Transfusion associated complications in cardiac surgery : the swan song of the allogeneic leukocytes ?

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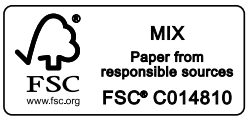
TRANSFUSION-ASSOCIATED COMPLICATIONS IN CARDIAC SURGERY: THE SWAN SONG OF THE ALLOGENEIC LEUKOCYTES?

Memiş Yavuz Bilgin



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TRANSFUSION-ASSOCIATED COMPLICATIONS IN CARDIAC SURGERY: THE SWAN SONG OF THE ALLOGENEIC LEUKOCYTES?

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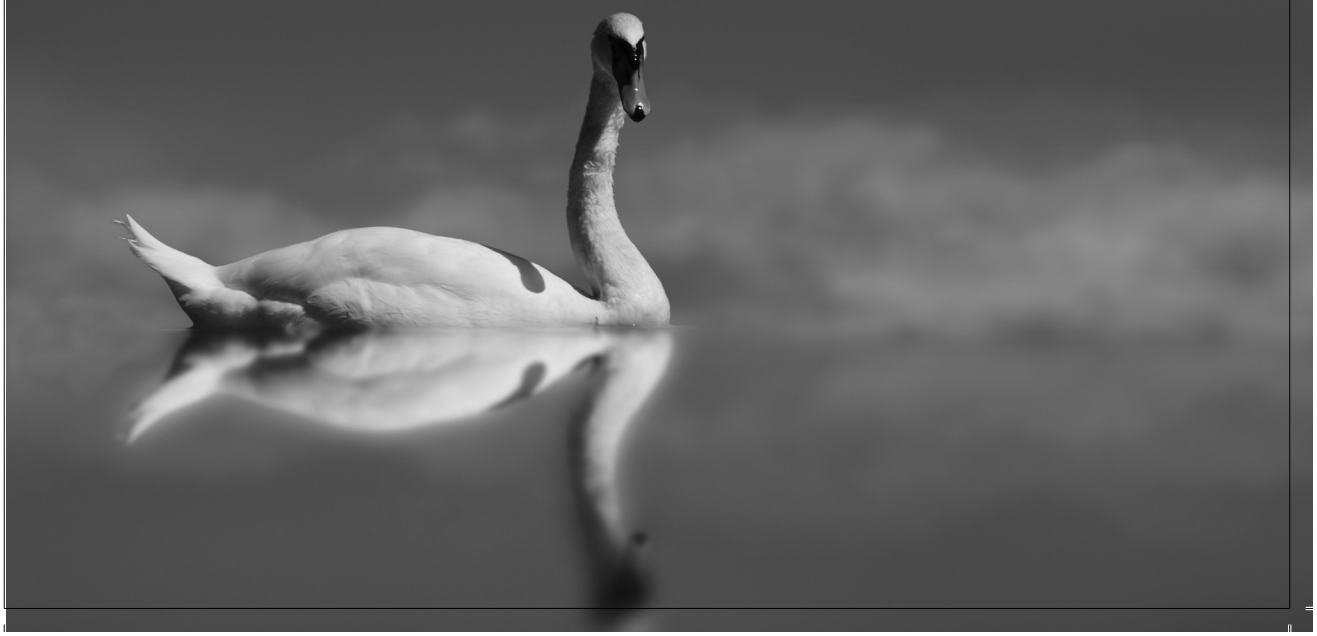
Contents

Chapter 1

General Introduction



Until the discovery of the AB0-bloodgroups in the early 1900s blood transfusions were a high-risk procedure: more than 50% of the recipients of blood died. The discovery of the AB0-bloodgroups followed by the development of citrate as anticoagulant to prevent clotting of blood enabled the start of transfusion medicine. Both World Wars and other disasters in the 20th century had a large impact on further development and structural organization of transfusion medicine. Before the introduction of centrifugation techniques in the 1960s whole blood transfusions were used. Since blood component transfusion of red blood cells, platelet concentrates and plasma became possible over time, red blood cell transfusions became gradually considered as a safe therapy for blood loss and other causes of anaemia. For decades a threshold of 6.2 mmol/l (10.0 g/dl) was used empirically as a transfusion trigger. In the 1970s the possibility of transfusion-induced immune suppression and in the 1980s the risk of transfusion transmitted infections (eg. human immunodeficiency virus or hepatitis) resulted in a more critical attitude towards blood transfusions and attention for the risk of blood transfusions [1]. Subsequently studies on evidence-based blood transfusion management were designed and surveillance registrations of side effects of blood transfusions were started; a process which is still ongoing.

Nowadays every year about 75 millions of blood units are collected and transfused worldwide, thereby yearly saving thousands of lives, facilitating more complex surgery and making transfusion of different blood components indispensibale for the treatment of many diseases [2]. The development of modern transfusion medicine represents one of the greatest achievements of medicine in the 20th century.

Of all donated units, 60-70% is transfused in surgical settings, in intensive care units or in acute situations. In the Western World yearly 50-70 per 1.000 patients receive a blood transfusion per year [3,4], while at the age of 80 years approximately one in five persons has been transfused [5]. It is expected that with aging, more complex surgery and increasing treatment options for hemato-oncological diseases the demand for allogeneic blood transfusions will increase.

Red blood cell (RBC) units are prepared by removing the plasma from the whole blood (with a volume of 450-500 ml) collected from voluntary donors. To maintain cell viability during storage, as replacement of plasma, an additive solution is used. RBC units have a volume ranging between 250-300 ml and have a hematocrit of 55-65%, equal to 135-180 ml of erythrocytes. Transfusion of one unit of RBC increases the hemoglobin (Hb) concentration of the recipient by approximately 0.6 mmol/l (1.0 gr/dl). Obviously this increase shows a huge variation depending on patient's clinical condition, weight and height and the conditions of RBCs in the unit [6]. The rationale of blood transfusion is to increase

oxygen transport capacity by increasing the Hb concentration aiming an adequate tissue oxygenation. To compensate for anemia the body has adaptive systems such as an increase of the cardiac output, redistribution of the blood flow and increase of the 2,3-diphosphoglycerate (2,3-DPG) in red blood cells, which causes a shift to the right of the oxygen dissociation curve [7,8]. If these compensatory mechanisms fail, the patient's outcome without red blood cell transfusions can be fatal. In children an increase in mortality was found when the Hb concentration fell below 2.5 mmol/l (4.0 g/dl) [9]. A retrospective cohort of adult surgical patients refusing blood transfusions for religious reasons showed only a small risk of death of 1.3% when preoperative Hb concentration was more than 7.5 mmol/l (12.0 g/dl), while the risk of death increased to 33.3% when the preoperative Hb concentration was less than 3.8 mmol/l (6.0 g/dl). This risk of death increased most in anemic patients with cardiovascular diseases and older age [10]. However, despite ample sophisticated research the precise margin between beneficial and deleterious effects of allogeneic blood transfusions, their mechanisms and the exact threshold for transfusions of blood components on morbidity and mortality are still not well defined. Clinical and laboratory studies in transfusion medicine are still ongoing to solve these important questions.

The Risks of Allogeneic Blood Transfusions

The safety of blood transfusions is optimal, although will never be maximal and there will always be risks. In well-resourced countries blood is collected from voluntary donors. They undergo a risk profile by a health questionnaire, which aims to identify any potential risks as well for the donor as for the recipient. Due to stringent donor selection, improved mandatory tests and close surveillance of new emerging infections the risk of transfusion-transmissible infections is very low. Therefore in well-resourced countries the concern regarding adverse transfusion effects shifted to non-infectious complications. With the aim to show transfusion safety at one hand and at the other hand to get insight in transfusion complications, in several countries the adverse reactions and events associated with blood transfusions are reported in national surveillance systems. Since 2003 the Dutch Foundation for Hemovigilance, Transfusion Reactions In Patients, (TRIP) collects all information about transfusion-associated reactions in the Netherlands. In 2009 2.384 transfusion reactions were reported to TRIP. That year the national (and only) blood supply organisation in the Netherlands, the Sanquin Blood Supply Foundation, delivered 699.720 blood products (559.976 RBC units, 49.354 platelet units and 90.390 plasma units). This means an average incidence of transfusion reactions of 3.4 per 1.000 blood components [11]. The incidence of more serious events was 98 (0.14 per 1.000 blood components). Transfusion reactions can

be divided into non-immune-mediated and immune-mediated reactions. The incidences of these reactions are summarized in Table 1.

Non-Immune Mediated Transfusion Reactions

Non-immune mediated transfusion reactions involve transmission of infectious agents (bacteria, viruses, parasites or prions) and complications such as transfusion-associated circulatory overload (TACO) and hemosiderosis.

Table 1 | Incidences of Adverse Effects of Allogeneic Blood Transfusions

Type of transfusion reactions	Estimated incidences
Non-immune-mediated	
Transfusion-transmitted infections	
Bacteria	RBC 1:1.000; platelets 1:2.000
Transfusion-associated sepsis	RBC 1:250.000; platelets 1:25.000
Viruses	
HIV	1:7.800.000
Hepatitis B	1:800.000
Hepatitis C	1:3.000.000
Parvo B19	<1:1.000.000
Parasites	<1:1.000.000
Prions	<1:1.000.000
Transfusion-associated circulatory overload (TACO)	2-8:100
Iron-deposition; hemosiderosis	Starts after >20 units RBCs
Immune-mediated	
Acute hemolytic transfusion reactions	1:18.000
Delayed hemolytic transfusion reactions	1:2.500-4.000
Febrile non-hemolytic transfusion reactions	1-7:100
Allergic transfusion reactions	1-3:100
Anaphylactic reactions	1:20.000-47.000
Transfusion-associated graft versus host disease (TA-GVHD)	<1:1.000.000
Post-transfusion purpura (PTP)	<1:1.000.000
Transfusion-related acute lung injury (TRALI)	1:1.000-5.000

Transfusion-transmitted infections through bacteria are mainly caused by contamination of blood products from skin-commensal bacteria derived from the donor's puncture site. Occult bacterial infections in the donor rarely cause blood-borne infections [12]. The incidence of bacterial contamination estimates 0.4% of RBC transfusions, while transfusion-associated

sepsis (TAS) occurs in 1 per 250.000 RBC transfusions [13]. Platelet concentrates are more prone for bacterial contamination, because they are stored at a higher temperature than RBCs (20-24°C versus 2-6°C). Approximately 1 per 2.000 platelet concentrates is contaminated by bacteria. In the literature, bacterial contamination accounts for 10-18% of transfusion-related fatalities [13]. In 2009, total 43 cases of bacterial contamination were reported to TRIP, only one of them caused TAS due to a blood product which was contaminated [11]. Because routine bacterial culture of platelet products may miss some contaminants, more sophisticated detection techniques or pathogen-inactivation methods are in development to further reduce bacterial contamination of blood products [14].

Due to genomic amplification testing, transfusion-transmission of human immunodeficiency virus (HIV) or hepatitis viruses has declined over the last decades. The estimated residual risk for transfusion-transmitted HIV infection is 1 per 7.8 million donations, for hepatitis B infection 1 per 800.000 donations and for hepatitis C infection 1 per 3 million donations [15]. In 2009 in the Netherlands 6 post-transfusion viral infections were reported to TRIP, of them one case of hepatitis B infection could be directly related to blood transfusions [10]. Prestorage leukocyte-depletion of blood transfusions can reduce the transmission of leukotrophic viruses, such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Since the implementation of leukocyte-depleted blood transfusions, no infections due to transmission of these viruses have been reported in the Netherlands [11]. Due to depression of the hematopoiesis Parvovirus B19 infection can be deleterious in patients with hemolytic anemia, immunocompromised patients and pregnant women because transmission to the foetus. Transmission of Parvovirus B19 is not common and is prevented by selection of donors with neutralizing antibodies, these B19-safe blood products are indicated for patients at risk [16]. Due to increased travelling more viruses and parasites are imported to non-endemic areas. Transfusion-transmission of import pathogens, such as West Nile virus (WNV), Chikungunya virus, malaria and Chagas disease (endemic in South and Central America caused by the parasite Trypanosoma cruzi), is prevented by stringent donor screening and testing on indication [17]. In the Netherlands there are as yet no reports of transfusion-transmitted infections from import viruses or parasites.

Since the 1990s there is concern about transfusion-transmission of prions leading to variant Creutzfeld-Jakob disease (vCJD). The incidence of vCJD is mainly concentrated in the United Kingdom where more than 75% of the known cases of vCJD have been recorded [18]. Three probable cases (1.7% of all cases of vCJD) have been reported in the United Kingdom in patients who had received blood products from donors who later developed vCJD [19]. As a preventive measure donors who resided until 1985 in the United Kingdom

for more than six months are refused as blood donors in the Netherlands. To date no transfusion-related transmission of vCJD is known in the Netherlands [11].

Besides transfusion-transmittable infections, an important non-immune mediated transfusion complication is transfusion-associated circulatory overload (TACO). TACO refers to pulmonary edema after transfusion of blood products. Recipients with renal or cardiac diseases and older patients are more susceptible for TACO, which is a serious underreported complication of blood transfusions. A retrospective analysis in elderly patients who underwent orthopedic surgery revealed an incidence of 2% after red blood cell transfusions and this could be up to 8% dependent on co-morbidity and age, with a fatality rate varying between 5 to 20% [20]. Because TACO is an underreported transfusion complication, the exact incidence is not known. The treatment of TACO consists of volume reduction with eventually ventilatory and/or circulatory support. More important is to prevent the risk of TACO by a restrictive transfusion strategy or the use of diuretics in patients with underlying cardiac and/or renal disease or elderly patients. Discrimination between TACO and transfusion-related acute lung injury (TRALI) can be difficult.

Hemosiderosis is inevitable in patients who chronically receive blood transfusions over years. Each RBC unit contains about 200 mg of iron, while from daily food only 1-2 mg iron is absorbed from the intestines. Each year adds 5-8 gram iron to the body stores of chronically transfused patients. The deposition of iron results in damage to the heart, liver and endocrine organs. Two-thirds of the patients with transfusion-related hemosiderosis die from heart failure [21]. Iron chelation therapy is advised after 20 units of RBCs or when the concentration of ferritine exceeds 1.000 mcg/l and the patient requires repeated transfusions with a life expectancy of more than 1 year.

Immune-Mediated Transfusion Reactions

Most reported transfusion reactions are immune-mediated [11]. These reactions are often distinguished in acute and delayed haemolytic transfusion reactions, febrile febrile non-hemolytic transfusion reactions, allergic transfusion reactions, transfusion-associated graft versus host disease, post-transfusion purpura and transfusion-related acute lung injury.

Current estimates show that acute hemolytic transfusion reaction (AHTR) occur in 1 per 18.000 transfused blood units and the mortality rate ranges between 1 per 600.000-1.800.000 units. AHTR develops within 24 hours after transfusion and is due to intravascular and/or extravascular destruction of erythrocytes [22]. Incorrect blood transfusion due to ABO-incompability is the main cause of fatal AHTR, which can occur in patients with high

antibody titres after administration of only 5-20 ml of blood [23]. In the Netherlands 60 cases of various kinds of incorrect blood transfusions were reported in 2009; fortunately none of these reactions led to death [11]. Delayed hemolytic transfusion reactions (DHTR) occur 24 hours to 28 days after blood transfusion and are caused by boosted RBC allo-antibodies in patients sensitized by previous blood transfusions or pregnancy. These antibodies are too weak to be detected by the compatibility testing prior to transfusion. In the literature, DHTRs occur in 1 per 2.500-4.000 RBC transfusions and mostly these reactions have a mild course; however fatal DHTRs can occur [24]. In the Netherlands 8 cases of DHTRs are reported to TRIP in 2009; while 753 newly diagnosed antibodies after blood transfusions were reported [11].

Febrile non-hemolytic transfusion reactions (FNHTR) are the most common adverse effect of blood transfusion. FNHTR is defined as a raise in body temperature of 2°C or more which occurs within 2 hours after a blood transfusion. FNHTR has an incidence of between 1 to 7% and one of the causes is a reaction between antibodies of the recipient against incompatible human leukocyte antigens (HLA) from the donor. This reaction is largely prevented by leukocyte-depletion of cellular blood products. Other identified causes are a response of recipient's leukocytes to cytokines in blood from the donor or accumulation of pyrogenic mediators during storage of blood products [25]. Observational studies documented since the implementation of leukocyte-depleted blood transfusions a significant reduction of almost 50% in the incidence of FNHTRs [26,27]. In 2009, 35% of the reported transfusion reactions were due to FNHTR, which is stable since the start of TRIP in 2003 [11].

Mild allergic transfusion reactions are common as well (1-3% of blood transfusions) and are mostly self-limited. Although severe anaphylactic transfusion reactions are rare, with an estimated incidence of 1 per 20.000-47.000 transfusions. In 2009, 69 cases of anaphylactic reactions were reported in the Netherlands [11]. Causes of allergic or anaphylactic reactions are hardly unravelled. Although IgA-deficiency associated with anti-IgA antibodies in the recipient who is transfused with IgA-containing blood products is often presumed, this cause of severe anaphylactic reactions is seldom identified. Despite IgA-deficiency with presence of IgA-antibodies having a high incidence of 1:1.200; IgA-antibodies have been found in less than 20% of suspected cases with severe allergic reactions [28]. As a preventive measure patients these patients should receive washed blood transfusions or blood products from IgA-deficient donors and should be transfused under appropriate prophylactic conditions.

Graft versus host disease (GVHD) is a common complication of hematopoietic stem cell transplantation. GVHD has an incidence of approximately 50% and its presentation can

be varying from temporary inconvenience to serious and life-threatening, while transfusion-associated graft versus host disease (TA-GVHD) is extremely rare and has a mortality rate over 90%. Since the start of TRIP in 2003 no case of TA-GVHD is reported in the Netherlands [11]. Immunocompromised patients are at increased risk of TA-GVHD and in immune competent recipients TA-GVHD can occur when an HLA homozygous donor shares one haplotype with the patient. The viable lymphocytes in the blood product then respond to the foreign host HLA antigens [29]. TA-GVHD is prevented by irradiation of blood products to patient populations at risk. Leukocyte-depletion of blood products reduces the risk of TA-GVHD, but is considered inadequate for prevention.

Another extremely rare complication with only one reported case since 2003 in the Netherlands is post-transfusion purpura (PTP), which is characterized by the development of severe thrombocytopenia occurring 1-24 days after an allogeneic blood transfusion [11]. PTP can develop in patients who are sensitized (by previous transfusion or pregnancy) to a human platelets antigen (HPA) [30]. Most patients with PTP recover in approximately two weeks, although PTP can result in severe bleeding. In emergency the first choice of treatment in PTP is with high-dose intravenous immunoglobins. Patients with a documented history of PTP are advised to receive cellular blood components that are antigen-negative for the platelet antibody.

A leading cause of transfusion-associated mortality is transfusion-related acute lung injury (TRALI), which is estimated to occur after 1:1.000 to 5.000 blood transfusions with an estimated mortality rate of 5-10% [31]. Since the start of registration of transfusion reactions by TRIP the reported cases of TRALI raised from 7 in 2003 to 21 in 2008, which is mainly the result of awareness of physicians [11]. TRALI is a serious life-threatening condition and is probably still underreported. According to an international agreed definition, the onset of TRALI is within 6 hours after blood transfusion [32]. The pathophysiology of TRALI has not been completely clarified yet and is the final result of a cascade of neutrophil priming, activation and endothelial damage [33]. One of the causes is passively transfused anti-leukocyte antibodies in the donor's plasma, which bind to antigens on patient's neutrophils and initiate priming and activation with release of cytokines, proteases and free oxygen radicals. Neutrophil sequestration in the lung is finally leading to endothelial damage and capillary leakage. Such antibodies, mostly directed against HLA class I and II or human neutrophil-specific antigens (HNA), are mainly found in multiparous female donors [34]. As a preventive method since end 2007 in the Netherlands, fresh frozen plasma is only derived from non-transfused male donors. Since this implementation the reported cases of TRALI was reduced from 21 to 12 in 2009 [11]. Besides leukocyte antibodies there is circumstantial

evidence that other insults such as bio-active lipids accumulating in stored erythrocytes, CD40 ligand in platelet products and cytokines involved in infections can prime neutrophils to adhere to the vascular endothelium [34]. Consequently, TRALI occurs more often in patients in whom leukocytes are already primed due to an underlying condition associated with pro-inflammatory mediators in which other factors can act as a second hit [35]. The clinical presentation of TRALI can be very similar to TACO [35,36]. It has been suggested that markers in plasma as elevated pro B-type natriuretic peptide (pro-BNP) levels may be helpful to discriminate TACO from TRALI [37]. Other possible differences between both underreported serious transfusion reactions are summarized in Table 2.

