ-dysfunction-syndrome (MODS), the primary endpoint of this study, developed in 12% of the children in both groups. It is tentative to speculate that leukocytes in blood products may have contributed to higher mortality in low-risk adults who had been liberally transfused [30]. Another 3 Hb-trigger studies had been conducted in neonates and infants and also used leukoreduced transfusions. The results raised concern about the safety of a lower Hb on brain damage in children undergoing cardiac surgery [8], or in very low birth weight premature infants [9,10].

RCTs on Leukodepletion of Red Blood Cell (RBC) Products

To date, 19 RCTs investigated clinical effects of allogeneic leukocytes in RBC products [11-29]. These studies in different clinical settings compared pre- or post (bedside)-storage filtered leukocyte-depleted RBCs (less than 10^6 leukocytes per unit) with various standard components, e.g. whole blood or RBCs in plasma or additive solution, with or without buffy-coat. Often the number of residual leukocytes was not stated. Patient and transfusion characteristics, study designs and the main results of these trials are summarized in Tables 1 and 2.
Studies on Immunosurveillance of Cancer

Only 2 RCTs, with 697 and 640 patients respectively, compared buffy-coat-poor RBC with filtered RBC on the cancer recurrence rate after colorectal surgery with curative intent. Both studies evaluated long-term outcome after at least 5 years follow-up and found no difference in colorectal cancer recurrence [12,17,31]. However, colorectal cancer is a weakly immunogenic tumour and the malignant cells can down-regulate expression of specific HLA-alleles and co-stimulatory molecules, allowing the tumour to escape an immune attack, whether or not the immune response is impaired by transfusions [32]. A possible TRIM effect of allogeneic leukocytes on surveillance of more immunogenic types of cancer remains undetermined.