Table 2 | Possible Differences between Transfusion Related Acute Lung Injury (TRALI) and Transfusion Associated Cardiac Overload (TACO)

Symptoms	**TRALI**	**TACO**
Onset of symptoms	<6 hours	Mainly <6 hours
Respiratory symptoms	Dyspnea	Dyspnea
Central venous pressure	Normal	Increased
Pulmonary wedge pressure	Normal	Increased
Fluid balance	Positive or negative	Positive
X-ray thorax	Bilateral infiltrates	Bilateral infiltrates with signs of fluid overload
Echocardiography	Normal ejection fraction	Decreased ejection fraction
B-type natriuretic peptide	Low or normal	High

TRALI, AHTR and TAS were the leading causes of reported transfusion-associated mortality in the UK and USA [38]. However, besides these clinically manifest transfusion reactions, several studies observed that allogeneic blood transfusions lead in selected clinical circumstances to higher morbidity and mortality compared to non-transfused patients with the same conditions. If a relationship with transfusions would be causal, the fatality rate of these occult complications would greatly exceed the hitherto reported rates by surveillance registries. These unsolved questions opened in different clinical settings new and more evidence-based and multi-disciplinary clinical research, in particular in patients undergoing cardiac surgery or staying in the intensive care unit (ICU), because these patients receive large amounts of blood products.

Allogeneic Blood Transfusions in Cardiovascular Disease and in Cardiac Surgery

Due to a more critical oxygen delivery to the myocardium, patients with cardiovascular disease are less tolerant to anemia than others. Blood transfusions for anemic patients with ischemic heart disease are intended to improve oxygen delivery to the myocardium and thereby the patient survival [39,40]. Cohort studies in patients with cardiovascular diseases, documented that at one hand anemia was associated with an increase in mortality and at the other hand that RBC transfusions may cause more congestive heart failure [41,42]. In a large cohort of 78.974 patients older than 65 years with acute myocardial infarction, patients with lower hematocrit (Ht) levels had a higher 30-day mortality rate and RBC transfusions significantly reduced the mortality rate in patients with a Ht level of less than 30% at admission [43]. In contrast, a post hoc analysis derived from three large cardiovascular studies showed that patients with an acute coronary syndrome who had received RBCs had (after adjustment for other predictive factors) significant higher 30-day mortality than non-transfused patients [44].

Coronary artery bypass graft (CABG) surgery is a frequently performed intervention to re-vascularise the myocardium. Worldwide more than 800.000 patients are undergoing cardiac surgery annually. In the Netherlands, with 16.000.000 inhabitants, approximately 17.000 patients (38% of them are valve replacements) are undergoing cardiac surgery every year [45]. The current mortality and morbidity rate after cardiac surgery is low and cardiac surgery has become a routine procedure also for older patients with more co-morbidities. Although the number of patients that receive blood transfusions and the numbers of transfused blood products became lower in time, patients undergoing cardiac surgery still receive more blood transfusions compared to most other surgical settings. Due to hemodilution and consumption of coagulation factors and platelets in the extracorporal circuit patients undergoing cardiac surgery can develop severe bleeding complications. Despite blood-sparing developments, reducing the need of blood transfusions, cardiac surgery still consumes a large proportion of RBC transfusions, estimated approximately 20% of the total blood supply [46].

In cardiac surgery preoperative as well as postoperative anemia are important prognostic factors for outcome. A pre-operative Hb level below 6.2 mmol/l (10.0 g/dl) is associated with higher mortality rate compared to patients with higher Hb values [47]. It has been observed that preoperative anaemia is associated with increased risk of stroke or kidney failure [48,49]. Furthermore, the nadir of the Hb concentration during cardiac surgery is related with worse adverse outcome [47] and massive blood loss is associated with an 8-fold increase in mortality [50].

Maybe influenced by different clinical risk factors, such as age, co-morbidity and preoperative Hb values the transfusion rates for CABG used to show great variability between hospitals with a mean number of transfused units varying between 0.4 to 6.3 units per patient [51,52]. Several observational studies showed that the peroperative administration of RBCs was the most constant factor associated with mortality and morbidity and was dose-dependently associated with postoperative infections and higher mortality [53,54]. In a prospective study in cardiac surgery, 4.8% of patients who did not receive RBCs, suffered from postoperative infections contrasting with 29% in patients who received 6 or more RBC units [55]. Not only short-term (30-and 90-day) mortality was influenced by transfusion of RBCs, also 1-year, 5-and 10-year postoperative mortality was found to be increased in transfused compared to non-transfused patients undergoing the same type of cardiac surgery [56-59]. However all these studies were retrospectively designed and provide by no means proof of a causal role of allogeneic RBC transfusions on postoperative morbidity and mortality after cardiac surgery, where many other factors influence the outcome.

Patients undergoing cardiopulmonary bypass develop systemic inflammatory reactions. Its magnitude and the capacity for reversal may determine outcome. Generation of inflammatory mediators may be associated with more complex and longer surgery, whereas these patients receive also larger amounts of blood transfusions. It is postulated that allogeneic blood transfusions could play an additional role (second-hit) in the development of postoperative complications.

Anemia and Blood Transfusions in the Intensive Care Unit

After cardiac surgery, patients generally stay at an intensive care unit (ICU) for as long as mechanical ventilation and cardiac inotropic drug support is needed. Anemia is often encountered in the ICU in surgical patients and is of multifactorial origin. Multiple blood sampling, blood losses due to gastro-intestinal tract bleeding or surgical reasons are the main reasons [60]. Daily multiple blood sampling amounts on average 41 to 66 ml at medical-ICUs and up to 377 ml/day at cardiothoracic ICUs [61-63]. The amount of blood loss drawn for diagnostic sampling turned out to be the most significant predictor for the receipt of blood transfusions [64]. Besides, hemodilution by abundant intravenous infusions, decreased RBC production due to iron-deficiency and inappropriate erythropoietin-response due to inflammatory mediators in critically ill patients, reduced RBC survival and increased (drug-induced) hemolysis may contribute further to postoperative anemia in ICU patients [65-67].

Lacking evidence-based transfusion triggers, anemia is often corrected with RBC transfusions. Several large observational studies investigated the incidence of anemia and the

trends of blood transfusions therapy at the ICU. In the USA an observational multicenter study comprising 4.892 patients across 284 ICUs found that more than 60% of the patients developed an Hb value less than 7.5 mmol/l (12.0 gr/dL), of which 44% received one or more RBCs. Low Hb value, without evidence of active blood loss, was in 90% the reason to transfuse with RBCs. In particular older patients and patients with a longer ICU-stay were at risk for transfusions [68]. In 1995, 85% of patients staying longer than 1 week at the ICU received a mean of 9.5 RBC units per patient [69]. Despite several blood-sparing developments this transfusion practice hardly changed in the following ten years [68]. A prospective observational study in several ICUs in Europe comprising 3.534 patients found comparable results [70]. Approximately 29% of the patients reached a Hb value of less than 6.2 mmol/l (10.0 gr/dl) with a transfusion rate of 37%. Of the patients with an ICU-stay longer than 7 days, 73% had received RBC transfusions. Overall mortality was almost twice as high in patients who received allogeneic RBC transfusions compared to patients who were not transfused.

In critically ill patients, blood transfusions have been associated with mortality, ventilator-associated pneumonia, acute respiratory distress syndrome (ARDS) and bloodstream infections [71-75]. Besides RBC transfusions, also fresh frozen plasma and platelet units were reported to contribute to complications at the ICU [76,77]. However, these patients represent heterogeneous patient population with complex illnesses. Studies performed in more homogenous patient cohorts, such as in 666 patients with major burn injury revealed that the number of RBCs was associated with mortality and the risk of infection increased with each unit of blood transfused [78]. In a large study in 9.539 trauma patients, blood transfusions within the first 24 hours were associated with systemic inflammatory response syndrome (SIRS) and mortality [79].

In the past, RBC transfusions were administered according to an arbitrary Hb trigger of 6.2 mmol/l (10 gr/dl) [80]. The Transfusion Requirements in Critical Care (TRICC) trial compared for the first time a restrictive transfusion strategy (maintaining hemoglobin level between 4.3-5.6 mmol/l [7.0-9.0 gr/dl]) with a liberal transfusion strategy (maintaining hemoglobin level level of more than 6.2 mmol/l [10.0 gr/dl]). The liberal strategy group had compared to the restrictive strategy group no benefit in 30-day and hospital mortality. Moreover pulmonary edema and ARDS occurred more often in the liberal group [81]. Also subgroup analysis in patients with coronary artery disease revealed similar 30-day mortality in restrictive and liberal transfusion strategy groups [82]. These findings changed the transfusion policy of red blood cells in the ICU completely. In contrast with this randomized controlled trial, a retrospective observational single-center study in 2.393 patients found an

association with a hematocrit level less than 25% upon admission to the ICU and 6-month and 1-year mortality [83]. Patients at ICU represent a very heterogeneous population with various complex pathology. Therefore it is difficult to determine the effects of anemia and blood transfusions in this population. To unravel an association between blood transfusions and outcome, research in more matched patient populations is important.

Clinical Relevance of Transfusion-Related Immunomodulation

In the 1970s, it was discovered that pre-transplantation blood transfusions improved a subsequent renal allograft survival [84]. It was presumed that a similar immune suppressive transfusion effect could lead to impaired cancer surveillance enhancing cancer recurrence after curative surgery and to susceptibility for postoperative infections [85-87]. Many observational studies reported that allogeneic blood transfusions were indeed associated with increased bacterial infections in patients undergoing abdominal surgery. These possible adverse effects of blood transfusions, which are not registered in national surveillance systems, are referred as transfusion-related immunomodulation (TRIM). The presumed magnitude of TRIM would be much larger than the recognized adverse transfusion reactions reported to the national surveillance systems [88].

The existence and possible mechanisms of TRIM are not yet fully discovered. Many factors might contribute to TRIM. Allogeneic leukocytes and (leukocyte-derived) soluble mediators in blood products are considered as most important [89]. Through filtration the number of allogeneic leukocytes in donated blood can be reduced by more than 99.9% with a residual leukocyte count of less than $1x10^6$/L. Leukocyte-depleted (or leukocyte-reduced) blood transfusions were applied since the 1980s to reduce non-hemolytic febrile transfusion reactions, HLA allo-immunisation and CMV transmission in patients at risk. In early 2000s in several European countries and in Canada universal leukocyte-depletion of allogeneic blood transfusion was implemented. In the Netherlands in 2002 universal leukocyte-depletion for blood transfusions was introduced as precautionary measure assuming to prevent viral infections and vCJD. The benefits of universal leukocyte-depletion are still controversial.

To investigate the clinical effects of TRIM several studies have been performed comparing leukocyte-containing with leukocyte-depleted blood products in different, mainly surgical, clinical settings. In particular patients undergoing cardiac surgery form a more homogeneous population and are at risk to receive large amounts of blood transfusions. In 1998 a Dutch randomized controlled trial in patients undergoing CABG, with or without valve replacement, compared standard buffy-coat depleted RBCs (containing 20-30% of

the donor leukocytes) with by filtration leukocyte-depleted RBCs [89]. Patients receiving more than 3 units RBCs had a significant reduction in postoperative infections and 60-day mortality if transfused with leukocyte-depleted RBCs. Another Dutch study, published in 1999, found in patients who received non-leukocyte-depleted blood transfusions during cardiac surgery an increase in concentrations of inflammatory mediators [90]. The findings from both studies suggest that allogeneic blood transfusions during cardiac surgery could play a role in postoperative outcome by contribution to an inflammatory response. This opened a new field in transfusion medicine: the causes and effects of TRIM, especially in cardiac surgery.

SCOPE OF THIS THESIS

The predominant research question is whether and how the presence of leukocytes in allogeneic blood transfusions have a clinical deleterious effects in cardiac surgery patients. These questions form the basis of this thesis evaluating clinical adverse transfusion effects, their nature and possible mechanisms of leukocyte-containing blood transfusions in cardiac surgery. For this purpose we conducted a randomized controlled trial (RCT) to confirm the results found in subgroup analysis in a former study. For this study, patients undergoing complex cardiac surgery, who are more extensively transfused, were selected. After confirming an adverse role of allogeneic leukocytes in red blood cell transfusions, we aimed to identify mechanisms how transfusion-related factors could influence the outcome after cardiac surgery.

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Chapter 2

Double-Blind Randomized Controlled Trial on the Effect of Leukocyte-Depleted Erythrocyte Transfusions in Cardiac Valve Surgery

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ABSTRACT

Background: Leukocytes in allogeneic blood transfusions are believed to be the cause of immunomodulatory events. A few trials on leukocyte removal from transfusions in cardiac surgery have been conducted, and they showed inconclusive results. We found in a previous study a decrease in mortality rates and number of infections in a subgroup of more heavily transfused patients.

Methods: Patients (n=496) undergoing valve surgery (with or without CABG) were randomly assigned in a double-blind fashion to receive standard buffy coat-depleted (PC) or prestorage, by filtration, leukocyte-depleted erythrocytes (LD). The primary end point was mortality at 90 days, and secondary end points were in-hospital mortality, multiple organ dysfunction syndrome, infections, intensive care unit stay, and hospital stay.

Results: The difference in mortality at 90 days was not significant (PC 12.7% versus LD 8.4%; odds ratio [OR], 1.52; 95% confidence interval [CI], 0.84 to 2.73). The in-hospital mortality rate was almost twice as high in the PC group (10.1% versus 5.5% in the LD group; OR, 1.99; 95% CI, 0.99 to 4.00). The incidence of multiple organ dysfunction syndrome in both groups was similar, although more patients with multiple organ dysfunction syndrome died in the PC group. LD was associated with a significantly reduced infection rate (PC 31.6% versus LD 21.6%; OR, 1.64; 95% CI, 1.08 to 2.49). In both groups, intensive care unit stay and hospital stay were similar, and postoperative complications increased with the number of transfused units.

Conclusions: Mortality at 90 days was not significantly different; however, a beneficial effect of LD in valve surgery was found for the secondary end points of in-hospital mortality and infections.

INTRODUCTION

Despite blood-sparing developments, blood transfusions still play a pivotal role in many large operations. Because of advances in transfusion medicine, allogeneic blood transfusions carry minimal risks for transmission of diseases. However, as it has been shown that allogeneic blood transfusions impair the immune response against cadaver kidney grafts [1], there is concern that blood transfusions could also suppress the recipients immune response against cancer and infections [2,3]. These effects are often referred to as transfusion-related immunomodulation (TRIM) [4]. Many factors in transfusions might contribute to TRIM, but leukocytes and soluble factors mediated by leukocytes in blood transfusions are considered of possible importance [5,6]. The depletion of leukocytes by filtration of blood products has been applied for many years to reduce human leukocyte antigen (HLA) alloimmunization and cytomegalovirus transmission for patients at risk. A limited number of clinical studies investigated whether leukocyte-depleted erythrocyte concentrates diminish TRIM [6]. The only prospective, randomized controlled trial (RCT) that compared the recurrence of colorectal cancer after perioperative transfusions with leukocyte-depleted versus buffy coat-depleted erythrocytes found no benefit [7]. The effect of leukocyte-depleted erythrocytes on postoperative infections after abdominal surgery has been studied in five RCTs, with conflicting results [8-12].

Generally, in complex cardiac surgery, more erythrocyte concentrates are transfused than in abdominal surgery [13]. Two RCTs on cardiac surgery have been performed, and they showed inconclusive results on the incidence of postoperative infections [14,15]. Two other studies were not focused on cardiac surgery [16,17]. In one study, a nonsignificant beneficial difference of leukocyte-depleted erythrocytes in severe infections was found [16]; the other found no decrease in antibiotic usage, their parameter for infections [17]. We observed in one RCT comparing leukocyte-depleted versus buffy coat-depleted erythrocytes in patients undergoing coronary artery bypass grafting (CABG), with or without valve replacement, a significant reduction in postoperative infections only in the patients receiving 4 or more units [15]. Unexpectedly, in this study, a reduced mortality rate in the group receiving leukocyte-depleted erythrocytes was found. This difference was due to the near absence of death by multiple organ dysfunction syndrome (MODS) in the patients receiving leukocyte-depleted erythrocytes. These results were the reason to initiate a prospective double-blind, 2-center RCT to detect possible differences in postoperative complications. This trial was performed in adults undergoing cardiac valve surgery (with or without CABG) because these patients

do have a high probability of receiving 4 or more erythrocyte units and have an increased risk on postoperative complications [15].

MATERIAL AND METHODS

Patients and Design

A prospective, randomized, double-blind controlled trial was conducted between June 1999 and May 2001 in adult patients older than18 years undergoing valve surgery (with or without CABG) in 2 university hospitals (Academic Medical Center and Leiden University Medical Center) in The Netherlands. The ethics review boards of both hospitals approved the trial protocol. The local trial coordinator collected written informed consent. Patients with a medical indication for leukocyte-depleted erythrocytes (LDs) and patients who had received blood transfusions within the previous 3 months were ineligible. When blood for compatibility testing of the participating patients was sent to the hospital transfusion service, the technicians randomly assigned the patients by opening a sealed and numbered envelope. The patients were randomly assigned into 2 groups: when there was an indication for transfusions, one group received buffy coat-depleted packed cells (PCs), which was at that time the standard product in The Netherlands, and the other group received prestorage by filtration of LDs. The patients, surgeons, anesthesiologists, and the trial coordinators were blinded to the random assignment, as the technicians placed uniform study labels on the description on the erythrocyte bags. In the hospital electronic information system, a code was used during the study period to hide the random assignment.

For the assessment of the preoperative risk of the patients, the score model described by Parsonnet was applied [18]. Surgical and anesthetic procedures were performed according to the standards of the hospitals. The hospitals used similar transfusion triggers for erythrocytes, plasma, and platelets. Not all patients underwent induced hypothermia (29° to 33°C). In one hospital, aprotinin was used in some patients; this hospital had a medium-care ward. Prophylactic antibiotics were given to all patients for 48 hours. After surgery, the patients were monitored at the intensive care unit (ICU); they were discharged from the ICU when there was no more need for inotropes and intubation.

Blood Products

PCs were prepared by centrifugation of whole blood at 3000 rpm for 10 minutes within 20 hours after withdrawal. Buffy coat and plasma were removed, and erythrocytes were

reconstituted with 100 mL saline-adenine-glucose-mannitol. The average leukocyte content was $0.7 \pm 0.4 \times 10^9$ per unit. The average hemoglobin content was 59 ± 5 g/unit. LDs were prepared before storage within 24 hours after withdrawal by passage of the buffy coat-depleted erythrocytes through a leukocyte filter (Cellselect-Optima, NPBI International-Fresenius HemoCare). The average leukocyte content was $0.15 \pm 0.02 \times 10^6$ per unit and the average hemoglobin content was 54 ± 4 g/unit. The quality controls of the blood products were according to the requirements of the blood product specifications in The Netherlands. Platelet concentrates were prepared from pooled buffy/coats and were all leukocyte-depleted by filtration (less than $1x10^6$ leukocytes per product) before storage.

Data Collection

Preoperative and postoperative clinical and laboratory data and transfusion data were registered daily and collected by the local trial coordinator from the patient records and the hospital electronic information systems. The scoring of organ dysfunction was assessed retrospectively on the basis of the daily records. We used parameters for organ dysfunction as described by Knaus et al [19]. MODS was defined as the failure of less than 2 organ systems. Infections were scored according to the criteria of the Centers for Disease Control and Prevention [20]. Causes of in-hospital mortality were obtained from the hospital patient records, report of mortality at 90 days from the referring cardiologist, or the general practitioner.

End Points and Statistics

The primary end point of the study was mortality at 90 days after surgery. Secondary end points were incidence of in-hospital mortality and the causes of death, incidence of MODS, infections during the hospital stay, duration of ICU-stay, and hospitalization. An independent safety committee monitored the interim results of the primary end point.

Based on the results of the subpopulation in a previous study [15], the trial was designed to detect a significant difference in mortality between both trial arms of 10% (15% versus 5%). To reach statistical significance ($p<0.05$) and a power of 90%, 210 evaluable patients were needed in each arm. To compensate for non-evaluable patients, the target number of included patients was 500.

Patients were stratified by the type of surgery (valve with or without CABG) and by hospital. Within each of these strata, a straightforward randomization was performed by using a fixed block size (n=24) to ensure a balance between the randomization groups. The final analysis incorporated the stratification structure (2x2) when estimating the relation

between the randomization variable and the various outcomes. The analysis for all end points was on an intention-to-treat basis. All patients were analyzed according to the original random assignment, and no patients were re-assigned. Based on the results of the previous study [15], subgroup analysis was performed according to transfusions. For comparison of qualitative parameters, the Fisher's exact test or χ^2 test was used, and for comparison of quantitative parameters, the *t* test or Mann-Whitney *U* test was used. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to compare the differences of mortality, infections, and MODS, calculated according to the Mantel-Haenszel procedure. The CI obtained by the Mantel-Haenszel procedure is based on a normal approximation. ICU-stay, hospital-stay, and duration of MODS were analyzed by means of the Student's *t* test. Multivariate analysis of the risk factors was performed with the use of an enter/backward logistic regression model. For statistical analysis, the SPSS program was used.

RESULTS

In total, 496 patients were enrolled in the study. Twenty-two patients were excluded for various reasons: 8 withdrew consent before the surgery, 6 cancelled surgery, 3 had no valve replacement performed, 3 had received blood transfusions in the past 3 months, and 2 died before the operation. In each arm, 237 patients were evaluable for the primary end points. Two patients (both randomly assigned into the LD group) died during the initial operation. These 2 patients were ineligible for the analysis of the secondary end points. Three patients (all of them randomly assigned into the PC group) died in a second operation (performed more than 24 hours later); these patients remained in the study for the secondary end points. As shown in Table 1, both groups were comparable with respect to baseline characteristics, with an exception for the storage time of the erythrocytes. Based on their Parsonnet-score, the patients in both trial arms are considered to be at high-risk (mean ± SD: 13.5 ± 8.3). The majority (58.2%) of the patients received 4 or more erythrocyte units (mean ± SD: 6.1 ± 6.6). Forty-two (8.9%) of the patients did not receive any transfusion. Four (0.8%) patients received both types of blood products; these patients remained in their original randomization arm.

Table 1 | Baseline Characteristics

	PC (n=237)	LD (n=237)
Age, years	66.6 ± 12.5	65.3 ± 14.7
Hospital A/B, n	126/111	130/107
Female	102 (43.0)	113 (47.7)
Myocardial infarction	30 (12.7)	29 (12.2)
Hypertension	60 (25.3)	68 (28.7)
Diabetes mellitus	33 (13.9)	22 (9.3)
Heart failure	48 (20.3)	59 (24.9)
Parsonnet score	13.5 ± 8.6	13.5 ± 8.0
0 to 4	31 (13.1)	19 (8.0)
5 to 9	53 (22.4)	61 (25.7)
10 to 14	57 (24.0)	65 (27.4)
15 to 19	39 (16.5)	36 (15.2)
≥20	57 (24.0)	56 (23.6)
Surgery		
Valve	164 (69.2)	156 (65.8)
Valve+CABG	73 (30.8)	81 (34.2)
Aortic valve	160 (67.5)	172 (72.6)
Mitral valve	79 (33.3)	64 (27.0)
Other valves	16 (6.8)	16 (6.8)
Multivalves	12 (5.1)	17 (7.2)
Reoperations	18 (7.6)	12 (5.1)
Cardiopulmonary bypass, min	143 ± 62	139 ± 60
Aortic cross-clamping, min	96 ± 45	99 ± 47
Rethoracotomy	36 (15.2)	26 (11.0)
Normothermia	44 (18.6)	48 (20.3)
Use of aprotinin	88 (37.1)	86 (36.3)
Erythrocyte transfusions		
Units, n	6.2 ± 7.1	5.9 ± 6.1
0	21 (8.9)	21 (8.9)
1 to 3	85 (35.9)	71 (29.9)
≥4	131 (55.3)	145 (61.2)
Storage time of the units, d	19.7 ± 5.4	17.4 ± 5.9*
Preoperative hematologic values		
Hemoglobin, g/dl	12.2 ± 4.1	12.4 ± 3.7
Platelets, $x10^9$/l	232 ± 72	232 ± 71
Leukocytes, $x10^9$/l	6.9 ± 2.9	6.7 ± 2.7

Values are mean ± SD or number (%). * $p<0.05$

Table 2 | Intention-to-Treat Analysis

	PC	LD	OR (95% CI)
Mortality at day 90	30 (12.7)	20 (8.4)	1.52 (0.84 to 2.73)
In-hospital mortality	24 (10.1)	13 (5.5)	1.99 (0.99 to 4.00)*
Causes of death			
During initial operation	0	2	
MODS	17 (7.2)	10 (4.2)	
Cardiac events	4 (1.7)	1 (0.4)	
During second operation	3 (1.3)	0	
Infections	75 (31.6)	53 (22.6)	1.64 (1.08 to 2.49)*
No. infections	79	58	
Type of infections			
Respiratory tract	39	25	
Urinary tract	14	14	
Wound	11	10	
Bacteremia	12	8	
Unknown	3	1	
MODS	49 (20.7)	48 (20.4)	1.07 (0.67 to 1.68)

Values are number (%). * $p \leq 0.05$

Intention-to-Treat Analysis

As shown in Table 2, in total, 50 (10.5%) patients died within the first 90 days after surgery. The total mortality rate at day 90 was higher in the PC group than in the LD group (12.7% versus 8.4%, respectively). This difference was not significant (OR, 1.52; 95% CI, 0.84 to 2.73; p=0.16). In the hospital, 37 (7.8%) patients died; in the PC group the in-hospital mortality rate was almost twice as high as in the LD group (10.1% versus 5.5%, respectively; OR, 1.99; 95% CI, 0.99 to 4.00; p=0.05).

In 128 of 472 patients (27.1%), 137 postoperative infections were diagnosed. In the PC group, 75 (31.6%) of 237 patients had infections, as did 53 (22.6%) of 235 patients in the LD group (OR, 1.64; 95% CI, 1.08 to 2.49; p=0.02). In total, 97 (20.6%) patients had MODS in the postoperative period. In both trial arms, there was a similar incidence of MODS (20.7% in PC versus 20.4% in LD group). The duration of MODS in days (mean±SD) was also not different in both groups (6.3 ± 8.8 in PC versus 6.1 ± 8.0 in LD; p=0.98).

ICU stay in the PC group was 5.6 ± 7.2 (mean ± SD) days and 5.5 ± 7.3 days in the LD group (p= 0.88). The median ICU stay in both groups was 3 days. The postoperative

hospital stay was 13.8 ± 10.7 days and 13.3 ± 13.7 days in the PC and LD groups, respectively (p=0.66). The median duration of the hospital stay in both groups was 10 days.

Analysis According to Transfusion

In both groups, the mortality rate at 90 days was higher in patients who had received ≥4 U: PC, 19.9% versus LD, 12.4%; OR, 1.82; 95% CI, 0.94 to 3.52; p=0.08 (Table 3). The causes of death after discharge in both groups were predominantly cardiac. Both for mortality at day 90 and in-hospital, there were no deaths in the non-transfused patients. In the patients who received 1 to 3 U, there was no difference in mortality rates between both groups. The in-hospital mortality rate increased markedly with transfusion of 4 or more units (Table 4) (PC, 17.6% versus LD, 8.3%; OR, 2.43; 95% CI, 1.16 to 5.12; p=0.02). In both groups, most of the patients with in-hospital death had MODS. Mortality rate associated with MODS was higher in the PC than in the LD group (Table 2).

Table 3 | Mortality at Day 90 According to Number and Type of Transfusions

	No.		Mortality at day 90			
	PC	LD	PC	LD	OR	95% CI (p)
Total	237	237	30 (12.7)	20 (8.4)	1.52	0.84 to 2.73 (0.16)
No. of transfusions						
0	21 (8.9)	21 (8.9)	0	0		
≥1	216 (91.1)	216 (91.1)	30 (13.9)	20 (9.3)	1.63	0.90 to 2.98 (0.11)
1 to 3	85 (35.9)	71 (29.9)	4 (4.7)	2 (2.8)		
≥4	131 (55.3)	145 (61.2)	26 (19.9)	18 (12.4)	1.82	0.94 to 3.52 (0.08)

Table 4 | In-Hospital Mortality Rates According to Number and Type of Transfusions

	No.		In-Hospital mortality			
	PC	LD	PC	LD	OR	95% CI (p)
Total	237	237	24 (10.1)	13 (5.5)	1.99	0.99 to 4.00 (0.05)
No. of transfusions						
0	21 (8.9)	21 (8.9)	0	0		
≥1	216 (91.1)	216 (91.1)	24 (11.1)	13 (6.0)	2.00	0.99 to 4.02 (0.05)
1 to 3	85 (35.9)	71 (29.9)	1 (1.2)	1 (1.4)		
≥4	131 (55.3)	145 (61.2)	23 (17.6)	12 (8.3)	2.43	1.16 to 5.12 (0.02)

Most patients with infections were in the group receiving 4 or more units (Table 5) (PC, 44.3% versus LD, 28.7%; OR, 1.93; 95% CI, 1.17 to 3.20; p=0.01). The types of infections are shown in Table 2. In both groups, the number of transfused erythrocyte concentrates was correlated with the incidence of MODS; however, this was not different between the groups (Table 6).

Table 5 | Postoperative Infections According to Number and Type of Transfusions

	No.		Infections			
	PC	**LD**	**PC**	**LD**	**OR**	**95% CI (p)**
Total	237	235	75 (31.6)	53 (22.6)	1.64	1.08 to 2.49 (0.02)
No. of transfusions						
0	21 (8.9)	21 (8.9)	2 (9.5)	1 (4.8)		
≥1	216 (91.1)	214 (91.1)	73 (33.8)	52 (24.3)	1.61	1.06 to 2.47 (0.03)
1 to 3	85 (35.9)	71 (30.2)	15 (17.6)	11 (15.5)		
≥4	131 (55.3)	143 (60.9)	58 (44.3)	41 (28.7)	1.93	1.17 to 3.20 (0.01)

Table 6 | MODS According to Number and Type of Transfusions

	No.		MODS			
	PC	**LD**	**PC**	**LD**	**OR**	**95% CI (p)**
Total	237	235	49 (20.7)	48 (20.4)	1.07	0.67 to 1.68 (0.79)
No. of transfusions						
0	21 (8.9)	21 (8.9)	2 (9.5)	2 (9.5)		
>1	216 (91.1)	214 (91.1)	47 (21.8)	46 (21.5)	1.05	0.65 to 1.68 (0.85)
1 to 3	85 (35.9)	71 (30.2)	5 (5.9)	6 (8.5)		
≥4	131 (55.3)	143 (60.9)	42 (32.1)	40 (28.0)	1.24	0.73 to 2.11 (0.42)

Multivariate Analysis

For the primary end point and the secondary end points, the following preoperative and peroperative risk factors were analyzed: Hospital, sex, age, Parsonnet score, type of surgery, duration of cardiopulmonary bypass and aortic-cross clamping, randomization arm, and number of erythrocyte transfusions. For mortality at day 90, Parsonnet score (p=0.001), the number of erythrocyte transfusions (p<0.001), and sex (p=0.04) were significant

prognostic factors; for in-hospital mortality, the number of erythrocyte transfusions ($p<0.001$), Parsonnet score ($p=0.01$), sex ($p=0.03$), and the randomization arm ($p=0.01$) were significant prognostic factors (Table 7). For postoperative infections, the number of erythrocyte transfusions ($p<0.001$) and randomization arm ($p=0.02$) were significant prognostic factors. For MODS, only the number of erythrocyte transfusions ($p<0.001$) was significant prognostic factor.

Table 7 | Multivariate Analysis for Mortality at Day 90 and In-Hospital Mortality Rates

	Mortality at day 90	**In-Hospital mortality**
	p	
No.erythrocyte transfusions	< 0.001	< 0.001
Parsonnet score	0.001	0.01
Sex	0.04	0.03
Randomization arm	0.16	0.01
Type of surgery	0.07	0.12
Hospital	0.12	0.85
Age	0.24	0.28
Duration of cardiopulmonary bypass	0.22	0.42
Duration of aortic cross-clamping	0.77	0.91

DISCUSSION

In this study, a large group of patients undergoing complex cardiac surgery were enrolled. These patients were heavily transfused with erythrocyte concentrates, which were randomly allocated between standard PCs and LDs. The overall mortality rate within the first 90 days after surgery was 10.5% and the in-hospital mortality rate was 7.8%. These rates were comparable with other studies in valve surgery [21,22]. In agreement with others [23], we found that most of the patients who died after discharge from the hospital died from cardiac causes. This was similar between both trial arms. We observed a high rate of rethoracotomy in this study with a difference between the hospitals: 7% in hospital A (256 patients) and 20% in hospital B (218 patients). A possible explanation is the policy of administration of aprotinin in one hospital. The use of aprotinin was equally distributed in both trial arms.

The major findings of this study were: 1) a nonsignificant difference for the primary outcome of the study, mortality at 90 days (12.4% versus 8.4%); 2) an in-hospital mortality rate in the PC group (10.1%) that was almost twice as high as that in the LD group (5.5%), largely caused by deaths associated with MODS; and 3) a significantly higher incidence of postoperative infections in the PC group (31.6%) compared with the LD group (22.6%). The incidence of MODS was not different, nor was the ICU stay or hospital stay between the groups. This lower in-hospital mortality rate in the LD group, as well as the strong relation with the number of transfusions, extends the results of the previous study [15]. This earlier study was primarily designed to evaluate the effect of leukocyte-depleted erythrocytes on alloimmunization and infections but revealed an unexpected difference in mortality rates in patients transfused with PCs. In a similarly designed study in patients with CABG (with infections as primary end point), a nonsignificant difference in mortality rate was observed; however, total mortality rate was low [14]. In 2 other randomized studies performed in different patient groups, the subgroup analyses of cardiac surgery patients failed to show a beneficial effect of LD on mortality rate [16,17].

The present study in high-risk cardiac surgery was primary designed to detect possible differences in mortality rates. Patients who received 1 to 3 U showed a similar mortality rate between both trial arms, whereas the difference was favourable for LD when 4 or more units were transfused. However, the difference in mortality rate at 90 days did not reach statistical significance between the 2 study populations. Based on results of 60-day mortality in the previous study [15], the present study was powered to detect a difference in mortality rate of 10%. We observed a smaller difference in mortality rates between the groups, in particular that caused by cardiac mortality after discharge, which was equal in both trial arms.

The reduction in postoperative infections in favour of LDs was solely present in patients who received 4 or more units. It has been shown that transfusion of whole blood during surgery is associated with prolonged impairment of macrophages and natural killer cell function. This effect appears to be dependent on the dosage of leukocytes or on the leukocyte-aggregates in the transfused concentrates [11].

This is the first prospective trial in which the development of MODS in relation to blood products was studied. Overall incidence of MODS was 20.7%; this was highly associated with the number of transfusions but not with the randomization groups. The mortality rate associated with MODS was higher in the PC group compared with the LD group; whether the beneficial effects of LD on MODS-related survival and to postoperative infections remains unclear and must be investigated further.

LD transfusions are widely implemented in Western Europe and Northern America. The clinical benefits other than prevention of HLA alloimmunization and cytomegalovirus transmission have been difficult to demonstrate [6]. Taking previous studies [6] into account, leukocytes appear to be particularly important as a risk for infections when larger numbers of erythrocyte concentrates are administered. In this study, a large, homogeneous and multiple transfused patient group was enrolled. We demonstrated in this population that LDs result in a decrease in postoperative infections and in-hospital mortality rates. Therefore, our study supports the transfusion of LD in patients at high risk for receiving multiple transfusions in cardiac surgery.

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Chapter 3

Is Increased Mortality Associated with Postoperative Infections after Leukocytes Containing Red Blood Cell Transfusions in Cardiac Surgery? An Extended Analysis

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ABSTRACT

Background: In two randomized trials in cardiac surgery we observed that leukoreduced allogeneic red blood cell (RBC) transfusions (LR) compared with standard buffy-coat-depleted RBC transfusions (BCD) resulted in lower rates of postoperative infections and mortality.

Methods: To unravel whether this comprises two independent side effects or could be related complications of allogeneic leukocytes, we performed a re-analysis on the patients of these two trials.

Results: For all analyses, homogeneity tests were shown not to be significant. Data on characteristics of postoperative infections, nature of microorganisms, number of transfusions and causes of death in both studies were subjected to an integrated analysis. In both studies combined, 1085 patients had been assigned to prestorage leukoreduced RBCs (LR, n=542) or standard buffy-coat-depleted RBCs (BCD, n=543). Postoperative infections were significantly higher in the BCD group [BCD: 34.2% vs. LR: 24.0%, common odds ratios (COR): 1.65, 95% confidence interval (CI): 1.27-2.15], whereas the species of cultured microorganisms and the type of the infections were similar in both randomization arms. Mortality with the presence of infections in the postoperative period was significantly higher in patients receiving BCD compared with LR (BCD: 5.5% vs. LR: 2.2%, COR: 2.59, 95% CI: 1.31-5.14), whereas mortality without infections in the postoperative period was similar in both arms (BCD: 3.9% vs. LR: 3.1%, COR: 1.24, 95% CI: 0.65-2.38). The only cause of death that differed significantly between BCD and LR was the combination of multiple organ dysfunction syndrome with infections in the postoperative period.

Conclusions: This re-analysis shows that transfusion of leukocytes containing RBCs during cardiac surgery may be associated with more infections with fatal outcome. This should be confirmed in a larger extended analysis or a prospective study.

INTRODUCTION

The outcome of patients undergoing cardiac surgery is closely related with the development and the severity of postoperative complications such as systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS) and infections [1,2]. Moderate SIRS often develops after cardiac surgery and usually resolves with supportive care. However, severe SIRS can evolve to MODS, which may cause mortality, dependent on the number of failed organs [3].

Although in supportive care of cardiac surgery allogeneic blood transfusions are commonly used, these may act as a two-edged sword. On the one hand, patients with coronary artery disease compensate less for anemia and need a higher hemoglobin transfusion trigger [4-6]. On the other hand, allogeneic blood transfusions may be associated with more complications [7,8]. Several studies on intensive care unit (ICU) patients and cardiac surgery showed a dose-dependent relationship between allogeneic blood transfusions and the development of postoperative infections [9-11] and hospital mortality [12,13].

A meta-analysis of the three fully published [14-16] studies in cardiac surgery revealed an increased short-term postoperative mortality after transfusions containing allogeneic leukocytes as compared with transfusions of blood leukoreduced by filtration [17]. However, in different surgical settings a meta-analysis for postoperative infections demonstrated no clear evidence in favour of leukoreduced blood transfusions [18].

We conducted two randomized controlled trials (RCTs) [14,16] in cardiac surgery and observed in both studies a transfusion dose-dependent increase of postoperative infections as well as a higher mortality rate associated with MODS after the transfusion of standard, buffy-coat-depleted red blood cells (RBCs) compared with RBCs leukoreduced by filtration. We wondered whether postoperative infections and mortality might reflect different mechanisms or a common mechanism induced by allogeneic leukocytes. For this purpose we re-analysed the original data of patients who participated in two RCTs for the nature of infections and the influence of these infections on the course of postoperative outcome.

MATERIAL AND METHODS

Both studies were double-blinded, prospective RCTs. In both studies, the ethical review boards of the hospitals approved the trial protocols, and the local trial coordinators obtained informed consent from the patients. The design and outcome of both studies are described elsewhere in detail [14,16]. Study 1 was a single-center study conducted between 1992 and 1994 [16]. Patients undergoing coronary artery bypass surgery (CABG), valve surgery or a combination of both were randomized into three study arms: leukoreduced RBCs were either prepared prestorage from freshly drawn blood or were filtered after storage prior to transfusion. For the current analysis, we only included the patients who received prestorage leukoreduced RBCs (LR, n=305) and those who received buffy-coat-depleted RBCs (BCD, n=306) because these products were the common products used in both studies. Study 2 was conducted between 1999 and 2001 in two hospitals and included patients undergoing valve surgery with or without CABG (14). In this study, buffy-coat-depleted RBCs (n=237) were compared with prestorage filtered RBCs (n=237). In both studies, all patients received prophylactic antibiotics postoperatively for 24 hours in patients undergoing CABG and 48 hours in patients undergoing valve surgery. Postoperatively the patients were monitored at the ICU until there was no need for positive inotropes and intubation. Surgical and anesthetical procedures were according to the standards of the hospital in both studies.

Blood Products and Transfusions

The blood products and the quality control of blood products for both hospitals in the two studies fulfilled the requirements and the specifications of the Dutch standards for blood banks. Within 24 hours of withdrawal BCD-RBCs were prepared by centrifugation of whole blood at 3000 *g* for 10 min. Buffy coat and plasma were removed, and RBCs were reconstituted with 100 ml saline-adenine-glucose-mannitol. The average leukocyte count was in the first study $0.8 \pm 0.5 \times 10^9$ per unit and in the second study $0.7 \pm 0.4 \times 10^9$ per unit. Prestorage filtration of RBCs (LR) was performed within 24 hours after collection of blood by passage through a leukocyte filter. In both studies the same filter (Cellselect-Optima, NPBI International-Fresenius HemoCare, The Netherlands) was used. The residual leukocyte count in the filtered units was measured in study 1 using Nageotte counting chamber, revealing a leukocyte count of [mean ± standard deviation (SD)] $1.2 \pm 1.4 \times 10^6$ per unit. In study 2, flow cytometry was used for quality control, more reliably detecting low leukocyte numbers. By this method the mean (±SD) residual leukocytes in the filtered units was $0.15 \pm 0.02 \times 10^6$ per unit. All platelet concentrates in both studies

were prestorage leukoreduced by filtration. In both studies, there were no by-study-defined transfusion triggers. The decision to transfuse blood products was based on local policies, hemoglobin level (less than 8-8·5 g/dl), platelet count (less than 100×10^9/l), blood loss and bleeding disorders. In one centre participating in study 2 more patients received aprotinin for prevention of blood loss.

Mortality

In study 1, mortality was a secondary endpoint and was registered until day 60 postoperatively; in study 2, mortality at 90 days after surgery was the primary endpoint. For both studies, death at 60 days was monitored and used for this analysis. The cause of death in both studies was retrieved from the patient hospital records or from the referring cardiologist or general practitioner.

Postoperative Infections

In both studies infections in the postoperative period had been a secondary endpoint and were recorded according to the criteria of Center for Disease Control and Prevention [19]. In both studies the following infections were scored: respiratory tract infection (defined as positive sputum culture and/or pulmonary infiltrate on the radiograph), urinary tract infection (defined as positive urine culture with clinical signs of urine tract infection), wound infection (defined as positive wound culture and/or local symptoms) and bacteremia (defined as positive blood culture and fever). All information on infections and the identified microorganisms was collected by the trial coordinators from the patient records or electronically from the hospital computer system.