Studies Evaluating Postoperative Bacterial Infections

Twelve RCT’s on leukodepletion of blood transfusions in different clinical settings evaluated postoperative infections as a primary or secondary endpoint [11-16,18-23]. These studies varied as to single- or multi-centre design, clinical diagnosis, methods to document and report infections, and the proportion of transfused patients (range 14%-95%). In abdominal and cardiac surgery several studies were performed investigating postoperative infections, which revealed different outcomes (Table 1). One RCT compared the effect of leukocyte-depleted and standard blood products in transfused trauma patients and reported no difference in occurrence of infections and acute lung injury (ALI) in an initial [18] and second re-analysis of these patients [33]. Recently, two meta-analyses of RCTs on leukocyte-depletion of blood products have been published and these initiated a methodological discussion [3,4]. Applying intention-to-treat analysis, Vamvakas concludes that there is no association between leukocyte-containing transfusions and the incidence of postoperative infections [3]. Blumberg et al analysed only subgroups of transfused patients, excluding 36% of the patients, and concludes that there is a significant and clinically relevant almost 50% reduction in postoperative infection rate after transfusion of leukocyte-depleted RBCs [4]. How such unequivocal conclusions could be reached was recently addressed in this journal [34]. Besides integration of studies that should not be integrated because of heterogeneity, the in- or exclusion of three recent publications (2 publications that appeared as abstracts and have not been published as complete studies in peer-reviewed journals [20,24]) were identified as causes for the discordant results. Full publication of the 2 studies in abstract form has to be awaited in order to conclude on an effect of leukocyte-depleted RBCs on postoperative infections after various types of surgery.
<table>
<thead>
<tr>
<th>Author; year</th>
<th>No. patients/ No. transfused (%)</th>
<th>Clinical setting</th>
<th>No. centers</th>
<th>Transfused patients</th>
<th>No. RBCs mean±SD or median (range)</th>
<th>Transfused patients with &gt; 3 RBC (%)</th>
<th>Main Endpoints</th>
<th>Results (LD vs BCD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>WB 56</td>
<td>WB 2 (1-5)</td>
<td></td>
<td>2)NK function</td>
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<td></td>
<td></td>
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<td></td>
<td>BCD 230</td>
<td>LD 3 (1-10)</td>
<td>LD 104 (31)</td>
<td>1)Cancer recurrence</td>
<td>130% vs 32%</td>
</tr>
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<td></td>
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<td></td>
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<td></td>
<td>BCD 3 (2-11)</td>
<td>BCD 94 (20)</td>
<td>2)Infections</td>
<td>2/3-5 vs 3.2%</td>
</tr>
<tr>
<td>Houibiers et al.; 1994 [12]</td>
<td>697/446 (64)</td>
<td>Colorectal cancer surgery</td>
<td>16</td>
<td>LD 216</td>
<td>LD 2 (1-5)</td>
<td>ND</td>
<td>1)Infections</td>
<td>30% vs 32%</td>
</tr>
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<td></td>
<td>BCD 230</td>
<td>BCD 2 (1-6)</td>
<td>ND</td>
<td>2)Mortality</td>
<td>2.3 vs 2.8%</td>
</tr>
<tr>
<td>Jensen et al.; 1995 [13]</td>
<td>586/260 (44)</td>
<td>Colorectal surgery</td>
<td>2</td>
<td>LD 118</td>
<td>LD 2 (1-5)</td>
<td>ND</td>
<td>1)Infections</td>
<td>3.0 vs 2.3%b</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BCD 142</td>
<td>BCD 2 (1-6)</td>
<td>ND</td>
<td>2)Mortality</td>
<td>2.3 vs 2.8%</td>
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<td></td>
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<td></td>
<td></td>
<td>BCC 34</td>
<td></td>
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</tr>
<tr>
<td>Tilestad et al.; 2001 [15]</td>
<td>279/ 112 (45)</td>
<td>Colorectal surgery</td>
<td>1</td>
<td>LD 48</td>
<td>LD 3 (2-3.3)</td>
<td>ND</td>
<td>Infections</td>
<td>45 vs 37%</td>
</tr>
<tr>
<td>van Hütten et al.; 2004 [16]</td>
<td>1051/545 (52)</td>
<td>Colorectal cancer surgery and aortic aneurism</td>
<td>19</td>
<td>LD 267</td>
<td>LD 3 (2-3.3)</td>
<td>LD 62 (23)</td>
<td>1)Infections</td>
<td>12.3 vs 2.3%</td>
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<tr>
<td></td>
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<td></td>
<td>BCD 278</td>
<td>BCD 3 (2-6)</td>
<td>BCD 58 (21)</td>
<td>2)Hospital stay</td>
<td>2-2.4 daysb</td>
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<td>3)MODS</td>
<td>3/4 vs 17%b</td>
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<td></td>
<td></td>
<td></td>
<td>4)Mortality</td>
<td>4/10.3 vs 8.4%</td>
</tr>
<tr>
<td>Skanberg et al.; 2007 [17]</td>
<td>642/298 (46)</td>
<td>Colorectal cancer</td>
<td>7</td>
<td>LD 137</td>
<td>LD 3.6 ± 0.3</td>
<td>ND</td>
<td>Respiratory support</td>
<td>1.36 vs 8.1%</td>
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<tr>
<td></td>
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<td></td>
<td>BCD 161</td>
<td>BCD 3.6 ± 0.3</td>
<td></td>
<td>2)Hospital stay</td>
<td>2/15.5 vs 15.5 days</td>
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<td></td>
<td></td>
<td></td>
<td>3)Mortality</td>
<td>3/5.2 vs 49.7%</td>
</tr>
<tr>
<td>Nathensset et al.; 2006 [18]</td>
<td>1864/ 268 (14)</td>
<td>Trauma patients</td>
<td>1</td>
<td>LD 136</td>
<td>LD 9.2 ± 9.6</td>
<td>ND</td>
<td>1)Infections</td>
<td>30 vs 36%</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>BCC 132</td>
<td>PC 8.6 ± 9.9</td>
<td></td>
<td>2)MODS</td>
<td>2/5.9 vs 6.6%</td>
</tr>
<tr>
<td>Watkins et al.; 2008 [19]</td>
<td>914/ 866 (95)</td>
<td>CABG ± valve surgery</td>
<td>1</td>
<td>FF 283</td>
<td>FF 5.3 ± 4.1</td>
<td>FF 164 (58)</td>
<td>1)Infections</td>
<td>17 vs 18 vs 23%b</td>
</tr>
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<td></td>
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<td></td>
<td>SF 280</td>
<td>SF 5.5 ± 5.6</td>
<td>SF 169 (60)</td>
<td>2)Mortality</td>
<td>2/3.6 vs 3.3 vs 7.8%b</td>
</tr>
</tbody>
</table>
## Transfusion-Related Immunomodulation (TRIM): A Second Hit in an Inflammatory Cascade?

<table>
<thead>
<tr>
<th>Author; year</th>
<th>No. patients/ No. transfused (%)</th>
<th>Clinical setting</th>
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<th>Transfused patients with &gt; 3 RBC (%)</th>
<th>Main Endpoints Results (LD vs BCD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bracey et al.; 2002 [20]</td>
<td>357/ 295 (83)</td>
<td>CAGB ± valve surgery</td>
<td>1</td>
<td>LD 136 BCC 159</td>
<td>LD 3 PC 3 ND</td>
<td>1)Infections 2)Mortality 3)ICU-/Hospital-stay</td>
<td>1)ns; data ND 2)5.9 vs 7.5% 3)ns; data ND</td>
</tr>
<tr>
<td>Wallis et al.; 2002 [21]</td>
<td>597/ 409 (69)</td>
<td>CAGB ± valve surgery</td>
<td>1</td>
<td>LD 176 BCC 175 PR 158</td>
<td>WBF 3.9 ± 3.9 BCD 3.5 ± 2.6 PC 2.9 ± 1.8 ND</td>
<td>1)Infections 2)Mortality</td>
<td>1)49 vs 38 vs 35% 2)0.5 vs 2.9 vs 2.5%b</td>
</tr>
<tr>
<td>Bilgin et al.; 2004 [22]</td>
<td>474/ 432 (91)</td>
<td>Valve surgery ± CAGB</td>
<td>2</td>
<td>LD 216 BCD 216</td>
<td>LD 6.2 ± 7.1 BCD 5.9 ± 6.1 LD 145 (67) BCD 131 (61)</td>
<td>1)Infections 2)MODS 3)Mortality</td>
<td>1)123 vs 32%b 2)20 vs 2.1% 3)8.4 vs 12.7%</td>
</tr>
<tr>
<td>Connery et al.; 2005 [23]</td>
<td>98/ 69 (70)</td>
<td>Primary CAGB</td>
<td>2</td>
<td>LD 38 BCC 31</td>
<td>LD(5F) 5.6± 13 PC 5.6±10 LD 16 (42) PC 15 (48)</td>
<td>1)Infections 2)Mortality</td>
<td>1)13 vs 26% 2)0.5 vs 13%b 3)2.6 vs 3.2%</td>
</tr>
<tr>
<td>Dzik et al.; 2002 [25]</td>
<td>27/50 (100)</td>
<td>All patients</td>
<td>1</td>
<td>LD 1355 BCC 1425</td>
<td>LD 2 (1-9) PC 2 (1-9) LD 498 (35) PC 474 (35)</td>
<td>1)Mortality 2)Hospital stay 3)Antibiotics</td>
<td>1)9 vs 8.5% 2)8.8 vs 8.9 days 3)31.5 vs 34%</td>
</tr>
<tr>
<td>Collier et al.; 2001 [26]</td>
<td>531/524 (99)</td>
<td>HIV-positive</td>
<td>11</td>
<td>LD 259 BCC 262</td>
<td>Mean 7.3 ND</td>
<td>1)Mortality 2)HIV RNA level</td>
<td>1)58% vs 53% 2)Similar</td>
</tr>
</tbody>
</table>