Multiple Organ Dysfunction Syndrome (MODS)

In study 1 MODS was not an endpoint. In this study, the causes of death were registered and MODS was diagnosed as cause of death by the treating physicians. In study 2 MODS was a secondary endpoint. The scoring of organ dysfunction was assessed by the local trial coordinators on the basis of the daily records according to a model described elsewhere [20]. The dysfunction of the following organ systems were scored: respiratory dysfunction (defined as respiratory frequency ≤5/min or ≥49/min, arterial $PaCO_2$ ≥6.65 kPa, $AaDO_2$ ≥46.7 kPa, or longer than 72 hours dependency of mechanical ventilation), cardiovascular dysfunction (defined as heart frequency ≤54/min or ≥ 150/min, mean arterial pressure ≤ 49 mmHg, serum pH ≤7.24 in combination with $PaCO_2$ ≤6.52 kPa, or dependency of positive inotropes), renal dysfunction (defined as urine production ≤ 479 ml per 24 hours or ≤159 ml per 8

hours, creatinine concentration ≥3.4 mg/dl, blood urea nitrogen concentration ≥50 mg/dl, or dependency of dialysis), hematological dysfunction (defined as white blood cell count ≤ 1.0×10^9/l, platelet count ≤20×10^9/l, or hematocrit ≤0.20) and insufficiency of the central nervous system (defined as Glasgow Coma Score ≤6). MODS was defined as the failure of 2 or more organ systems.

Statistics

These extended analyses were performed on intention-to-treat basis. Breslow-Day and Tarone's homogeneity tests were performed to verify that combined analyses were valid. Data were expressed as mean ±SD, number or percentage when appropriate. For comparison of qualitative parameters, the Fisher's exact test or χ^2 test was used, and for the comparison of quantitative parameters, the *t*-test or Mann-Whitney *U*-test was used. To analyse the differences, common odds ratios (COR) with 95% confidence intervals (95% CIs) were calculated using the Mantel-Haenszel estimation. Multivariate analysis of the risk factors was performed using logistic regression model. The survival was analysed with Kaplan-Meier curves. All p-values are two tailed. All analyses were performed in SPSS (SPSS Inc., Chicago, IL, USA).

RESULTS

In Table 1 the patient characteristics of both studies are summarized. Patients included in study 2 underwent CABG combined with valve surgery and were at higher risk with respect to age, gender, type of surgery and duration of surgery; this was reflected in longer ICU stay and hospital stay compared with study 1. In both studies more than 90% of the included patients received one or more RBC transfusions. Three patients in the LR group received solely BCD products, and in both groups 27 patients received both types of blood products. In the current analysis these patients remained in their original randomization arm. The homogeneity tests for all analyses were not significant.

In the BCD group 186 patients (34.2%) and in the LR group 130 patients (24.0%) had an infection. In total, 80 patients (7.4%) died, 51 patients (9.4%) in the BCD group and 29 patients (5.4%) in the LR group. The CORs for infections (COR: 1.65, 95% CI: 1.27-2.15, p<0.01) and for mortality (COR: 1.84, 95% CI: 1.15-2.96, p=0.01) showed significant differences between BCD and LR.

Table 1 | Characteristics of Included Patients

	Study 1		Study 2			Total	
	BCD	**LR**	**BCD**	**LR**	**p***	**BCD**	**LR**
Number	306	305	237	237		543	542
Female (%)	85 (27.8)	80 (26.2)	102 (43.0)	113 (47.7)	<0.01	187	193
Age (yrs±SD)	64.9 ± 9.4	63.5 ± 9.7	66.6 ± 12.5	65.4 ± 14.7	0.01	65.6 ± 10.9	64.3 ± 12.2
Type of surgery							
CABG (%)	234 (76.5)	225 (73.8)	-	-	<0.01	234 (43.1)	225 (41.5)
Valve (%)	49 (16.0)	58 (19.0)	164 (69.2)	156 (65.8)		213 (39.2)	214 (39.5)
CABG+valve (%)	23 (7.5)	22 (7.2)	73 (30.8)	81 (34.2)		96 (17.7)	103 (19.0)
Duration of surgery							
CPB (min±SD)	124 ± 48	123 ± 53	143 ± 62	139 ± 60	<0.01	132 ± 55	130 ± 57
Ao.clamping (min±SD)	66 ± 29	68 ± 33	95 ± 45	99 ± 47	<0.01	79 ± 39	82 ± 42
Transfusion characteristics							
Number transfused RBCs (mean±SD)	5.4 ± 5.6	5.4 ± 4.6	6.1 ± 7.1	5.9 ± 6.1	0.09	5.7 ± 6.3	5.6 ± 5.3
Median	4	4	4	4		4	4
Range	0-40	0-35	0-46	0-50		0-46	0-50
Storage time (days)	13.2 ± 6.0	12.9 ± 6.3	19.5 ± 5.4	17.4 ± 5.9	<0.01	16.1 ± 5.8	15.4 ± 6.2
Non-transfused (%)	12 (3.9)	20 (6.5)	21 (8.9)	21 (8.9)	0.02	33 (6.1)	41 (7.6)
Transfused plasma units (mean±SD)	4.3 ± 4.2	3.9 ± 3.1	4.5 ± 5.1	4.0 ± 4.5	0.83	4.4 ± 4.7	4.0 ± 3.8
Transfused platelet units (mean±SD)	1.3 ± 1.1	1.1 ± 0.9	1.2 ± 1.2	1.1 ± 1.3	0.89	1.2 ± 1.0	1.1 ± 1.1
ICU-stay (days)	4.2 ± 4.6	4.1 ± 4.7	5.5 ± 7.3	5.5 ± 7.2	<0.01	4.8 ± 5.9	4.7 ± 6.0
Hospital-stay (days)	11.1 ± 7.4	10.9 ± 7.9	13.9 ± 11.1	13.4 ± 14.5	<0.01	12.3 ± 9.3	12.0 ± 11.3

* p-values are calculated for differences between the study 1 and 2; BCD=Buffy-coat depleted red blood cells; LR=Leukocyte depleted red blood cells; CABG=Coronary artery bypass surgery; CPB=Cardiopulmonary bypass; Ao.Clamping=Aortic clamping; RBC=Transfused red blood cells

The most frequent type of infection was respiratory tract infection (48.7%) followed by urinary tract infection (33.2%), bacteremia (12.3%) and wound infection (11.1%). In the BCD group nine patients (4.8%) had two or more infections and in the LR group seven patients (5.4%). In the deceased patient group, respiratory tract infection was the most common type of infection (33.8%) followed by bacteremia (16.3%), urinary tract infection (8.8%) and wound infection (2.5%). The distribution of the types of infections was similar in both randomization groups (Table 2). There were more patients with more than 1 infection in the BCD group associated with mortality.

From 33 patients (17.7%) in the BCD group and 25 patients (19.2%) in LR group cultures revealed no microorganisms. The homogeneity test between the studies for type of microorganisms (p=0.49) was not significant. In the BCD group in 45 patients (24.2%) more than one microorganism was cultured. The isolated microorganisms in the BCD group were predominantly *Haemophilus influenzae* (16.8%), *Staphylococcus aureus* (16.8%) and *Escherichia coli* (15.4%). In the LR group, in 29 patients (22.3%) more than one microorganism was cultured. The most isolated microorganisms in this group were *E. coli* (23.1%), *H. influenzae* (18.5%), *Enterobacter* species (13.8%) and *S. aureus* (13.1%). There were more gram-negative (75.1%) than gram-positive (22.4%) microorganisms cultured in patients with infections. The cultured microorganisms in patients with infections and in deceased patients were not different between the randomization groups.

In Table 3 the causes of death are shown. The most common cause of death in the BCD group was MODS combined with infections in the postoperative period; this was significantly higher than that in the LR group. In the LR group cardiac complications (without MODS or infections) were the most common causes of death. Mortality, either from infections or other causes, tended to increase the more RBCs patients received (Table 4). The extent of the association between RBCs dose and mortality was not different between the trial arms for deaths not caused by infections but was significantly greater in the BCD arm compared with LR for deaths caused by infections (p=0.01 overall and p=0.02 if 8 or more units were transfused).

The association between infections in the postoperative period and mortality was analysed in a multivariate logistic regression model with pre-operative and per-operative risk factors. The following risk factors were found to be independently associated with mortality: cardiopulmonary bypass time (p<0.001), age at surgery (p=0.001), infections (p=0.004), type of surgery (p=0.012) and the randomization arm (p=0.03). Gender (p=0.07), study (p=0.99) and aortic clamping time (p=0.16) showed no independent association. Infections (p=0.43) and type of surgery (p=0.12) lost their significant association with

Table 2 | Types of Infections

	Infections			Infections associated with mortality			Not associated with mortality		
	BCD N (%)	LR N (%)	p	BCD N (%)	LR N (%)	p	BCD N (%)	LR N (%)	p
Patients with infections	186	130		30	12		156	118	
Patients with >1 infection	9 (4.8)	7 (5.4)		6 (20.0)	1 (8.3)		3 (1.9)	6 (5.1)	
Type of infections			0.73			0.31			0.44
Respiratory infections	90 (48.4)	64 (49.2)		20 (66.7)	7 (58.3)		70 (44.9)	57 (48.3)	
Urinary tract infections	61 (32.8)	43 (33.1)		5 (16.7)	2 (16.7)		56 (35.9)	41 (34.7)	
Bacteremia	25 (13.4)	14 (10.8)		9 (30.0)	4 (33.3)		16 (10.2)	10 (8.5)	
Wound infections	19 (10.2)	16 (12.3)		2 (6.7)	0		17 (10.9)	16 (13.5)	

p-values are calculated for differences between the randomization arms; BCD=Buffy-coat reduced red blood cells; LR=Leukoreduced red blood cells

Table 3 | Causes of Death

	BCD (N=543) N (%)	LR (N=542) N (%)	COR (95% CI)	p
MODS and infections (in the postoperative period)	20 (3.7)	7 (1.3)	2.92 (1.22-6.97)	0.02
Infections (without MODS)	9 (1.8)	5 (0.9)	2.01 (0.60-5.44)	0.42
MODS (without infections)	3 (0.6)	3 (0.6)	1.0 (0.20-4.97)	1.0
Cardiac complications (without MODS or infections)	16 (2.9)	11 (2.0)	1.47 (0.67-3.12)	0.44
Bleeding/Surgical complications	3 (0.6)	3 (0.6)	1.0 (0.20-4.97)	1.0
Total	51 (9.4)	29	1.84 (1.15-2.96)	0.01

p-values are calculated for differences between the randomization arms; BCD=Buffy-coat reduced red blood cells; LR=Leukoreduced red blood cells; MODS= Multiple-Organ-Dysfunction-Syndrome; COR=Common odds ratio

mortality when the number of erythrocyte transfusions was added to the model (p<0.001). Cardiopulmonary bypass time (p=0.01), age at surgery (p=0.005) and the randomization arm (p=0.03) remained independently associated with mortality.

Table 4 | Mortality, with and without Infection in the Postoperative Period Related to the Number of RBC Transfusions

		Mortality with Infection N (%)			Mortality without Infection N (%)		
No. of RBCs	**BCD/LR**	**BCD**	**LR**	**COR (95%CI) p**	**BCD**	**LR**	**COR (95% CI) p**
0	33/41	0	0		0	0	
1-3	208/188	2 (1.0)	1 (0.5)	1.82 (0.16-20.2) 1.0	3 (1.4)	3 (1.6)	0.90 (0.18-4.53) 1.0
4-7	188/198	7 (3.7)	2 (1.0)	3.79 (0.78-18.5) 0.10	8 (4.3)	4 (2.0)	2.16 (0.64-7.28) 0.25
≥8	114/115	21 (18.4)	9 (7.8)	2.66 (1.16-6.09) 0.02	10 (8.8)	10 (8.7)	1.01 (0.40-2.53) 1.0
Total	543/542	30 (5.5)	12 (2.2)	2.59 (1.31-5.14) 0.01	21 (3.9)	17 (3.1)	1.24 (0.65-2.38) 0.49

p-values are calculated for differences between the randomization arms; BCD=Buffy-coat reduced red blood cells; LR=Leukoreduced red blood cells;COR=Common odds ratios

The survival curves of patients with and without infections in the postoperative period are shown in Figure 1. The survival of patients without infections was not different between the randomization groups (p=0.28), and most deaths in this group occurred in the first 20 days after surgery. Most of the deaths in patients with infections occurred later; the difference between BCD and LR was mainly because of more deaths in the BCD group after an interval of 20 days or longer after surgery (p=0.09).

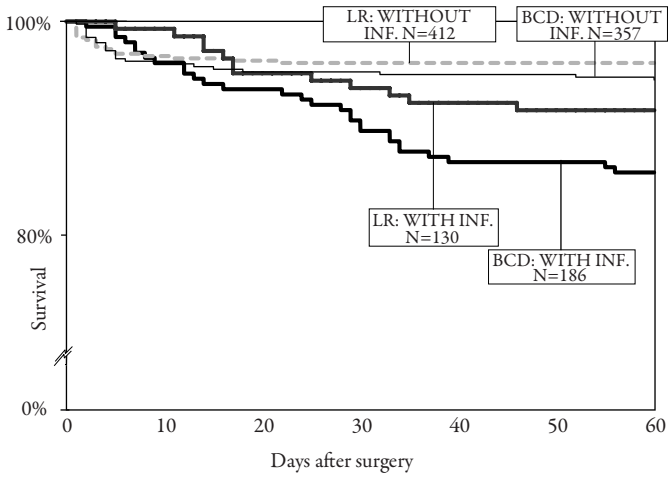

Figure 1 | The 60-day survival in after cardiac surgery in patients with or without infections in the postoperative period. Survival without infections was not different between BCD and LR (p=0.28). Difference in survival with infections between BCD and LR was mainly observed after 20 days (p=0.09).

DISCUSSION

To evaluate the role of leukocytes in blood products administered during cardiac surgery, three fully published RCTs [14-16] and two abstracts [21,22] are available. In four of the five studies, RBCs leukoreduced by filtration were associated with lower mortality. However, the exact mechanism associated with increased mortality has not been unravelled yet [23]. In all studies, postoperative infections had been evaluated as an independent endpoint with contradictory results. The current re-analysis was undertaken to explore whether infections in the postoperative period contributed to mortality in patients undergoing cardiac surgery after transfusion of leukocyte containing RBCs. This approach could be helpful to identify the possible role of infections in survival of patients receiving leukocytes containing RBCs. For this re-analysis we used the data of two previously published studies [14,16]. These studies were undertaken in cardiac surgery; however, there were also differences between the studies. Therefore, we tested the homogeneity of the results from both studies before analysing the

data. Because there were no significant differences between the studies in all homogeneity tests, we reported the results from the combined analysis.

Our finding that the mortality rate was increased in patients with the presence of infections in the postoperative period after cardiac surgery was in agreement with others [24]. The possible relationship between leukocytes containing RBCs and mortality associated with postoperative infections has not been described before. Mortality associated with or without infections increased with the number of units of RBCs transfused in both randomization arms. However, the difference in mortality between those receiving BCD or LR was more marked in patients receiving 8 or more units of RBCs, which suggests that transfusion-related immunomodulation may either be dose related or be more clinically significant in sicker patients. Because no differences in type of infections or type of microorganisms between BCD and LR were present, this points to an overall more reduced host resistance after non-leukoreduced RBC transfusions. No specific suspectibility for particular microorganisms, as has been suggested in an animal model, was seen [25]. Patients dying without infections did so mainly in the first 20 days after surgery, whereas patients with infections often died later. This was reflected in the growing difference in mortality between BCD and LR beyond 20 days. This is in agreement with other observations on a possible long-standing suppression of the innate immune system caused by leukocytes containing RBCs [26,27]. Patients with infections in the postoperative period appear to have a higher mortality associated with MODS when they receive leukocytes containing RBCs instead of leukoreduced RBCs. This difference in mortality associated with infections could be explained by increase of pro-inflammatory mediators by production or release induced by leukocytes containing RBCs. It has been shown that transfusion of RBCs in cardiac surgery leads to higher concentrations of pro-inflammatory mediators and is associated with more postoperative infections [28]. It is possible that such a postoperative inflammatory response (which could lead to MODS and a worse outcome) is more pronounced in patients receiving leukocytes containing RBCs compared with leukoreduced RBCs. More studies are necessary to investigate the role of allogeneic blood transfusions in the pathophysiology of postoperative inflammatory responses and organ dysfunction.

There are several restrictions in this analysis. First, this is not a new RCT but an extended analysis of two double-blind RCTs conducted in different patient populations. The RCTs were designed to investigate the potential effect of leukocytes containing RBCs in a highly transfused patient group. In the second study only patients undergoing valve surgery (with or without CABG) were included. This group was older, underwent longer surgery and stayed longer in ICU and in hospital. This high-risk patient group was selected because

the first study indicated the greatest deleterious effects of leukocytes containing RBC transfusions in the more heavily transfused patients who underwent valve surgery combined with CABG, which was confirmed in the second study. Second, the studies had different endpoints. The first published study found no difference in alloimmunization (primary endpoint). The secondary endpoints (postoperative infections and mortality) were higher in patients transfused with leukocytes containing RBCs compared with patients transfused with leukoreduced RBCs. These differences were associated with the number of RBC transfusions. The second study found a non-significant difference for mortality at 90 days (the primary outcome). However, in patients transfused with leukocytes containing RBCs, in-hospital mortality was twice as high and postoperative infections (secondary endpoints) were significantly higher compared with patients transfused with leukoreduced RBCs. For this re-analysis these outcomes were not double-checked or adjudicated by independent investigators. Third, both studies had been performed 7 years apart. Between the first study conducted in 1992-1994 and the second in 1999-2001, surgical procedures, application of aprotinin and transfusion practice changed, although cardiac surgery patients remained at risk to receive a large number of blood products. Fourth, the first study did not score MODS as an independent outcome but only as a cause of death. Therefore, exact incidence and severity of MODS in all participating patients is not known. Also, detailed data on pre-operative risk status of the patients were not recorded in the first study. Furthermore, the data had been collected in the same way by the same investigators in the same country. All these factors could have influenced this extended analysis. However, their influence affects both randomization arms equally because randomization had resulted in a balanced distribution of patients in both studies.

In summary, this extended analysis suggests a possible common pathway to postoperative death because of a higher incidence of presence of infections in the postoperative period associated with mortality, which might be elicited by long-standing immune suppression as a result of the presence of leukocytes in allogeneic blood transfusions. It is necessary to confirm these findings, preferentially by similar analysis on patients enrolled in all five RCTs conducted in cardiac surgery or (if possible) by a prospective study in countries that have not implemented universal leukoreduction.