*Data on ALI were re-analyzed and presented in another publication [33] than the initial publication [18];bStatistically significance (P<0.05) between BCD and LD (SF+FF);cThis RCT was interrupted early. Abbreviations Table 1: LD=Leukodepleted RBCs; FF=Fresh filtered RBCs; SF=Stored filtered RBCs; BCD=Buffly-coat depleted RBCs; BCC=Buffly-coat-containing RBCs; PR=plasmareduced RBCs; WB=Whole blood; WB=White blood cell filtered; ND=Not documented; PTI=Pulmonary tract infections.
Studies Evaluating Mortality

Short-term mortality was evaluated in 12 RCTs (Table 1). Under conditions of heterogeneity, Vamvakas found in a meta-analysis no overall adverse effect of leukocyte-containing products on short-term mortality (OR: 1.14; 95% CI: 0.89-1.45), with an exception in cardiac surgery (OR: 1.72; 95% CI: 1.05-2.81) [3]. As shown in Table 1, cardiac surgery patients received, in contrast to patients with other types of surgery, often more than three units of RBCs. Mortality after cardiac surgery is generally below 5%, however increases with complexity of the surgical intervention, increased blood loss, co-morbidity and older patient age to more than 10% [35]. In general extensively transfused cardiac surgery patients have more postoperative morbidity and mortality [36]. Platelet transfusions probably enhance both morbidity and mortality [37], but they are inextricably bound to larger numbers of RBC transfusions and more surgical bleeding.

Four RCTs performed in cardiac surgery are published as full articles [19,21-23]. Two of these trials randomised the patients for three different blood products. One compared buffy-coat-depleted (BCD)-RBCs with two filtered RBCs: fresh filtered RBCs before storage (FF) or stored filtered RBCs (SF) [19]. All products had a similar shelf-life of around 13 days. There was a higher mortality (7.8%) in the group who received BCD-RBCs as compared with 3.6% and 3.3% in those receiving FF or SF products respectively (p=0.015). This suggests that soluble mediators, still present in the SF products, caused no more adverse effects than FF-RBC, lacking leukocyte-derived soluble factors. In a subgroup analysis, the difference in mortality was present only in patients who received more than three RBC units. A second study using three types of blood products, assigned patients to filtered whole blood (stored <7 days before filtration), BCD-RBC or plasma-reduced RBCs. Short-term postoperative mortality was 0.5%, 2.9 % and 2.5% respectively, indicating no additional deleterious role of a higher number of leukocytes present in plasma-reduced RBCs as compared to BCD-RBCs [21]. In the study of van de Watering et al [19] the incidence of multiple-organ-dysfunction-syndrome (MODS) was not registered, however mortality due to MODS was the major cause of excess deaths after standard transfusions. We conducted another study in more complicated cardiac surgery with a higher probability of multiple RBC transfusions in order to explore the relationship with leukocyte-containing transfusions on MODS and mortality [22]. Surprisingly, the incidence of MODS (20%) was similar in the groups receiving standard BCD-RBC or pre-storage filtered RBC, however MODS as a cause of death occurred more often in patients who received BCD-RBC. Subgroup analysis showed that only patients who received more than 3 units suffered higher mortality in the group receiving BCD-RBC. A fourth small study in 69 low-risk CABG
patients compared bedside-filtered RBCs (containing soluble leukocyte-produced factors) with the same unfiltered RBC product [23]. There was no difference in mortality between both randomization arms. This study was preliminary stopped because interim analysis showed less respiratory tract infections in the filtered group (p=0.048). Two other studies in cardiac surgery are still available only as abstracts, mentioning limited data [20,24].

The observation that not the leukocyte load per transfusion [21], nor the soluble mediators released by leukocytes during storage [19], but rather the number of units transfused that entails the worse outcome [19,22], suggests that sicker patients in cardiac surgery requiring more RBC transfusions are more susceptible to TRIM. We analysed in more detail the causes of death in two RCTs in cardiac surgery [19,22]. This revealed that patients who received standard buffy-coat-poor RBCs, compared with before storage filtered leukodepleted RBCs, excessively died from a combination of infection and MODS (OR 2.92; 95% CI 1.22-6.97; p=0.02). Short-term mortality (60-days) from infections alone and from MODS without infections or from bleeding or surgical complications was equal in both transfusion arms [38]. Long-term mortality after transfusions of buffy-coat-poor RBCs or leukocyte-depleted RBCs, has been published in two studies (both after colorectal cancer surgery) which observed no difference in survival after 7 and 8 years [17,39].

Recently in an observational study Koch et al. investigated the effects of peri-operatively transfusion of RBCs either stored less than 14 or more than 14 days in cardiac surgery [40]. In this study one-year mortality was higher in patients receiving RBCs stored more than 14 days, however this association between storage time and mortality was only reported as unadjusted analysis. In the RCTs comparing buffy-coat-poor RBCs with leukocyte-depleted RBCs mortality increased in more heavily transfused patients. Consequently small imbalances in heavily transfused patients may be an important confounder, this was also suggested in the correspondence after the publication of Koch et al [41]. Currently, taking several studies in cardiac surgery investigating the storage time of RBCs into account [42-46], it is not possible to conclude that RBCs with limited storage time should be used particular in cardiac surgery patients. Because available databases used retrospectively in these studies investigated different storage times and used different blood products results from prospective studies have to be awaited.

Studies Outside Surgery and Trauma

Few studies on possible TRIM effects have been performed. Only one RCT addressed the effects of universal leukoreduction as a transfusion policy. For six months, in a tertiary hospital, 2,780 consecutive patients with a transfusion indication were randomised
between filtered and standard unmodified RBCs [25]. No difference in mortality, use of antibiotics, or hospital stay was found. The study was criticized because of a high (20%) percentage of product violations, many patients receiving the wrong, not assigned, blood products. Considering that not all clinical diagnosis groups may experience negative effects of leukocyte-containing products, the results of this large study reflect the absence of benefit of universal leukoreduction of blood products in non-selected patient groups.

The VA TS (Viral Activation Transfusion Study) was conducted after in-vitro and in-vivo observations that allogeneic leukocytes stimulate HIV replication [26]. In this study, 531 HIV-positive patients with a first indication for transfusion were randomised between pre-storage filtered and unmodified buffy-coat containing RBC transfusions. No difference in HIV-RNA level or in the number of CD4-positive cells was found between the study arms. Median survival time was 13 months in the filtered RBC group and 20.5 months in the group receiving the unmodified RBC group. This difference was not significant in intention-to-treat analysis, but after correction for various prognostic factors transfusion of unmodified RBCs was associated with better outcome (RR 1.35; 95% CI 1.06-1.72) [26].