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Chapter 4

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

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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 1 | Characteristics of Patients Participating in RCTs Comparing Leukodepleted Versus Leukocyte Containing RBCs

Author; year	No. patients/ No. transfused (%)	Clinical setting	No. centers	Transfused patients	No. RBCs mean±SD or median (range)	Transfused patients with > 3 RBC (%)	Main Endpoints	Results (LD vs BCD)
Jensen et al.; 1992 [11]	197/ 104 (53)	Colorectal surgery	3	LD 48 WB 56	LD 2 (1-4) WB 2 (1-5)	ND	1)Infections 2)NK function	1)0.2 vs 23%[b]
Houbiers et al.; 1994 [12]	697/446 (64)	Colorectal cancer surgery	16	LD 216 BCD 230	LD 3 (1-10) BCD 3 (2-11)	LD 104 (31) BCD 94 (26)	1)Cancer recurrence 2)Infections	1)30% vs 32% 2)36 vs 32%
Jensen et al.; 1996 [13]	586/ 260 (44)	Colorectal surgery	2	LD 118 BCD 142	LD 2 (1-5) BCD 2 (1-6)	ND	1)Infections 2)Mortality	1)3.0 vs 23% [b] 2)3.4 vs 2.8%
Tartter et al.; 1998 [14]	221/ 59 (27)	Colorectal surgery	1	LD 25 BCC 34	ND	ND	Infections	15 vs 44% [b]
Titlestad et al.; 2001 [15]	279/ 112 (45)	Colorectal surgery	1	LD 48 BCD 64	LD 3 (2-4.3) BCD 3 (2-6)	ND	Infections	45 vs 37%
van Hilten et al.; 2004 [16]	1051/ 545 (52)	Colorectal cancer surgery and aortic aneursm	19	LD 267 BCD 278	LD 3.5 BCD 3.5	LD 62 (23) BCD 58 (21)	1)Infections 2)Hospital stay 3)MODS 4)Mortality	1)23 vs 23% 2)–2.4 days[b] 3)14 vs 17%[b] 4)10.3 vs 8.4%
Skanberg et al.; 2007 [17]	642/298 (46)	Colorectal cancer	7	LD 137 BCD 161	LD 3.6 ± 0.3 BCD 3.6 ± 0.3	ND	1)Respiratory support 2)Hospital stay 3)Mortality	1)3.6 vs 8.1% 2)15.5 vs 15.5 days 3)52.5 vs 49.7%
Nathens et al.; 2006 [18] Watkins et al.: 2008 [33]	1864/ 268 (14)	Trauma patients	1	LD 136 BCC 132	LD 9.2 ± 9.6 PC 8.6 ± 9.9	ND	1)Infections 2)MODS 3)Mortality 4)ALI	1)30 vs 36% 2)5.9 vs 6.6% 3)22 vs 19% 4)42 vs 43%
van de Watering et al.; 1998 [19]	914/ 866 (95)	CABG ± valve surgery	1	FF 283 SF 280 BCD 303	FF 5.3 ± 4.1 SF 5.5 ± 5.6 BCD 5.4 ± 5.1	FF 164 (58) SF 169 (60) BCD 175 (58)	1)Infections 2)Mortality	1)17 vs 18 vs 23% 2)3.6 vs 3.3 vs 7.8%[b]

Author; year	No. patients/ No. transfused (%)	Clinical setting	No. centers	Transfused patients	No. RBCs mean±SD or median (range)	Transfused patients with > 3 RBC (%)	Main Endpoints	Results (LD vs BCD)
Bracey et al.; 2002 [20]	357/ 295 (83)	CAGB ± valve surgery	1	LD 136 BCC 159	LD 3 PC 3	ND	1)Infections 2)Mortality 3)ICU-/Hospital-stay	1)ns; data ND 2)5.9 vs 7.5% 3)ns; data ND
Wallis et al.; 2002 [21]	597/ 409 (69)	CABG ± valve surgery	1	LD 176 BCC 175 PR 158	WBF 3.9 ± 3.9 BCD 3.5 ± 2.6 PC 2.9 ± 1.8	ND	1)Infections 2)Mortality	1)49 vs 38 vs 35% 2)0.5 vs 2.9 vs 2.5%[b]
Bilgin et al.; 2004 [22]	474/ 432 (91)	Valve surgery ± CABG	2	LD 216 BCD 216	LD 6.2 ± 7.1 BCD 5.9 ± 6.1	LD 145 (67) BCD 131 (61)	1)Infections 2)MODS 3)Mortality	1)23 vs 32% [b] 2)20 vs 21% 3)8.4 vs 12.7%
Connery et al.; 2005 [23][c]	98/ 69 (70)	Primary CABG	2	LD 38 BCC 31	LD(SF) 5.6± 13 PC 5.6±10	LD 16 (42) PC 15 (48)	1)Infections 2)Mortality	1) 13 vs 26% (PTI: 0 vs 13%[b]) 2) 2.6 vs 3.2%
Boshkov et al.; 2006 [24]	1227/ 562 (46)	CABG ± valve surgery	3	LD 304 BCC 258	ND	ND	Mortality	4.9 vs 9.7% [b]
Dzik et al.; 2002 [25]	2780 (100)	All patients	1	LD 1355 BCC 1425	LD 2 (1-9) PC 2 (1-9)	LD 498 (35) PC 474 (35)	1)Mortality 2)Hospital stay 3)Antibiotics	1)9 vs 8.5% 2)8.8 vs 8.9 days 3)31.5 vs 34%
Collier et al.; 2001 [26]	531/524 (99)	HIV-postive	11	LD 259 BCC 262	Mean 7.3	ND	1)Mortality 2)HIV RNA level	1)58% vs 53% 2)Similar

[a]Data on ALI were re-analyzed and presented in another publication [33] than the initial publication [18]; [b]Statistically significancy (P<0.05) between BCD and LD (SF+FF); [c]This RCT was interrupted early. Abbreviations Table 1: LD=Leukodepleted RBCs; FF=Fresh filtered RBCs; SF=Stored filtered RBCs; BCD=Buffy-coat depleted RBCs; BCC=Buffy-coat-containing RBCs; PR=plasmareduced RBCs; WB=Whole blood; WBF=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 VATS (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

Author; year	No. patients	No. transfused patients (%)	Diagnosis	No. centers	Endpoints	Patients (N)	Units (N)	Severe rejection	Graft survival
Sanfilippo et al.; 1985 [27]	52	52 (100)	Cadaveric renal transplantation	1	Graft survival	LD 30 BCC 22	3	ND	1-year: 50 vs 50%
Opelz et al.; 1997 [28]	423	205 (48.4)	Cadaveric renal transplantation	14	Rejection & graft survival at 1 and 5 years	BCC 205 NT 218	3	16 vs 25%*	1-year: 90 vs 82%* 5-years: 79 vs 70%*
Hiesse et al. ; 2001 [29]	144	97 (67.3)	Cadaveric renal transplantation	8	Rejection & graft survival	BCC (1 DR match) 49 BCC (0 DR match) 48 NT 47	1	19 vs 33 vs 33%	5-years: 92 vs 92 vs 90%

Abbreviations Table 2: LD= Leukodepleted RBCs; NT=non transfused; BCC=Buffy-coat-containing RBCs; [b]Statistically significancy (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 [TRLs]) 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].

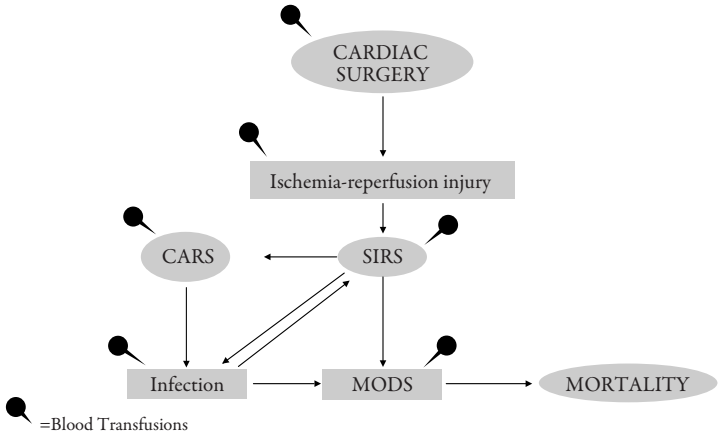

Figure 1 | Mechanism of Blood Transfusions and Complications after Cardiac Surgery. *Abbreviations* Figure 1: SIRS=Systemic inflammatory response syndrome; CARS=Compensatory anti-inflammatory response syndrome; MODS=Multiple-organ-dysfunction-syndrome

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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Chapter 5

The Effects of Allogeneic Leukocytes in Blood Transfusions during Cardiac Surgery on Inflammatory Mediators and Postoperative Complications

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ABSTRACT

Background: To investigate whether the higher incidence of postoperative complications in cardiac surgery after transfusion of leukocyte-containing red blood cells can be related with inflammatory mediators.

Methods*:* Analysis of inflammatory markers IL-6, IL-10, IL-12 and procalcitonin in patients participating in a randomized trial comparing leukocyte-depleted (LD-RBC) with leukocyte containing, buffy-coat-depleted, red blood cells (BCD-RBC).

Setting: Two university-affiliated hospitals in the Netherlands.

Subjects: 346 patients undergoing cardiac valve surgery with a complete series of pre-and postoperative blood samples.

Results: There were no differences in the cytokines and procalcitonin concentrations upon arrival at ICU between both study arms. In subgroups, patients transfused with 0-3 RBC transfusions showed similar cytokine concentrations in both study arms, whereas patients with 4 or more RBC transfusions had significantly higher IL-6 concentrations in the BCD-RBC group. Patients who developed postoperative infections and multiple-organ-dysfunction-syndrome (MODS) showed, respectively, increased concentrations of IL-6 and IL-12 in the BCD-RBC group. The interaction tests between infections and non-infections were also significant for IL-6 and for MODS and non-MODS for IL-12. Multivariate analysis showed that high IL-6 concentration with MODS and both high IL-6 and IL-10 concentrations with hospital mortality.

Conclusions: Allogeneic leukocyte-containing blood transfusions compared with leukocyte-depleted blood transfusions induce dose-dependent significantly higher concentrations of pro-inflammatory mediators in the immediate post-operative period after cardiac surgery. High concentrations of IL-6 are strong predictors for development of MODS; whereas both IL-6 and IL-10 are associated with hospital mortality. These findings suggest that leukocyte-containing RBCs interfere with the balance between postoperative proinflammatory response, which may further affect the development of complications after cardiac surgery.

INTRODUCTION

Cardiac surgery is associated with tissue trauma, ischemia-reperfusion injury and blood surface contact. These conditions induce systemic effects and 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 [1,2]. Moderate SIRS often develops after cardiac surgery and usually resolves with supportive care. However severe SIRS can evolve to MODS, which cause higher morbidity and mortality after cardiac surgery [3,4].

During cardiac surgery, despite blood-saving developments, allogeneic red blood cells (RBCs) are often transfused. Allogeneic RBC transfusions are dose-dependently associated with increased risk of postoperative infections and mortality after cardiac surgery [5-9]. However, it is not clear whether this relationship is causal or what the possible mechanisms could be [10].

In two randomized controlled trials in patients undergoing cardiac surgery we found a transfusion-dose dependent increased rate of postoperative infections and mortality (due to multiple-organ-dysfunction-syndrome) in the patient group receiving buffy-coat-depleted red blood cells (BCD-RBCs) as compared to filtered leukocyte-depleted red blood cells (LD-RBCs) [11,12]. These findings suggest that allogeneic leukocytes in BCD-RBCs may have played a causal role, possibly by enhancement of the inflammatory response after cardiac surgery. In this study we compare blood samples collected from patients randomized to either BCD-RBCs or to LD-RBCs. The aim of this laboratory analysis is to investigate a possible relationship between the presence of leukocytes in blood transfusions and some inflammatory mediators with postoperative complications as morbidity and mortality.

We selected four key mediators that represent the inflammatory response after surgery. The pro-inflammatory cytokine IL-6 has been shown to be an early predictor for non-surviving patients in cardiac surgery and it has been previously reported that intra-operative blood transfusions in cardiac surgery caused an increase of IL-6 levels [13,14]. IL-10, an anti-inflammatory cytokine, has been found to be increased after per-operative allogeneic blood transfusions in orthopaedic surgery in association with prolonged hospital stay [15]. IL-12 reflects activation and proliferation of lymphocytes and natural killer cells, which are relevant for the defence against nosocomial infections [16,17]. Procalcitonin has been shown to be an early marker for sepsis and bacterial infections after major surgery and has been found increased on the first postoperative day after cardiac surgery in patients who developed organ dysfunction and severe complications [18].

MATERIAL AND METHODS

Study Design

In a randomized controlled trial performed in two university hospitals in the Netherlands, after written informed consent was obtained, 474 patients undergoing cardiac valve surgery with or without coronary-artery-bypass-graft (CABG) were randomized to receive BCD-RBCs or LD-RBCs. The endpoints of the study were: postoperative infections, multiple-organ-dysfunction-syndrome (MODS) and 90-days and in-hospital mortality. The local ethical committees of both hospitals approved the trial protocol. The design and outcome of the study has been described elsewhere in detail [12]. For the assessment of preoperative risk of the patients the score model described by Parsonnet was applied [19]. Anesthetical and surgical procedures were according to the standards of the hospitals. Following endotracheal intubation, patients were ventilated to normocapnia with an air-oxygen mixture. Heparin sulphate was administered before start of cardiopulmonary bypass at a dose of 3 mg/kg and subsequently at doses to maintain the activated clotting time above 400 seconds. After arterial and venous cannulation, cardiopulmonary bypass was commenced using a membrane oxygenator (Baxter, Uden, the Netherlands). After termination of cardiopulmonary bypass, heparin was antagonized by protamine sulphate at a 1:1 ratio. A nonpulsatile roller pump was used for all operations. The nonpulsatile flow rate at about 2.4 l/min/m^2 were set to maintain arterial pressure. Patients were cooled to 27-30°C. For myocardial protection cardioplegia with cold crystalloids was administered. In one hospital, patients considered to be at high risk for bleeding received aprotinin. All patients received prophylactic antibiotics postoperatively for 48 hours. Postoperatively the patients were monitored at the intensive care unit (ICU) until there was no need for positive inotropes and mechanical ventilation.

Postoperative Complications

Postoperative infections were defined according to the criteria of Centers of Disease Control and Prevention (CDC) [20]. The following infections were scored: respiratory tract infection (defined as: positive sputum culture and pulmonary infiltrate on the X-ray), urinary tract infection (defined as: positive urine culture with clinical signs of urine tract infection), wound infection (defined as: positive wound culture with clinical symptoms) and bacteremia (defined as: positive blood culture and fever). The development of postoperative organ dysfunction was assessed on the basis of the daily medical records at the ICU using the model described by Knaus [21]. The dysfunction of following organ systems were scored postoperatively: respiratory dysfunction (defined as: respiratory frequency $\leq$5/min or

≥49/min, or arterial $PaCO_2$ ≥50 torr [6.5 kPa], or $AaDO_2$ ≥349 torr [46.5 kPa], or longer than 72 hours dependency of mechanical ventilation), cardiovascular dysfunction (defined as: heart frequency ≤54/min or ≥150/min, or mean arterial pressure ≤49 mm Hg, or serum pH ≤7.24 in combination with $PaCO_2$ <50 torr [6.6 kPa], or dependency of positive inotropes), renal dysfunction (defined as: urine production ≤479 mL/24 hours or ≤159 ml/8 hours, or creatinine concentration ≥3.4 mg/dl, or blood urea nitrogen concentration ≥50 mg/dl, or dependency of dialysis), hematological dysfunction (defined as: white blood cell count $\leq 1.0\times10^9$/l, or platelet count $\leq 20\times10^9$/l, or hematocrit ≤0.20) and insufficiency of the central nervous system (defined as Glasgow Coma Score ≤6). MODS was defined as the failure of 2 or more organ systems. The trial coordinators collected all information on infections, MODS and hospital mortality from the patient records or electronically from the hospital computer system.

Blood Products

The blood products had been prepared and controlled according to the procedures of the Dutch blood banks. Within 20 hours of withdrawal, RBCs were prepared by centrifugation of whole blood at 3000 × g for 10 minutes. Buffy-coat and plasma were removed from donated blood and BCD-RBCs were reconstituted with 100 mL saline-adenine-glucose-mannitol (SAG-M). The average leukocyte count (±SD) in BCD-RBCs was $0.7 \pm 0.4\times10^9$ per unit. LD-RBCs were prepared by prestorage filtration of RBCs within 24 hours after collection of blood. BCD-RBCs were subsequently filtered through a leukocyte filter (Cellselect-Optima, NPBI International-Fresenius HemoCare, the Netherlands) resulting in a mean residual leukocyte count in the LD-RBCs of $0.15 \pm 0.02\times10^6$ per unit. All platelet concentrates were prestorage leukoreduced by filtration. The mean storage time of all transfused RBCs was calculated in days.

Inflammatory Markers

Blood samples were taken pre-operatively, at the end of perfusion, upon admission to the ICU and in case of prolonged ICU-stay, in total two days during ICU-stay. Blood samples were immediately centrifuged and alliquotted, the serum was stored at -80°C. The concentrations of procalcitonin, IL-6, IL-10 and IL-12 were measured in all patients pre-operatively and at arrival at ICU; and in case of prolonged ICU-stay (longer than 2 days) additionally at day 1 and 2 of ICU-stay. All concentrations of the mediators were measured in duplicate using enzyme-linked immunoassay technique (PeliKline Compact, Sanquin Diagnostics, Amsterdam, the Netherlands). The lower detection limit was for all cytokines 5.0 pg/mL.

Procalcitonin was measured by an immunoluminometric assay (LumiTestPCT, Brahms Diagnostica, Berlin, Germany). The analytic sensitivity of the assay was 0.1 ng/mL.

Statistical Analysis

All patients with at least a pre-operative blood sample and a blood sample at arrival at ICU were analyzed in this study. Descriptive results of continuous variables were expressed as mean (±SD). Categorical variables were reported as frequency distributions (%). Complications were defined as postoperative infections, MODS or hospital mortality. For comparison of qualitative parameters Fisher's exact test or χ^2 test was used and for comparison of quantitative parameters t test or Mann-Whitney U test was used. The concentrations of the cytokines were expressed as the median with interquartile ranges (IQR) and were compared between randomization arms and correlated with complications. Differences between groups analyzed by the Mann Whitney U test were reported as odds ratios (OR) with 95% confidence intervals (95% CI). Interaction test was performed to measure the relation between the randomization arms and the development of complications on the concentrations of cytokines. Multivariate analysis of the risk factors was performed using a logistic regression model to estimate the risk factors for each of the postoperative complications. For this purpose the following variables were included in the model: gender, type of surgery, Parsonnet-score, use of pre-operative statins, cardiopulmonary bypass time, use of peri-operative aprotinin and corticosteroids, randomisation arm, number of RBC transfusions, storage times of transfused RBCs. The risk factors from the univariate analysis for the composite postoperative complications with $p<0.20$ were entered into the model, the measured concentrations of IL-6, IL-10 and IL-12 at the arrival at ICU and the postoperative increase of leukocytes were forced into the final model. For this analysis the concentrations of the cytokines, the Parsonnet-score and cardiopulmonary bypass time were transformed into quartiles. The storage time was divided into three groups: patients receiving only RBCs stored for less than 17 days, patients receiving only RBCs stored for 17 or more days and patients receiving RBCs with storage times less than 17 and 17 or more days. In the final model the independent risk factors for infections, MODS and hospital mortality were analyzed separately in a stepwise elimination process. The exponent with odds ratios and 95% confidence intervals were reported. All p-values were two-tailed and are reported more as a measure of strength of associations than probabilistic assessment. All analyses were performed by using SPSS version 15.0 for Windows (SPSS Inc., Chicago, USA).

RESULTS

Patient Characteristics

From the 474 evaluable patients randomized within the previously described study [12], due to logistic reasons (mostly this regarded procedures outside office hours) from 128 patients complete serial blood samples could not be collected or processed properly (from 106 patients no pre-or postoperative blood samples could be collected and from 22 patients no pre-and postoperative blood samples were collected). As shown in Table 1, the patient and transfusion characteristics of the 346 included patients were balanced between the BCD-RBC and LD-RBC groups. In the BCD-RBC group significantly more postoperative infections were found as compared to the LD-RBC group (p=0.02). In the cohort available for laboratory analysis the in-hospital mortality was not significantly different between both randomization arms (p=0.19). The incidence of MODS was similar in both randomization arms (Table 1). The patient characteristics of the subgroup of 54 patients with prolonged ICU-stay were also well balanced between both study arms (Table 1). There were no differences in patient characteristics in the subgroups of patients with postoperative complications between both randomisation arms (data not shown).

Inflammatory Markers on ICU-arrival

The pre-operative concentrations of all cytokines and procalcitonin levels were all below the detection limits. The concentrations of cytokines and procalcitonin at arrival at ICU were not associated with patient age, Parsonnet-score, use of pre-operative statines and peri-operative aprotinin, duration of cardiopulmonary bypass and aortic clamping times and the analysis according to storage times of transfused RBCs. Patients who received corticosteroids peri-operatively had at ICU-arrival lower concentrations of IL-6 than patients who did not (median 70; interquartile range [IQR] 39-152 versus median 118; IQR 52-245 pg/ml, p<0.01); whereas the concentrations of IL-10 were at ICU-arrival higher in the patient group who received corticosteroids (median 54; IQR 14-93 pg/ml versus median 18; IQR 6-76 pg/ml, p<0.01). The concentrations of procalcitonin were increased at ICU-arrival (median 0.16; IQR 0.13-0.21 ng/ml). A postoperative increase of the leukocyte count was observed at ICU-arrival in all patients (median 2; IQR 1.4-3.6×10^9/l).