Studies on Pre-Transplantation Blood Transfusion
Pre-transplantation third-party blood transfusion reducing kidney graft rejection has been investigated in only 3 randomised studies of different design (Table 2) [27-29]. One study compared in 52 patients the effect of standard unmodified RBCs compared with buffy-coat-poor or washed RBC on the development of HLA antibodies and graft survival. No difference in outcome was observed, but the leukoreduced products did not meet the standards (<10^6 leukocytes/unit) and all products may have been equally effective [27]. In a multi-centre randomised study in 423 prospective cadaver kidney transplantation patients, a better 1-year (90 versus 82%; p=0.02) and 5-year (79 versus 70%; p=0.025) graft survival was observed after 3 random pre-transplantation transfusions of unmodified RBCs compared with no transfusions [28]. Also severe rejections were significantly reduced in patients received RBCs. In a third multi-centre study, 144 patients were randomly assigned to one HLA-DR shared transfusion (n=49), one HLA-DR mismatched transfusion (n=48) or no transfusion (n=47). Blood transfusion consisted of unmodified RBCs stored less than 72 hours. There was no difference in graft survival at 1 year (90, 92 and 92%) or at 5 years (79, 84 and 80%) respectively. The incidence of acute rejections in patients who had received an HLA-DR shared transfusion was not significantly lower than observed in the other 2 groups (19 versus 33%), but the study was not powered to detect possible differences of this order [29]. The results of this latter study do not confirm previous observational studies, suggesting that
**Table 2 | Characteristics of Renal Transplantation Patients Participating in RCTs Evaluating Pre-transplantation Blood Transfusions**

<table>
<thead>
<tr>
<th>Author; year</th>
<th>No. patients</th>
<th>No. transfused patients (%)</th>
<th>Diagnosis</th>
<th>No. centers</th>
<th>Endpoints</th>
<th>Patients (N)</th>
<th>Units (N)</th>
<th>Severe rejection</th>
<th>Graft survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanfilippo et al.; 1985 [27]</td>
<td>52</td>
<td>52 (100)</td>
<td>Cadaveric renal transplantation</td>
<td>1</td>
<td>Graft survival</td>
<td>LD 30</td>
<td>ND</td>
<td>1-year: 50 vs 50%</td>
<td></td>
</tr>
<tr>
<td>Opelz et al.; 1997 [28]</td>
<td>423</td>
<td>205 (48.4)</td>
<td>Cadaveric renal transplantation</td>
<td>14</td>
<td>Rejection &amp; graft survival at 1 and 5 years</td>
<td>BCC 205</td>
<td>NT 218</td>
<td>16 vs 25%*</td>
<td>1-year: 90 vs 82%* 5-years: 79 vs 70%*</td>
</tr>
<tr>
<td>Hiesse et al.; 2001 [29]</td>
<td>144</td>
<td>97 (67.3)</td>
<td>Cadaveric renal transplantation</td>
<td>8</td>
<td>Rejection &amp; graft survival</td>
<td>BCC (1 DR match) 49 BCC (0 DR match) 48</td>
<td>NT 47</td>
<td>19 vs 33 vs 33%</td>
<td>5-years: 92 vs 92 vs 90%</td>
</tr>
</tbody>
</table>

Abbreviations Table 2: LD= Leukodepleted RBCs; NT= Non-transfused; BCC= Buffy-coat-containing RBCs. Statistically significance (P<0.05) between BCD and LD (SF+FF).
pre-transplant transfusion from a donor sharing one HLA-DR antigen protects against graft rejection [47]. The three studies do not allow a combined analysis, because of heterogeneity in design, different immunosuppressive protocols and blood products used. Although the largest study found a protective effect of TRIM on renal graft survival [28], a smaller study designed on the presumed mechanism of allograft induction by HLA-DR sharing blood transfusions was not supportive [29]. Lacking more confirmatory studies an evidence based conclusion on graft-tolerizing effect of pre-transplant allogeneic leukocytes in blood products is as yet not possible.

Possible Mechanisms of TRIM

Many factors, soluble and cellular, present in leukocyte containing blood products have been proposed to modulate the immune system [34].

Leukocyte-containing RBCs contain viable and apoptotic leukocytes, erythrocytes, residual platelets depending on the type of product, and factors released by these cells during storage. Soluble immune response modifiers accumulating during storage of blood products include elastase, histamine, soluble HLA, soluble Fas-ligand, TGF-β1 and pro-inflammatory cytokines IL-1β, IL-6, and IL-8 [48]. In-vitro, soluble leukocyte derived factors from stored RBC products induce immediate up-regulation of expression of inflammatory genes in third party leukocytes [49,50]. Interleukin-8 may be the cause of transient post-transfusion leukocytosis in critically ill patients, possibly by mobilisation of cells from the bone marrow [51]. In a multivariate analysis Heddle et al identified the number of contaminating leukocytes and the storage duration of RBCs as the most significant factors associated with febrile non-haemolytic transfusion reactions [52]. However, although investigated in just one RCT, stored and then filtered RBC, expected to contain leukocyte mediators, was associated with a similar reduction of postoperative mortality as pre-storage filtered blood, suggesting a causal role for leukocytes [19].

Apoptosis of leukocytes begins immediately after blood withdrawal. The speed of leukocyte deterioration in RBCs during storage at 2-6°C varies and can be distinguished in functional lesions and gradual apoptosis and necrosis, first of granulocytes, then monocytes, while lymphocytes can remain viable for more than 25 days. Apoptotic cells engage the phosphatidylserine (PS)/annexin V receptor on macrophages, inducing release of prostaglandin E-2 and TGF-β, factors suppressing macrophages and natural killer cells and impair the antigen-presenting capacity [53].
Viable allogeneic leukocytes in blood components can act as responder cells or as stimulator cells inducing cellular immunity and antibody production in the recipient. A functional phosphorylation defect, described after 3-5 days storage, impairs protein synthesis of T cells upon signalling of the T-cell receptor and reduces the proliferative responder capacity of donor lymphocytes against recipient cells [54]. After 10-14 days of storage, the capacity of donor antigen presenting cells to stimulate recipient T-helper cells is abrogated in vitro by reduction of co-stimulatory molecules [55]. After transfusions stored for a couple of days, a two-way interaction between donor and recipient cells is reflected by the appearance of circulating proliferating lymphoblasts a few days after blood transfusion [56]. In general, donor DNA becomes undetectable one week after transfusion, but persistence of donor cells for years, even after transfusion of filtered and stored leukocyte depleted transfusions, has been described in approximately 25% of recovered trauma survivors [57]. Utter et al showed that trauma induced immune suppression and impaired the patient's proliferative reaction against donor lymphocytes, which could establish donor microchimerism [58]. These trauma survivors are apparently healthy and this long-term chimerism is unlikely an explanation for postoperative infections and short term mortality after cardiac surgery.

Despite convincing in vitro and animal studies supporting TRIM, it is difficult to demonstrate clinical counterparts of such effects after blood transfusion in humans. Dzik et al proposed the existence of two different categories of TRIM effects: on the innate immune response and on the adaptive antigen-driven immune system, to separate the mechanism in surgery patients and transfusion-induced tolerance in organ transplantation [53]. Although there is increasing knowledge that the innate and adaptive immune systems do not act independently and are linked by (subsets of) natural killer and dendritic cells [59], the clinical condition of a surgery patient compared to a patient in steady state disease may be crucial.

Tissue damage and other trauma such as burns, mechanical ventilation and hypovolemia generate products and expose structures of degraded tissue (e.g. heat-shock proteins, proteases) interacting with sensors (Toll Like Receptors [TLRs]) on macrophages leading to immediate release of stress hormones, inflammatory cytokines and chemokines [60]. Besides release of cortisol, serotonin, TNF-α, IL-1 β, IL-6 and IL-8, the coagulation and complement systems are activated [61]. These factors may cause a systemic inflammatory response syndrome (SIRS) and are immediately counteracted by a compensatory anti-inflammatory response syndrome (CARS) [62]. An overwhelming SIRS causes a dormant state of cell metabolism, referred to as multiple-organ-dysfunction-syndrome (MODS) [63]. CARS has an immune paralysing effect and is characterised by anti-inflammatory cytokines,
such as TGF-β1, IL-4 and IL-10 and inhibition of the IL-12-IFN-γ pathway, impairing natural defence against invading micro-organisms [64,65].