Table 1 | Characteristics of Patients

	All patients			Patients staying > 2 days at ICU		
	BCD-RBC (n=171)	**LR-RBC (n=175)**	**p**	**BCD-RBC (n=24)**	**LR-RBC (n=30)**	**p**
Pre-operative characteristics						
Age in years (mean±SD)	67.1 ± 11.8	66.8 ± 13.9	0.81	73.3 ± 6.1	71.5 ± 9.5	0.42
Valve + CABG, n (%)	60 (35.0)	63 (36.0)	0.90	15 (62.5)	15 (50.0)	0.42
Female, n (%)	71 (41.5)	82 (46.8)	0.33	10 (41.6)	17 (56.6)	0.41
Parsonnet-score	13.1 ± 8.3	13.9 ± 8.0	0.33	16.5 ± 8.3	15.5 ± 8.2	0.66
Use of statins, n (%)	39 (22.8)	46 (26.2)	0.46	6 (25.0)	6 (20.0)	0.75
Peri-operative characateristics						
Corticosteroid use, n (%)	52 (30.4)	55 (31.4)	>0.90	10 (41.6)	12 (40.0)	>0.90
Aprotonin use, n (%)	79 (46.1)	80 (45.7)	0.90	14 (58.3)	20 (66.6)	0.58
Cardiopulmonary bypass, in minutes (mean±SD)	134 ± 51	139 ± 58	0.37	159 ± 67	160 ± 70	>0.90
Transfusion characteristics						
RBC transfusions, median (IQR)	3 (2-7)	3 (2-6.5)	0.56	7.5 (3.2-9.7)	7 (4-12.2)	0.10
Storage time of all transfused RBCs in days, (mean±SD)	17.1 ± 5.7	17.0 ± 5.3	>0.90	16.7 ± 4.7	15.4 ± 4.6	0.35
Number of patients receiving only <17 days stored RBCs (%)	58 (33.9)	57 (32.5)	0.82	7 (29.1)	12 (40.0)	0.56
Number of patients receiving only <17 days stored RBCs	63 (36.8)	65 (37.1)	0.90	8 (33.3)	7 (23.3)	0.54
Number of patients receiving RBCs stored for < and ≥ 17 days	29 (16.9)	35 (20.0)	0.49	8 (33.3)	11 (36.6)	>0.90
Number of RBCs, n (%)						
0	21 (12.3)	18 (10.3)	0.61	1 (4.2)	0	0.44
1-3	66 (38.6)	59 (33.7)	0.37	4 (16.7)	8 (26.7)	0.52
≥4	84 (49.1)	98 (56.0)	0.24	19 (79.2)	22 (73.3)	0.75

	All patients			Patients staying > 2 days at ICU		
	BCD-RBC (n=171)	LR-RBC (n=175)	p	BCD-RBC (n=24)	LR-RBC (n=30)	p
Postoperative characteristics						
All complications, n (%)	66 (38.6)	55 (31.4)	0.18	18 (75.0)	17 (56.6)	0.25
Infections, n (%)	51 (29.8)	33 (18.9)	0.02	16 (66.7)	11 (36.7)	0.05
MODS, n (%)	29 (16.9)	33 (18.9)	0.68	9 (37.5)	9 (30)	0.58
Hospital mortality, n (%)	14 (8.2)	8 (4.6)	0.19	7 (29.2)	2 (6.7)	0.06

[a]Data presented as mean ±SD, n (%) or median with 25th and 75th quartiles (IQR); BCD-RBC, Buffy-coat depleted red blood cells; LD-RBC, leukocyte-depleted red blood cells; MODS, multiple-organ-dysfunction-syndrome; RBC, red blood cells

Inflammatory Markers and Randomization to Different Blood Products

Between patients randomized to receive BCD-RBC or LD-RBC no differences were measured on arrival at ICU in the concentrations of IL-6, IL-10, IL-12 and procalcitonin. In the subgroup of patients receiving 0-3 units RBCs the concentrations of cytokines were not different (Table 2). There were 182 (52.6%) patients who had received 4 or more units RBCs: 84 (49.1%) patients in the BCD-RBC group and 98 (56.0%) patients in the LR-RBC group. In this subgroup, only the concentration of IL-6 was significantly higher in the BCD-RBC group as compared to the LD-RBC group (median 152; IQR 74-340 pg/ml versus median 96, IQR 61-249 pg/ml, p=0.02). The concentrations of IL-10 and IL-12 were at ICU-arrival not different in the analysis according to the type of blood products and the number of RBC transfusions (Table 2).

The concentration of IL-6 at ICU-arrival was higher in the BCD-RBC group than in the LD-RBC group in patients who developed postoperative infections. The concentration of IL-12 was higher in patients who developed MODS in the BCD-RBC group. The concentrations of procalcitonin, postoperative increase of the leukocyte count (data not shown) and IL-10 were not different between BCD-RBC and LD-RBC groups, also not in subgroups with or without postoperative complications (Table 2). The interaction tests showed a significant difference for IL-6 in the groups with complications and infections compared with non-complications and non-infections, respectively. The interaction test for IL-12 was significant in the MODS group between non-MODS group (Table 2).

In patients who had received 4 or more units of RBCs, 90 (49.4%) patients had postoperative complications: 49 (58.3%) patients in the BCD-RBC group and 41 (41.8%) patients in the LR-RBC group. Patients receiving 4 or more units RBCs and who developed complications had significantly higher concentrations of IL-6 in the BCD-RBC group as compared to the LD-RBC group (median 258; IQR 124-367 pg/mL versus median 196, IQR 75-264 pg/ml, p=0.04). In the subgroup of patients receiving 4 or more units RBCs without complications, the concentrations of IL-6 levels were not different between both study arms (median 90; IQR 52-159 pg/ml versus median 75, IQR 47-137 pg/ml, p=0.66). There were no differences in the concentrations of IL-10 and IL-12 between both blood products according to number of transfusions in patients with and without transfusions (data not shown).

Table 2 | Cytokine Concentrations at ICU-Arrival in Patients with and without Complications

	BCD-RBC	LD-RBC	p	p for interaction[b]
IL-6 (pg/ml)[a]				
All patients (n=346)	113 (50-250)	85 (45-228)	0.07	
Transfusions: 0-3 RBCs (n=164)	77 (36-167)	72 (37-194)	0.64	0.14
≥4 RBCs (n=182)	152 (74-340)	96 (61-249)	0.02	
Complications: None (n=225)	71 (39-152)	72 (40-146)	>0.90	0.04
Any (n=121)	198 (98-360)	183 (72-267)	0.10	
Infections: No (n=262)	85 (40-184)	77 (45-212)	0.83	0.006
Yes (n=84)	190 (97-390)	99 (52-250)	0.03	
MODS: No (n=284)	97 (47-190)	73 (41-188)	0.20	0.17
Yes (n=62)	258 (120-350)	237 (90-278)	0.23	
Hospital mortality: No (n=324)	99 (47-215)	81 (44-217)	0.26	0.07
Yes (n=22)	303 (159-381)	206 (73-260)	0.06	
IL-10 (pg/ml)[a]				
All patients (n=346)	25 (6-77)	25 (9-93)	0.35	
Transfusions: 0-3 RBCs (n=164)	18 (6-56)	19 (9-94)	0.35	0.74
≥4 RBCs (n=182)	43 (7-84)	33 (9-90)	0.75	
Complications: None (n=225)	25 (6-66)	26 (10-82)	0.30	0.52
Any (n=121)	24 (7-91)	22 (7-115)	0.74	
Infections: No (n=262)	26 (7-69)	26 (9-91)	0.40	0.92
Yes (n=84)	17 (6-85)	24 (10-110)	0.24	
MODS: No (n=284)	25 (6-74)	25 (10-82)	0.29	0.68
Yes (n=62)	19 (9-128)	32 (6-165)	>0.90	
Hospital mortality: No (n=324)	24 (6-77)	24 (9-83)	0.34	0.71
Yes (n=22)	64 (10-123)	161 (62-210)	0.20	
IL-12 (pg/ml)[a]				
All patients (n=346)	40 (23-64)	37 (23-58)	0.48	
Transfusions: 0-3 RBCs (n=164)	36 (21-54)	31 (18-50)	0.66	0.82
≥4 RBCs (n=182)	44 (26-87)	44 (27-64)	0.47	
Complications: None (n=225)	34 (21-63)	34 (22-51)	>0.90	0.22
Any (n=121)	48 (30-75)	45 (25-63)	0.29	
Infections: No (n=262)	37 (21-64)	34 (21-51)	0.70	0.76
Yes (n=84)	51 (30-75)	49 (30-64)	>0.90	
MODS: No (n=284)	37 (21-64)	38 (23-58)	0.80	0.03
Yes (n=62)	50 (39-88)	36 (24-60)	0.02	
Hospital mortality: No (n=324)	39 (22-64)	37 (23-58)	0.56	0.65
Yes (n=22)	55 (44-89)	38 (24-49)	0.10	

[a]Data presented as median with 25th and 75th quartiles (IQR); [b]p for interaction test between the differences of cytokines in patients with and without complications; BCD-RBC, Buffy-coat depleted red blood cells; LD-RBC, leukocyte-depleted red blood cells; MODS, multiple-dysfunction-syndrome.

Multivariate Analyses of Risk Factors for all Postoperative Complications

The association between cytokine concentrations upon arrival at the ICU and subsequent development of postoperative complications was analysed separately for each complication (infections, MODS and hospital-mortality) in a multivariate logistic regression model. Based on the results of the univariate analysis for the postoperative complications (Table 3), the risk factors with a $p<0.20$ were included in the multivariate analysis. The multivariate analysis showed that all complications were independently associated with number of RBC transfusions (Table 4). Furthermore the concentration of IL-6 was independently associated with the composite of postoperative complications. Postoperative infections were also independently associated with the study arm in favour of LD-RBCs and the cardiopulmonary bypass time. MODS was also independently associated with IL-6 concentrations and the hospital mortality was independently associated with the Parsonnet-score and with the concentrations of IL-6 and IL-10.

Table 3 | Results of Univariate Analyses of Risk Factors Associated with Postoperative Complications

	All postoperative complications	Infections	MODS	Hospital mortality
	p	p	p	p
Type of surgery (valve or valve+CABG)	0.05	0.15	0.69	0.30
Gender (male or female)	0.91	0.58	0.49	0.14
Pre-operative statin use	0.85	0.85	0.94	0.47
Parsonnet-score	<0.001	0.003	0.04	<0.001
Randomization arm (BCD-RBC or LR-RBC)	0.10	0.01	0.64	0.16
Peri-operative corticosteroid use	0.16	0.79	0.96	0.29
Peri-operative aprotinin use	0.29	0.51	0.48	0.69
Cardiopulmonary bypass time (in minutes)	0.001	0.001	0.01	0.53
Number of RBC transfusions	<0.001	<0.001	<0.001	<0.001
Storage time of transfused RBC units (< 17 days, ≥ 17 days, or both)	0.01	0.02	0.01	0.07

BCD-RBC, Buffy-coat depleted red blood cells; LD-RBC, leukocyte-depleted red blood cells; MODS, multiple-organ-dysfunction-syndrome

Table 4 | Multivariate Analyses of Risk Factors Associated with Postoperative Complications

	All postoperative complications OR (95% CI)	p	Infections OR (95% CI)	p	MODS OR (95% CI)	p	Hospital mortality OR (95% CI)	P
Parsonnet-score	1.18 (0.95-1.46)	0.13	1.16 (0.92-1.47)	0.20	0.98 (0.76-1.31)	0.98	1.70 (1.04-2.78)	0.04
Gender							2.09 (0.70-6.22)	0.19
Randomization arm	1.46 (0.87-2.49)	0.15	2.01 (1.15-3.54)	0.01			1.92 (0.67-5.51)	0.22
Type of surgery	1.08 (0.60-1.92)	0.80						
Use of corticosteroids	0.54 (0.29-1.05)	0.06						
Cardiopulmonary bypass time	1.25 (0.95-1.64)	0.10	1.46 (1.09-1.96)	0.01	0.95 (0.69-1.31)	0.74		
Number of RBC transfusions	1.22 (1.13-1.31)	<0.001	1.14 (1.08-1.21)	<0.001	1.26 (1.17-1.36)	<0.001	1.12 (1.05-1.19)	0.001
Storage time of transfused RBC units	0.97 (0.68-1.37)	0.84	0.99 (0.68-1.45)	>0.90	0.79 (0.49-1.27)	0.33	1.05 (0.50-1.93)	>0.90
Postoperative increase of leukocytes	0.96 (0.86-1.07)	0.47	0.92 (0.82-1.04)	0.17	0.85 (0.55-1.33)	0.48	1.01 (0.81-1.25)	>0.90
Procalcitonin concentration at ICU-arrival	0.28 (0.05-1.57)	0.15	0.99 (0.24-4.11)	>0.90	0.87 (0.15-5.05)	0.87	0.02 (0.01-20.2)	0.27
IL-6 concentration at ICU-arrival	1.54 (1.21-1.94)	<0.001	1.05 (0.79-1.39)	0.74	1.87 (1.36-2.57)	<0.001	2.18 (1.27-3.71)	0.004
IL-10 concentration at ICU-arrival	1.14 (0.87-1.50)	0.34	0.92 (0.69-1.22)	0.54	1.31 (0.93-1.85)	0.12	1.70 (1.06-2.73)	0.03
IL-12 concentration at ICU-arrival	1.03 (0.77-1.37)	0.84	1.31 (0.97-1.76)	0.07	0.92 (0.63-1.33)	0.64	1.17 (0.66-2.10)	0.59

BCD-RBC, Buffy-coat depleted red blood cells; LD-RBC, leukocyte-depleted red blood cells; MODS, multiple-organ-dysfunction-syndrome; RBC, red blood cells

Kinetics of Cytokines in Patients with Prolonged ICU-stay

In this group of 346 patients, 54 patients had a prolonged ICU-stay of at least two days. From these patients from the end of perfusion until postoperative day 2 the kinetics of the cytokines IL-6, IL-10 and IL-12 were measured. These patients had a higher age, had longer duration of surgery, received more blood transfusions and had higher hospital mortality than the whole study group of 346 patients (Table 1). The concentration of IL-6 increased at the end of perfusion and reached its peak upon arrival at ICU after which it declined. This peak was higher in patients who received BCD-RBC as compared to patients who received LD-RBC transfusions (median 188; IQR 111-330 pg/ml versus median 104; IQR 66-193 pg/ml, p=0.02). The increase of IL-10 preceded the increase of IL-6 and reached immediately after the end of perfusion significantly higher concentrations in the LD-RBC group than in the BCD-RBC group (median 168; IQR 81-270 pg/ml versus median 94; IQR 19-234 pg/ml, p=0.05). The IL-10 concentrations had already decreased upon arrival at ICU. IL-12 concentrations only showed a transient rise upon the arrival at ICU and were similar in both study arms (Figure 1).

DISCUSSION

In a randomized study comprising 474 patients with high risk heart valve surgery with or without CABG we found leukocyte-containing transfusions (BCD-RBC) dose-dependently associated with more postoperative infections and higher in-hospital mortality compared to patients receiving leukocyte-depleted (LD-RBC) transfusions [12]. We assumed that inflammatory mediators associated with transfusion of allogeneic leukocytes during cardiac surgery might play a role. We investigated for the concentrations of inflammatory cytokines (IL-6, IL-10 and IL-12) and procalcitonin in patients undergoing cardiac valve surgery in relation with transfusion of BCD-RBCs or LD-RBCs. From 346 representative patients pre- and post-operative blood samples were available to investigate cytokine and procalcitonin levels after surgery. In the analysis of the total patient population we found no differences in the levels of the selected cytokines IL-6, IL-10 and IL-12 or procalcitonin upon arrival at the ICU. However, significantly higher IL-6 levels at arrival at ICU were found in patients after transfusion of 4 or more units BCD-RBCs compared with LD-RBCs. Higher IL-6 and IL-12 after leukocyte-containing transfusions were present in patients who developed infections and MODS respectively. IL-10 and procalcitonin were not associated with number and type of transfusions in patients with or without complications, although higher IL-10 levels were

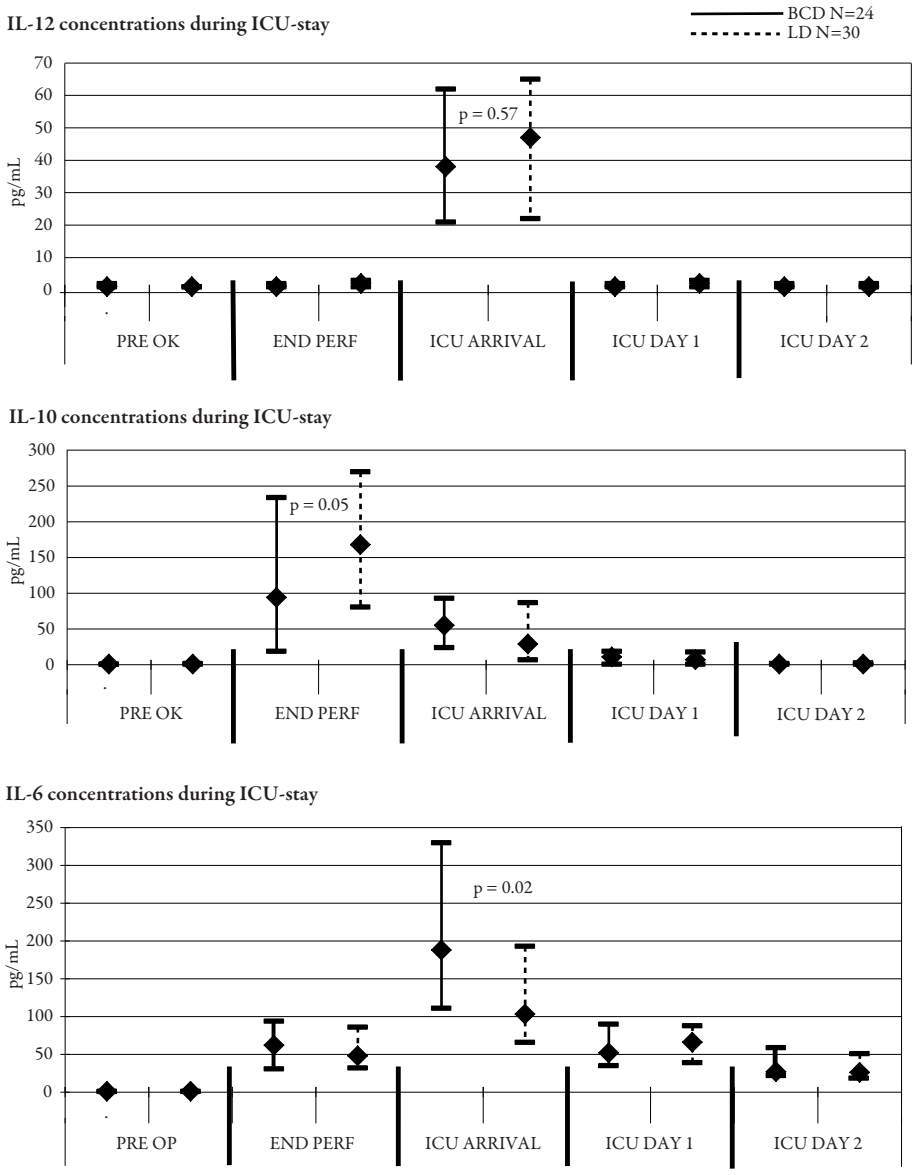

Figure 1 | The cytokines IL-6, IL-10 and IL-12 concentrations (pg/mL) in patients measured pre-operatively (PRE OP), at end of perfusion (END PERF), arrival at ICU (ICU ARRIVAL), at ICU-stay day 1 (ICU DAY 1) and day 2 (ICU DAY 2) in patients staying longer than 2 days at ICU. The comparisons are made between buffy-coat depleted red blood cells (BCD-RBC) and leukocyte-depleted red blood cells (LD-RBC).

associated with hospital mortality in both randomisation arms. This is the first study showing higher pro- inflammatory markers after leukocyte-containing transfusions in cardiac surgery, in particular in subgroups later developing serious clinical complications.

In several studies IL-6 concentrations have been found increased in association with a pro-inflammatory response after cardiac surgery [22,23]. In these studies the possible relationship with allogeneic blood transfusions was not analyzed. Furthermore other studies found an association between allogeneic blood transfusions and postoperative morbidity and mortality [5-9], however these studies did not perform laboratory analysis. Only one study reported that blood transfusions contributed to the release of inflammatory markers in cardiac surgery [13]. The purpose of the laboratory analysis was to evaluate a possible relationship between the concentrations of inflammatory markers after cardiac surgery and leukocyte containing blood products and whether an association with postoperative complications, such as infections, MODS and hospital mortality could be found. The interaction between allogeneic blood transfusions and postoperative complications has been questioned in the literature [24], which has not been solved by our study, however our results suggest that stimulation of a pro-inflammatory response by allogeneic leukocytes in transfusions enhances susceptibility for complications. To identify the relation between transfusion of type of blood products and the development of complications on the concentrations of cytokines we performed an interaction test. This showed that the differences in the concentrations of IL-6 and IL-12 in patients who developed infections and MODS, respectively, is related to the transfusion of leukocyte-containing blood products, while no differences were measured in the concentrations of IL-6 and IL-12 in patients without infections and MODS. The multivariate analysis in this patient cohort revealed that, besides the strong effect of the number of blood transfusions on all postoperative complications, an association existed between the type of blood product (BCD-RBC) and the development of postoperative infections. This was in accordance with clinical observations of detailed analysis of causes of deaths observed in two randomised studies [11,12], which revealed that the higher mortality in patients receiving BCD-RBCs was caused by a combination of infections and MODS. While death due to cardiac complications and non-infectious reasons were comparable in patients after BCD-RBC or LD-RBC transfusions [25].

In contrast to prior reports, we found no differences in procalcitonin concentrations immediately after cardiac surgery predicting complications in patients [26]. On the other hand, our study confirms that higher concentrations of IL-6 are associated with complications after cardiac surgery [27,28] and that higher IL-6 and IL-10 concentrations are related to mortality [29,30]. Furthermore we observed that the higher the number of transfusions,

the more outspoken differences in cytokine concentrations between randomisation arms emerged. We found in two randomized trials a transfusion-dose dependent association with postoperative infections and mortality with leukocyte-containing blood transfusions [11,12]. Our laboratory analysis suggest that the presence of leukocytes in blood transfusions are transfusion-dose dependent associated with the concentrations of some inflammatory mediators, which could be further related with the development of more postoperative complications in highly transfused patients.