The innate immune system in SIRS or CARS phase of the cascade stimulates or suppresses antigen presenting cells and may skew the adaptive immune response towards T-helper 1, T-helper 2 or to regulatory T-cells. The clinical condition of the patient receiving blood transfusions may determine to a large extent the type of TRIM effect.

Cardiac Surgery and the Inflammatory Cascade

During cardiac surgery blood is exposed to the extra-corporeal circuit, hypothermia, ischaemia/reperfusion injury. These insults are potent inducers of a stress response. After cardiac surgery a post-perfusion SIRS occurs, with leukocytosis, capillary leakage, and organ dysfunction. 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 [Figure 1] [66].

Figure 1 | Mechanism of Blood Transfusions and Complications after Cardiac Surgery. 
*Abbreviations:* Figure 1: SIRS=Systemic inflammatory response syndrome; CARS=Compensatory anti-inflammatory response syndrome; MODS=Multiple-organ-dysfunction-syndrome

Sablotzki et al [67] measured the cytokine pattern up to 48 hours after CABG surgery in 24 patients who all recovered uneventfully. After the start of bypass, soluble
IL-2 receptor, IL-2 and IL-12 decrease and incompletely restore themselves, respectively 6-48 hours after surgery. The levels of IL-6 and IL-10, undetectable before surgery, increase at the end of bypass and reperfusion. The very high IL-10 peak fades away after 6 hours, while IL-6 remains high up to 48 hours. Such a cytokine pattern shows that cardiac surgery immediately evokes a biphasic cytokine response. This response includes platelet activation and macrophage de-activation with decrease of TLRs [68,69]. Activated platelets cause semi-maturation of dendritic cells, which produce IL-10 [70]. The first day after cardiac surgery there is a profound reduction in dendritic cells with impaired IL-12 and IFN-γ production, depressing T-helper-1 and natural killer cells, inhibiting the immune response against microbial invasion [68,69].

In 114 patients undergoing cardiac surgery Fransen et al [71] found an association between allogeneic blood transfusions and postoperative increase of concentrations of inflammatory mediators. Furthermore, patients developing MODS after cardiac surgery often show a higher and longer increase of pro-inflammatory factors from the first post surgical day onwards and in particular high IL-8 and IL-6 are early predictors for non-survival after cardiac surgery [72].

Allogeneic blood transfusions are given at different times during and after cardiac surgery. Any intervention by biological response modifiers during an already existing inflammatory cascade, which include leukocyte-containing RBC transfusions, can be inappropriately timed and lead to increased morbidity and mortality. It is possible that by leukocyte-containing RBC transfusions to patients with an activated inflammatory response, this further imbalances the SIRS-CARS equilibrium in favour of SIRS. This (second-hit) response may exacerbate a pro-inflammatory stimulus leading to aggravation of MODS and could finally result in death (Figure 1).

CONCLUSIONS

Allogeneic leukocyte-containing RBC transfusions may have immunomodulatory effects that are presumed beneficial for organ transplantation, but harmful for surveillance of cancer and for resistance to postoperative infections. This concept initiated hundreds of studies, and 19 randomised controlled trials in various clinical conditions. However, important questions remain as to the nature and magnitude of clinical benefits and complications ascribed to TRIM, its mechanism and the putative causal factors in allogeneic blood components. The clinical counterpart of transfusion-induced effects on the cellular immune system is difficult
to demonstrate. This may be the result of differences in the composition of leukocyte-containing RBC transfusions used in the various trials and whether blood transfusions are administered to patients in a steady state or during an activated or suppressed innate immune response. A TRIM effect due to leukocyte-containing RBCs has yet only been shown in cardiac surgery patients. These patients often need multiple transfusions, which are administered during an activated (anti)-inflammatory cascade. Leukocyte-containing blood transfusions interfering in this cascade by induction of an additional inflammatory insult as well as by immunomodulatory effects may disturb a delicate balance, leading to fatal complications in patients at risk.
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