Additionally we evaluated the time course of the cytokine concentrations in 54 multiple transfused patients with prolonged ICU-stay. In this more heavily transfused (median 7 units RBCs) subgroup with a prolonged stay in the ICU (longer than 2 days), at arrival at ICU, IL-6 levels were higher and IL-10 levels significantly lower in patients who received BCD-RBCs as compared to LD-RBCs. The increased concentrations of IL-6, IL-10 and IL-12 occurred immediately after cardiac surgery and generally decreased within 24 hours. The anti-inflammatory cytokine IL-10 was the first to increase immediately after perfusion and was higher in LD-RBC group compared to BCD-RBC group. In both study arms the concentration of IL-10 decreased during ICU-stay. The increase of inflammatory cytokine IL-6 was observed later and reached higher levels in the BCD-RBC group than in LD-RBC group. This pattern of waves of pro-and anti-inflammatory cytokines seems typical for cardiac surgery and has been reported also in patients with uneventful recovery [13,29,30].

Cardiac surgery causes an initially anti-inflammatory response followed by a pro-inflammatory response leading to production and release of inflammatory mediators [13,29,30]. The balance between these pro-inflammatory and anti-inflammatory responses determines the clinical course of the post-surgical systemic inflammatory response. The higher pro-inflammatory cytokine IL-6 and lower anti-inflammatory cytokine IL-10 in the BCD-RBC group suggests that leukocyte-containing blood transfusions aggravate this pro-inflammatory pattern, which is more pronounced than in the leukocyte-depleted blood transfusions. These findings support that leukocyte-containing allogeneic blood transfusions amplify an inflammatory response in addition to an ongoing systemic inflammatory response after cardiac surgery. This transfusion-related immunomodulation could enhance the development of postoperative adverse events, which may result in severe infections and aggravation of MODS. These adverse events could influence recovery and could eventually lead to higher incidence of mortality.

CONCLUSIONS

This laboratory analysis derived from a large patient population undergoing cardiac surgery provides for the first time detailed information about differences between leukocyte-depleted and leukocyte-containing transfusion-related immune responses. The results supports that leukocyte-containing transfusions interfere with the balance between postoperative pro-and anti-inflammatory responses towards the pro-inflammatory direction. The complex balance between pro-inflammatory and anti-inflammatory mediators leading to postoperative complications is difficult to discriminate based on our data. Additionally to blood transfusions, multiple factors may influence inflammatory responses that could contribute to the development of postoperative complications. Future studies are needed to investigate the more long-term effects of allogeneic blood transfusions in cardiac surgery.

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Chapter 6

Mannose-Binding Lectin is Involved in Multiple-Organ-Dysfunction-Syndrome after Cardiac Surgery: Effects of Blood Transfusions

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ABSTRACT

Background: Serum levels of mannose-binding lectin (MBL), a recognition molecule of the lectin pathway of complement, are highly variable, based on genetic variation. After cardiac surgery, extracorporeal circulation and ischemia/reperfusion injury initiate a systemic inflammatory response, which can evolve to multi-organ dysfunction syndrome (MODS). Peroperative transfusions of allogeneic leukocytes contribute to infectious and inflammatory complications. The present study investigates the role of MBL in relation to blood transfusions and complications following cardiac surgery.

Methods: In cardiac surgery patients who participated in a randomised trial comparing leukoreduced with buffy-coat-depleted red blood cell transfusions, circulating MBL was measured pre- and post-operatively by ELISA. Data were related to the incidence of complications and to the transfusions the patients received.

Results: Patients with high pre-operative serum MBL levels (>400 ng/ml) show a significant (52 ± 12%) decrease of serum MBL post-operatively, while patients with low serum MBL levels (<400 ng/ml) show a significant increase of serum MBL levels after surgery (140 ± 106%), which was further enhanced by fresh-frozen plasma (FFP) transfusions. MBL levels were not associated with infections, sepsis, or death. Patients with MBL deficiency (MBL <80 ng/ml) were protected against development of MODS (p=0.016), whereas FFP transfusion abolished this protection (p=0.048).

Conclusions: Cardiac surgery is associated with MBL consumption, independent of the transfusion of allogeneic leukocytes. Patients with MBL deficiency develop no MODS, unless they have been transfused with FFP, which is associated with MBL reconstitution. Therefore, sustained MBL deficiency may be a favourable status for patients undergoing cardiac surgery.

INTRODUCTION

Cardiac surgery associated with ischemia/reperfusion injury is often followed by a systemic inflammatory response syndrome (SIRS). Moderate SIRS usually resolves with supportive care, although severe SIRS can evolve to multiple-organ-dysfunction-syndrome (MODS). The outcome of cardiac surgery is closely related with the severity of MODS and development of severe infections [1].

One of the components of the inflammatory response which is activated during cardiac surgery is the complement system [2,3]. The complement system can be activated by three pathways: the classical pathway, the alternative pathway 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) [4]. Polymorphisms in the MBL gene result in a wide range of functional MBL levels. Roughly 30% of the Caucasian population has reduced levels of MBL, due to single nucleotide polymorphisms in exon 1 of the *MBL2* gene, and approximately 5-10% has a functional MBL deficiency [5].

MBL deficiency in itself does not lead to clinical problems, but several studies have shown that MBL deficiency confers an increased susceptibility for infections in immune-compromised patients [6-9]. The role of MBL deficiency on the development and outcome of SIRS and sepsis syndrome is controversial. Worse outcome in patients with sepsis is described [10,11]. However, in animal models, inhibition of the lectin pathway was shown to protect against ischemia-reperfusion injury and diminished neutrophil accumulation and cytokine release [12], and MBL-deficient mice were protected against severe ischemia/reperfusion injury in various organ systems [13-16]. These studies suggest that MBL deficiency could have favourable effects on tissue injury following ischemia and reperfusion.

In cardiac surgery, red blood cell transfusions dose-dependently increase the risk of morbidity and mortality. In randomized trials we observed that red blood cells containing leukocytes compared with filtered leukoreduced red blood cells were associated with increased postoperative infections and mortality with MODS [17]. After complicated cardiac surgery, approximately 20-25% of the patients develop MODS and the combination of MODS and infections is associated with increased mortality [18]. However the role of blood component transfusions on activation of the complement system, especially on the lectin pathway, is not known. Cellular interactions of MBL have been described *in vitro* for several cell types. Interaction of polymorphonuclear leukocytes with ligand-bound MBL *in vitro* was shown to induce cell aggregation and superoxide production [19]. Direct

interactions between leukocytes and the complement system may provide an explanation for the beneficial effect of leukocyte depletion in red cell transfusion. We hypothesize that MBL and the lectin pathway may contribute to complications after cardiac surgery, both via activation of the complement cascade and via cellular interactions.

In the present study, we evaluated the effect of cardiac surgery and blood transfusions on pre- and postoperative MBL levels and we assessed whether MBL levels were associated with postoperative complications after cardiac surgery.

MATERIALS AND METHODS

Patients

A randomized, double-blinded controlled trial had been conducted in two hospitals and included patients undergoing valve surgery with or without coronary artery bypass graft (CABG). Patients were randomized to receive (when needed) standard buffy-coat depleted or pre-storage leukoreduced red blood cells. Patient samples were collected after informed consent was obtained. The endpoints of the study were: postoperative infections, MODS and mortality (90-days and in-hospital). The design and clinical outcome of the study has been described elsewhere [17]. In this trial infections were defined according to the criteria of the Center for Disease Control (CDC) [20]. The following infections were taken into account: respiratory tract, urinary tract, sepsis and wound infections. The incidence and duration of organ dysfunction was described as defined by Knaus [21]. MODS was defined as failure of two or more organ systems. The blood products used in this study were prepared and controlled according to the Dutch standards for blood banks.

Blood samples were taken pre-operatively before the start of the surgical procedure and at admission on the ICU, immediately centrifuged, and the sera stored at -80°C.

Measurement of MBL

The concentration of MBL was measured by sandwich enzyme linked immunosorbent assay (ELISA) as described previously [22]. In summary, 96-well ELISA plates were coated with mAb 3E7 (monoclonal anti-MBL antibody kindly provided by Dr. T. Fujita, Fukushima, Japan). After blocking residual binding sites with PBS containing 1% BSA and washing, serum samples were diluted and incubated, followed by detection with digoxygenin (dig-) conjugated 3E7 and HRP-conjugated sheep Fab anti-Dig antibodies (from Roche Applied Science, Mannheim, Germany), respectively. Enzyme activity was developed using ABTS.

The optical density was measured using a microplate reader, and results were calculated on basis of a calibration line using pooled normal human serum with a known concentration of MBL.

To estimate the effect of the surgical procedure on the MBL serum concentration, the ratio of the post- and preoperative MBL serum level was calculated.

Statistics

Categorical characteristics among MBL groups were compared using cross-tables with calculation of p-values. Continuous variables were analyzed using Student t-test or, when appropriate Mann-Whitney test was used. The differences in MBL levels before and after surgery were analyzed by the Wilcoxon's signed rank test. Spearman Rank correlation coefficients were used for correlation. A logistic regression model was used to evaluate the effect of per-operative factors on the postoperative/preoperative MBL ratio. P-values <0.05 were considered as statistically significant. All analyses were performed in SPSS (SPSS Inc, Chicago, IL, USA).

RESULTS

Patients Characteristics

Of the 474 patients included in the trial, serum for MBL determination was available from 400 patients prior to surgery. From these 400 patients, postoperative MBL levels could be assessed in 330 patients. The main characteristics of the patient population are presented in Table 1.

Serum MBL Levels

Pre-operative serum levels of MBL were highly variable as expected in the human population (range 15-3709 ng/ml; Table 1). In Figure 1A the post-/ preoperative MBL ratios are shown. The ratio of post-operative and pre-operative MBL concentration was negatively correlated with the pre-operative MBL concentration ($R = -0.73$, $p<0.0001$). Patients with high serum MBL levels before surgery (>400 ng/ml) showed an average decrease of $52 \pm 12\%$ of the serum MBL level after the procedure (range ratio 0.19-0.93; $p < 0.0001$, Figure 1B). In contrast, patients with low serum MBL levels before operation (< 400 ng/ml) generally showed an increase of serum MBL levels, as represented by a ratio significantly higher than 1 ($p<0.0001$, Figure 1B).

Table 1 | Patient Characteristics

	Total	Randomization*		P value
		Buffy-coat depleted red blood cells	Leuko-reduced red blood cells	
Patient number	400	194	206	
Age (mean ±SD)	65.8 ± 13.9	66.5 ± 12.5	65.1 ± 15	0.36
Type of surgery (N (%))				
Valve	269 (67.3%)	133 (68.6%)	136 (66.0%)	0.60
Valve + CABG	131 (32.8%)	61 (31.4%)	70 (34.0%)	
Female (N (%))	178 (44.5%)	85 (43.8%)	93 (45.1%)	0.84
Parsonnet score	13.8 ± 8.2	13.8 ± 8.6	13.8 ± 7.8	0.98
Blood transfusions during surgery†				
Red blood cells (mean units per patient ±SD)	3.2 ± 3.6	3.4 ± 4.7	3.0 ± 2.1	0.23
FFP (mean units per patient ±SD)	3.2 ± 4.1	3.6 ± 5.4	2.8 ± 1.7	0.22
Patients without FFP transfusions (N (%))	224 (56.0%)	103 (53.1%)	121 (58.7%)	0.27
Patients without red blood cell transf. (N (%))	114 (28.5%)	54 (27.8%)	60 (29.1%)	0.82
Cardiopulmonary bypass time (minutes; mean ±SD)	140 ± 62	145 ± 66	136 ± 57	0.13
Aortic cross clamping time (minutes; mean ±SD)	96± 46	96 ± 47	96 ± 44	0.98
Serum total protein (gram/l) (mean ±SD)				
Pre-operative	74.5 ± 4.3	74.3 ± 4.0	75 ± 4.6	0.55
Post-operative	49.9 ± 6.2	49.6 ± 6.6	50.2 ± 5.8	0.50
Infections (N (%))	113 (28.3%)	66 (34.0%)	47 (22.8%)	0.02
MODS (N (%))	89 (22.3%)	45 (23.2%)	44 (21.4%)	0.72
Sepsis (N (%))	17 (4.3%)	10 (5.2%)	7 (3.4%)	0.46
Hospital mortality (N (%))	34 (8.5%)	23 (11.9%)	11 (5.3%)	0.04
Mean MBL (ng/ml) (mean ±SD)				
Pre-operative (N=400)	837 ± 796	820 ± 802	854 ± 792	0.68
Post-operative (N=330)	453 ± 350	449 ± 356	457 ± 346	0.82
Median MBL (range)				
Pre-operative	571 (15-3709)	534 (21-3619)		
Postoperative	339 (35-1934)	329 (35-1934)		
Pre-operative MBL <80 (ng/ml) (N (%))	38 (9.5%)	21 (10.8%)	17 (8.2%)	0.40
Pre-operative MBL <400 (ng/ml) (N (%))	164 (41.0%)	81 (41.7%)	83 (40.3%)	0.84

* Patients were randomized to receive either buffy-coat-depleted red blood cells or leukocyte depleted red blood cells, when needed. † Units per patients transfused during surgery. Only patients who received red blood cells and FFP transfusions are included for calculation of mean and SD.

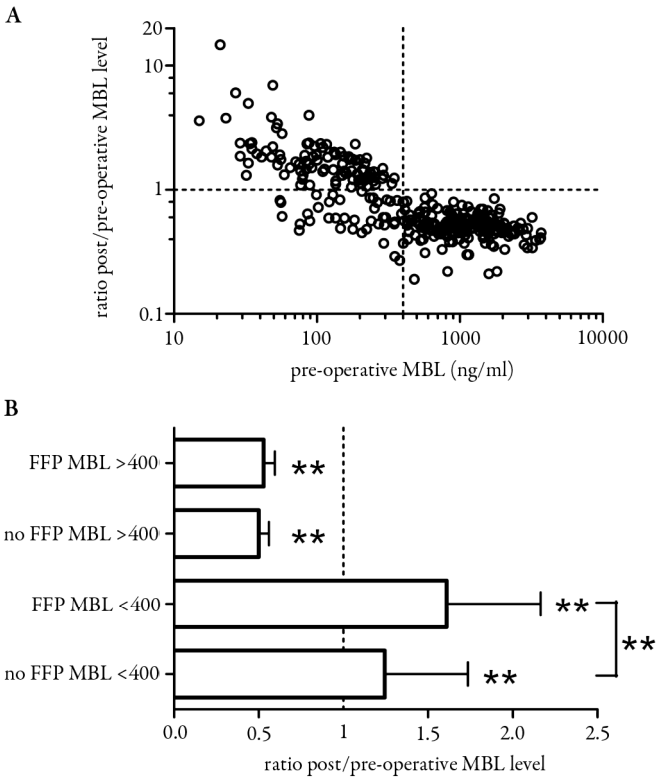

Figure 1 | The effect of plasma transfusion on levels of serum MBL in patients who undergo cardiac surgery. **A.** Ratios of post/pre-surgery MBL levels were plotted against pre-operative serum MBL levels in all patients (N=330). The dashed line indicates a serum MBL level of 400 ng/ml. **B.** Mean ratios post/pre-surgery MBL levels ± SD were calculated for patients above and below 400 ng/ml who did or did not receive FFP during the operation, as indicated. ** indicates statistical significance. Ratios in all groups were significantly different from 1 ($P<0.0001$; Wilcoxon signed rank test). The difference between patients with serum MBL level below 400 ng/ml who did or did not receive plasma was tested with the Mann Whitney test ($P=0.0039$).

Ratios of MBL levels were not different between recipients of leukoreduced or buffy-coat depleted red blood cells. In both groups the MBL level decreased or increased with the same ratios determined by the preoperative MBL level (not shown). In contrast, FFP transfusions were associated with an additional increase of post-operative serum MBL in patients with a pre-operative MBL level below 400 ng/ml (median post/pre MBL ratio 1.25 versus 1.61,

p=0.0039; Figure1B), whereas FFP transfusion did not show an effect in patients with MBL levels above 400 ng/ml.

Following surgery, all patients displayed a dilution effect, as shown by a decreased serum total protein concentration (average ratio total protein post/pre-surgery =0.67 ± 0.08; range 0.46-0.86). However, this dilution factor was not correlated to post/pre-surgery MBL ratios, also not when patients with pre-MBL levels above 400 ng/ml were analysed only ($R<0.1$).

In a logistic regression model we further evaluated the contribution of various per-operative factors on the post/pre-surgery MBL ratio. The type of surgery (p=0.23), duration of cardiopulmonary bypass (p=0.19), duration of aortic crossclamping (p=0.25), number of red blood cell transfusions (p=0.19), randomization arm (p=0.74) and the decrease in total protein concentrations (p=0.43) did not show a significant effect on the post/preoperative MBL ratio.

MBL Serum Levels and Complications of Cardiac Surgery

Following cardiac surgery 113 of 400 patients developed infections (28.3%), 89 patients developed MODS (22.3%), 17 patients had sepsis (4.3%) and 34 patients died (8.5%; Table 1). Infections and mortality were significantly higher in patients receiving leukocyte-containing transfusions than in patients randomized for leuko-reduced red blood cells (p=0.02 and p=0.04, respectively). Patients who developed MODS had a significantly longer duration of cardiopulmonary bypass (median 148 versus 124 minutes; p=0.0002) and aortic crossclamping (median 98 versus 90 minutes; p=0.008).

Since data as presented above show that the surgical procedure and plasma transfusions have an effect on serum MBL levels, the relation between serum MBL and complications of cardiac surgery were further evaluated based on pre-operative MBL levels in patients who did not receive plasma. Using an MBL level of 400 ng/ml as a cut-off value, no significant association could be identified between higher or lower pre-operative MBL levels and infections, sepsis, MODS or mortality in the 224 patients who did not receive FFP transfusions (Table 2). However, patients with an MBL level below 80 ng/ml, indicating overt MBL deficiency, did not develop MODS (Figure 2 and Table 2; p=0.016). The incidence of infections, sepsis and mortality was not significantly different in patients with pre-operative MBL serum levels below or above 80 ng/ml.

In total 176 patients received FFP transfusions. In patients with pre-operative MBL levels below 80 ng/ml, the FFP transfusions increased the risk for development of MODS until the same degree as for patients with preoperative MBL concentrations above 80 ng/ml

(Table 3; p=0.048). In patients with pre-operative serum MBL levels above 80 ng/ml or above 400 ng/ml, FFP transfusion did not show any effect on the development of MODS.

Table 2 | Complications in Relation to Pre-operative MBL Levels in Patients who did not Receive Plasma

MBL (ng/ml)*	All	≤80	>80	p-value§	≤400	>400	p-value§
N†	224	18	206		93	131	
Infection (%)‡	25.0	22.2	25.2	1.0	28.0	22.9	0.43
Sepsis (%)‡	3.6	0	3.9	1.0	5.4	2.3	0.28
MODS (%)‡	21.0	0	22.8	0.016	19.4	22.1	0.74
Mortality (%)†	6.3	0	6.8	0.61	7.5	5.3	0.58

*MBL level before surgery; †Number of patients in each category; ‡Percentage of patients who developed this complication; §P value (Fisher exact test; comparison between MBL-low and MBL-high patients); MODS = multiple-organ-dysfunction-syndrome

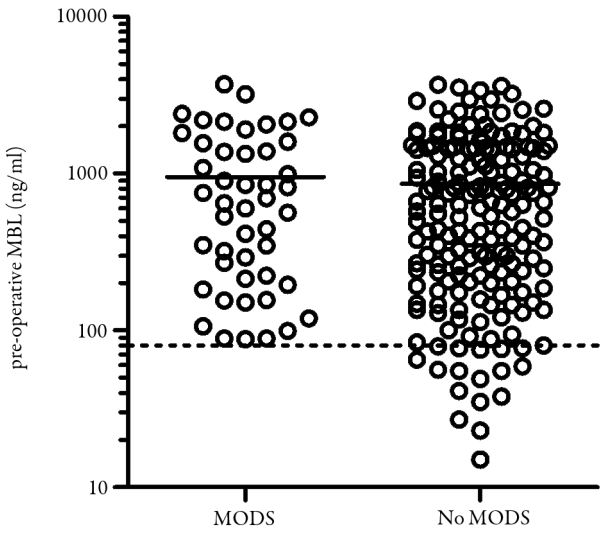

Figure 2 | Low serum MBL levels protect against MODS in patients who did not receive plasma during operation. Pre-operative serum MBL levels are shown in patients who did or did not develop MODS. Only patients who did not receive FFP were plotted.

Table 3 | Plasma Transfusion is a Risk Factor for MODS in Patients with Low Pre-operative MBL Levels

MBL level (ng/ml)*	No plasma (N)†	% MODS‡	Plasma (N)†	% MODS‡	p-value§
All	224	21	176	24	0.55
≤80	18	0	20	25.0	0.048
>80	206	22.8	156	23.7	0.90
≤400	93	19.4	7 1	25.3	0.45
>400	131	22.1	105	22.9	1.00

* MBL level before surgery; †Number of patients who did or did not receive plasma during the procedure; ‡Percentage of patients who developed multi-organ dysfunction syndrome; §P value (Fisher exact test; comparison between patients who did or did not receive plasma)

CONCLUSIONS

In a randomised study in cardiac surgery patients we observed a lower incidence of postoperative infections and mortality associated with MODS after transfusion of filtered leukoreduced red blood cell transfusions compared with standard buffy-coat depleted red blood cells [17]. Pre-operative and postoperative blood samples had been taken for investigation of mechanisms, which could explain differences between both randomization arms, should these occur. Because in previous studies low MBL levels have been associated with infections and high levels with ischemia/reperfusion injury [6-12], both important complications of cardiac surgery, we hypothesized that leukocytes in red blood cell units may play a role by activation of the innate immune system via the lectin pathway.

The results of this study show that absence or presence of allogeneic leukocytes in erythrocyte products had no effect on MBL concentrations after surgery. In both randomisation arms, patients with higher (>400 ng/ml) pre-operative MBL levels show post-surgical decrease by almost 50%. Although patients with preoperative MBL values below 400 ng/ml increased their postoperative MBL levels, especially if FFP had been administered during the procedure. If the preoperative MBL value was above 80 ng/ml, preoperative and postoperative MBL values were not associated with postoperative infections, MODS or mortality. However, none of the 18 patients with MBL deficiency below 80 ng/ml who did not receive per-operative FFP developed MODS. In contrast, the incidence of MODS in 20 MBL-deficient patients who received FFP was comparable with the incidence in patients with higher preoperative MBL concentrations.

This is, to the best of our knowledge, the first study that reports MBL serum levels after cardiac surgery. In a previous study in a small number of 23 patients undergoing abdominal aneurysm aorta surgery, a postoperative decrease of approximately 40% of MBL was observed, whereas in a control group, undergoing biliary and pancreatic surgery, there was no effect on MBL levels after the operation [23]. In patients undergoing abdominal surgery for respectable esophagus carcinoma a clear increase of serum MBL was observed, with a slow kinetics starting on day 5 postoperatively [24]. In gastric surgery patients, no effect on post-surgery MBL levels was observed as assessed on day 3 [25]. These variable results in patient cohorts undergoing different surgical interventions, in studies with relatively small cohort sizes, indicate interplay of different factors modifying the MBL serum level. In our large patient population undergoing cardiac surgery, we observed an increase as well as a decrease of MBL concentrations. The change in MBL concentration after surgery was significantly associated with the patient's MBL status prior to surgery. Because the MBL level in the population shows a wide range, which is genetically based, this relationship is easily missed when small groups of patients are evaluated. The measured decrease of 50% in the MBL concentration was somewhat overestimated because of postoperative hemodilution, reflected by a decrease of the total protein concentration. In reverse, due to this hemodilution, the increase of MBL after cardiac surgery is somewhat underestimated. When calculation of MBL post/pre ratios was corrected for this hemodilution effect, we confirmed a median 24% decrease of serum MBL levels ($P<0.0001$, not shown) in patients with pre-operative MBL levels above 400 ng/ml, suggesting an independent cause for the decrease of serum MBL.

The reduction in serum MBL in patients with higher preoperative MBL levels is most likely the result of consumption by activation of the lectin pathway during surgery, presumably mainly by interaction of MBL with ischemic and injured tissue. Accordingly, MBL consumption was previously observed in patients undergoing aorta aneurism operation [23], a procedure also associated with extended ischemia, but not in patients undergoing other surgical interventions [23-25]. The finding that this decrease was not compensated in our cardiac surgery patients, neither by endogenous production nor by FFP transfusions, suggests a stronger MBL consumption in these patients than in patients with lower preoperative MBL levels. In patients with preoperative MBL concentrations below 400 ng/ml we do not know whether (compensated) per-operative MBL consumption may occur, but after surgery endogenous production is increased and the level further increases after FFP transfusions. The latter observation, i.e. MBL reconstitution of MBL-deficient patients upon FFP transfusion, has also been reported for patients undergoing aortic aneurysm repair [3]. It is well conceivable that MBL consumption is more efficient in patients with a high MBL

level before transplantation, since levels of MBL above 400 ng/ml are strongly associated with the MBL wildtype genotype and normal lectin pathway function, whereas MBL from low-MBL patients may show impaired interaction with ligand [26]. Further studies with sampling during the various phases in cardiac surgery are needed revealing the kinetics of MBL during surgery to explain the mechanisms of this dichotic MBL behavior.

In the present study no association was observed between pre- or post-surgery MBL levels and postoperative infections after cardiac surgery, in contrast to studies performed in immunocompromised patients showing an association of MBL deficiency with increased risk of infections [6-9]. However, patients with an obvious MBL deficiency before surgery were significantly protected against the development of MODS. Only in these patients, FFP transfusions, resulting into (partial) reconstitution of the MBL status, reversed this protection against MODS. This is in agreement with observations in experimental renal and myocardial ischemia/reperfusion injury and septicaemia in rats and mice, which suggest that low MBL ameliorates ischemia/reperfusion injury and sepsis-induced organ damage [12,13,27,28]. Lower MBL levels have also been associated with less graft loss in kidney transplantation in humans [22]. In the setting of aortic aneurism repair, one patient has been presented who was MBL deficient and who did not respond to the surgical procedure with production of soluble complement activation products and cytokines, in contrast to all MBL-sufficient patients who developed an evident inflammatory reaction [3]. MBL may mediate an inflammatory response via activation of the complement cascade and probably also by direct interaction with and activation of leukocytes [19]. However we observed a beneficial effects of transfusion with leukoreduced red blood cells in cardiac surgery [17], no effects of allogeneic leukocytes could be observed in this study in postoperative MBL levels. Taken together, results now presented in our study support a role for MBL in the activation of the complement system and induction of a systemic inflammatory response upon ischemia and reperfusion injury, which may evolve into multiple organ dysfunction. MBL deficiency have probably a protective role in development of MODS, which disappears with transfusions of FFP and is not directly related with allogeneic leukocytes.

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Chapter 7

Cost-Effectiveness of Leukocyte-Depleted Erythrocyte Transfusion in Cardiac Valve Surgery

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ABSTRACT

Background: Cost-effectiveness of leukodepleted erythrocytes (LD) over buffy-coat-depleted packed cells (PC) is estimated from the primary dataset of a recently reported randomized clinical trial involving valve surgery (± CABG) patients.

Methods: Data on the patient level of 474 adult patients undergoing valve surgery (± CABG) who were randomized double-blind to LD or PC was used to calculate the healthcare costs and longevity per patient. The incremental cost-effectiveness ratio (ICER) in net costs per life-year gained was established from the healthcare perspective. Bootstrapping and cost-effectiveness acceptability curves were used to determine the confidence interval of the ICER.

Results: The longevity of patients in the PC and LD group was 10.6 and 11.4 years respectively. Relative to PC, LD yielded an estimated 0.8 (95% CI-0.27 to 1.84) life-year in baseline. Adjusted for age and gender differences health gains for LD are 0.4 life-year gained (95% CI-0.67 to 1.44]. Healthcare costs per patient averaged US$ 10163 per patient in the PC group and US$ 9949 in the LD group. Average cost-savings were US$ 214 (95% CI-1964 to 1536) per patient. Acceptability curves constructed from bootstrap simulations showed a probability of being cost saving of 59% for universal leukodepletion from the healthcare perspective. The probability of adopting leukodepletion regardless the costs reaches 92.7%. LD in patients receiving 4 or more transfusions showed the highest cost-savings and health gains.

Conclusions: Leukodepletion of erythrocytes is a cost-saving strategy in cardiac valve (± CABG) patients. However, acceptability analysis failed to show a significant difference with buffy-coat-depleted packed cells.

INTRODUCTION

The clinical benefit and application of leukodepletion of cellular blood products such as erythrocytes or platelets remains a strongly debated issue in transfusion medicine [1,2]. Observational and prospective clinical studies show results ranging from a positive clinical outcome to no effect. Observational "before-after" studies summarised in a meta-analysis suggested a reduction in risk of post-operative infections, but no decrease in mortality was observed [3].

A meta-analysis of randomised clinical trials reporting on the effect of leukodepletion showed no effect on mortality across all included clinical settings. However, subgroup analysis for open heart surgery patients showed an increase in mortality for patients receiving standard RBCs instead of leukodepleted RBCs [4]. Recently a double-blind randomized clinical trial involving valve surgery (with or without CABG) patients found a reduction in infections and in hospital mortality as secondary endpoints. No significant reduction of the primary endpoint mortality was observed after three months, the primary data on the patient level of this is used for the present cost-effectiveness analysis [5]. Only the secondary outcome hospital mortality was considered in the previously mentioned meta-analysis of randomised clinical trials.

Up to date formal cost-effectiveness analyses on leukodepletion are scarce and are mainly derived from observational, retrospective data. However, the few studies that are available show a favorable economic profile for selected clinical indications. For instance, utilizing observational clinical data in an economic model, leukodepletion of platelets appears to be cost-saving when applied in adult acute myelogenous leukaemia [6]. Furthermore, leukodepletion of platelets in acute myelogenous leukemia and lymphoma patients, and leukodepletion of whole blood in colorectal surgery showed lower hospital costs compared to unfiltered blood products [7,8]. In CABG patients leukodepletion of erythrocytes was cost-saving [9]. The Canadian Co-ordinating Office for Health Technology Assessment concluded that universal leukodepletion would not be cost-saving but that selective leukodepletion, that is, applying it to all patients who had an established indication for its use (such as frequently transfused patients), might be cost-saving [10].

In this study the cost-effectiveness ratio of pre-storage leukodepleted (by filtration) erythrocytes incremental to standard buffy-coat-depleted packed cells was established from the healthcare perspective for patients undergoing valve surgery with or without CABG using clinical outcomes and cost data per patient from a prospective randomized double blind clinical trial [5].

MATERIAL AND METHODS

Patients, Design and Data Collection

The design of the clinical trial is described in detail by Bilgin et al [5]. Briefly, a prospective, randomized, double-blind, controlled trial was conducted in 2 university hospitals in the Netherlands in adult patients >18 years undergoing valve surgery (with or without CABG). Patients with a medical indication for leukodepletion and patients who had received blood transfusions within the previous 3 months were ineligible. The patients were randomized into two groups: when there was an indication for transfusions, one group received buffy-coat-depleted packed-cells (PC), which was at that time the standard product in the Netherlands, and the other group received pre-storage leukocyte-depleted (by filtration) erythrocytes (LD). The hospitals used similar transfusion triggers for erythrocytes, plasma and platelets. To all patients prophylactic antibiotics were given for 48 hours. Postoperatively the patients were monitored at the intensive-care-unit (ICU); they were discharged from the ICU when there was no more need for inotropes and intubation.

Primary endpoint was the rate of mortality 90 days after surgery. Secondary endpoints were incidence of in-hospital mortality, the incidence of postoperative Multiple-Organ-Dysfunction-Syndrome (MODS), infections, ICU and standard care stay. We used parameters for organ dysfunction as described by Knaus [11]. MODS was defined as the failure of >2 organ systems. Infections were scored according to the criteria of CDC (Centers for Disease Control and Prevention) [12]. Causes of in-hospital mortality were obtained from the hospital patient records, mortality at 90 days from the referring cardiologist or the general practitioner. Blood product use (erythrocytes, platelets and plasma) and prescriptions for antibiotics were registered. The analysis for all endpoints was on an intention-to-treat basis.

Cost-Effectiveness Analysis

The Incremental Cost Effectiveness Ratio (ICER) was expressed in net costs per life-year gained. Net costs were estimated by subtracting average costs per patient in the PC group ($C_{PC)}$ from those in the leukodepleted arm in the trial (C_{LD}). Table 1 lists the unit costs used to calculate the total costs from the trial data. To estimate the ICER for overall implementation of leukodepletion and only for cardiac surgery patients, excess costs of leukodepletion were calculated at two levels: Dutch Sanquin Blood Supply Foundations' estimate for universal leukodepletion (baseline) and estimation for selective leukodepletion for cardiac surgery patients solely. Costs of ICU stay and standard care were obtained from the reference costs for Dutch pharmaco-economic analyses and corrected for blood

product use [13]. Costs of erythrocytes, fresh frozen plasma and platelets were obtained from Sanquin Blood Supply Foundation. Antibiotics costs were derived from the Dutch Price Index (Z-index, The Hague, The Netherlands) and corrected for the average discount for hospital pharmacies (-20%). As outlined in the introduction net cost (CLD-CPC =DC) were considered from the healthcare perspective. Incremental health gains were expressed in life-years gained (DE) and were derived by linking survival, derived from the mortality at day 90, to age and gender specific remaining life expectancies in the Netherlands (source: Statistics Netherlands, Heerlen, The Netherlands). To account for increased mortality in cardiac surgery patients survival was corrected by an annual excess death rate of 1% [14]. Because the study duration was less then one year, discounting of costs and monetary benefits was not necessary. Life-years gained were discounted at 3% (0% and 5% in the sensitivity analysis) according to international guidelines [15,16].

Table 1 | Unit Costs Used to Calculate Costs from Trial Data

Cost component	Cost (US$) per unit	Unit
Leukodepletion (baseline, universal)	20	RBC
Leukodepletion (selective)	36	RBC
ICU stay	1024	Day
Standard care stay	305	Day
Antimicrobial therapy	Reference price	Dose
RBCs	130.84	Unit
Fresh Frozen Plasma	164.45	Unit
Platelets	342.15	Unit

Clinical trials contain both costs and health information at the patient level and therefore inferences can be made for the confidence interval around the point estimate cost-effectiveness ratio. However, being a ratio, standard statistics do not apply for the cost-effectiveness ratio and other approaches such as bootstrapping must be applied [17]. Bootstrapping as a non-parametric approach avoids the difficulties related to distribution depended statistics and estimates an empirical sampling distribution for the cost effectiveness ratio [18]. In bootstrapping a number of random cost and effect pairs equivalent to the number of observations in the original data set are taken with replacement from the original data set. Next, the bootstrap estimates for the mean costs and health effects of both the LD ($\overline{C}^{*}_{LD}, \overline{E}^{*}_{LD}$) and

pC ($\overline{C}^{*}_{PC}, \overline{E}^{*}_{PC}$) group are calculated, enabling to calculate the bootstrap estimate of the incremental cost effectiveness ratio (*ICER**), given by:

$$ICER^{*} = \frac{\overline{C}^{*}_{LD}, \overline{C}^{*}_{PC}}{\overline{E}^{*}_{LD}, \overline{E}^{*}_{PC}} = \frac{\Delta \overline{C}^{*}}{\Delta \overline{E}^{*}}$$

Repeating the bootstrapping process R times yields the empirical sample distribution of the cost effectiveness ratio, with the estimated mean cost effectiveness ratio $\overline{ICER}^{*}$ (we took R=5000), see Figure 1. Using the bootstrap replicates, an acceptability curve is constructed. In figure 1, for example, the percentage of bootstrap replicates below a given willingness to pay (line R) corresponds to the probability of acceptance for that specific willingness to pay. An acceptability curve is generated by plotting the percentage of bootstrap replicates below the willingness to pay line R, when varied from nought (points below x-axis) to infinity (points right-hand side of y-axis). The acceptability curve is tending towards 1 minus the one sided p-value for the effect difference if the willingness to pay goes to infinity (y-axis). The 95% confidence interval for the cost-effectiveness ratio is determined by the 2.5% and the 97.5% probabilities of acceptance. Due to the precautionary principle it is unlikely that interventions with a negative impact on health will be implemented in blood transfusion medicine, regardless of the potential cost-savings. The acceptability curve can be adjusted for the precautionary principle by disregarding bootstrap replicates falling in the Southwest quadrant.

The willingness to accept monetary compensation for health losses is greater (higher selling price of a life-year lost) than the willingness to pay for health gains [19]. The willingness to pay for blood transfusion safety is very high and due to political and societal pressure the willingness to accept monetary compensation for health losses is likely to approach infinity. Therefore, in the sensitivity analysis cost-effectiveness acceptability curves were constructed regarding bootstrap replicates with health losses regarded as unacceptable. For the cost-effectiveness analysis the statistical package Splus was used.

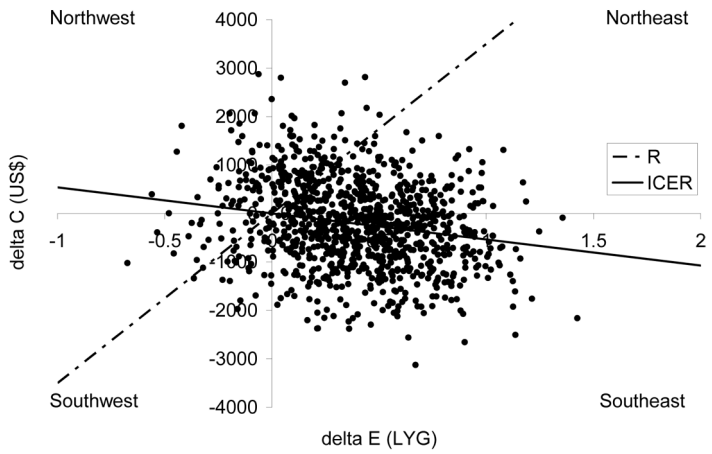

Figure 1 | Empirical sample distribution of the incremental cost-effectiveness ratio (ICER) for leukodepletion incremental to buffy coat depleted RBCs (1000 bootstrap replicates shown). For a willingness to pay of 3500 US$/LYG (line R) the percentage acceptability is the percentage of replicates below line R.

RESULTS

Clinical Trial

In total 496 patients were enrolled in the study. Twenty-two patients were excluded for various reasons: 8 patients had withdrawn consent before the surgery, 6 because surgery was cancelled, 3 because only CABG was performed, 3 had received blood transfusions in the past 3 months and 2 died before the operation. In each arm 237 patients were evaluable for the primary endpoints. Two patients (both randomized in the LD group) died during the initial operation. These two patients were ineligible for the analysis of the secondary endpoints. Three patients (all of them randomized in the PC group) died in a second operation (performed > 24 hours later), these patients remained in the study for the secondary endpoints. As shown in Table 2 both groups were comparable with respect to baseline characteristics, with an exception for the storage time of the erythrocytes. The majority (58.2%) of the patients received >4 units erythrocytes (mean ± SD: 6.1 ± 6.6). Forty-two (8.9%) of the patients did not receive any transfusion. Four (0.8 %) patients received both types of blood products, these patients remained in their original randomization arm.

Table 2 | Baseline Characteristics and Primary and Secondary Endpoints

	PC	LD	OR [95% CI]
Baseline characteristics			
Age, year	66.6 ± 12.5	65.3 ± 14.7	
Valve surgery + CABG	73 (30.8)	81 (34.2)	
Units erythrocytes transfused	6.2 ± 7.1	5.9 ± 6.1	
Storage time of the units, days	19.7 ± 5.4	17.4 ± 5.9*	
Endpoints			
Mortality at day 90 (primary endpoint)	30 (12.7)	20 (8.4)	1.52 [0.84 to 2.73]
In-hospital mortality	24 (10.1)	13 (5.5)	1.99 [0.99 to 4.00]*
Infections	75 (31.6)	53 (22.6)	1.64 [1.08 to 2.49]*
MODS†	49 (20.7)	48 (20.4)	1.07 [0.67 to 1.68]

*p≤0.05; values are numbers (%), †MODS indicates Multiple-Organ-Dysfunction-Syndrome.

As shown in Table 2, in total 50 (10.5%) patients died within the first 90 days post-operatively. Analyzed according to assignment in the PC group the total mortality at day 90 was higher than in the LD group (12.7% versus 8.4% respectively). This difference was not significant: OR=1.52; 95 % CI: 0.84-2.73, p=0.16. In the hospital 37 (7.8%) patients died, in the PC group the in-hospital mortality rate was almost twice as high as in the LD group (10.1% versus 5.5% respectively, OR=1.99, 95% CI: 0.99-4.00, p=0.05. In 128 of 472 patients (27.1%) in total 137 postoperative infections were diagnosed. In the PC group, 75 (31.6%) of 237 patients and in the LD group, 53 (22.6%) of 235 patients had infections (OR=1.64, 95% CI: 1.08-2.49, p=0.02). In total 97 (20.6%) patients developed MODS in the post-operative period. In both trial arms there was a similar incidence of MODS: PC: 20.7% versus LD: 20.4%. The duration of MODS in days (mean ± SD) was also not different in both groups (PC: 6.3 ± 8.8 versus LD: 6.1 ± 8.0, p=0.98). ICU-stay in the PC group was 5.6 ± 7.2 (mean ± SD) days and in the LD group 5.5 ± 7.3 days (p=0.88). The median ICU-stay in both groups was 3 days. The postoperative hospital stay was 13.8 ± 10.7 days and 13.3 ± 13.7 days in the PC and LD group respectively (p=0.66). The median duration of the hospital stay in both groups was 10 days.

Cost-Effectiveness Analysis

The remaining average life expectancies for the PC group and LD group utilizing 90 day post surgery survival rates are 11.4 and 10.6 year respectively, with a non-significant difference

(DE) of 0.8 life-years gained in baseline (see Table 3). Costs of care and blood products (all price levels) were consistently lower for the LD group compared to the PC group, Table 4. Only costs of antibiotics were on average slightly higher in LD group. Therefore, LD compared to PC displays negative net costs from the healthcare viewpoint (both the universal and the selective scenario), Table 3 and 4. However, the difference between the healthcare costs of PC and LD is not significant. Since LD compared to PC shows negative net cost in combination with positive health gains, LD is formally labelled as a cost-saving strategy and the formal calculation of the ICER point estimate is not necessary and non-informative.

Table 3 | Health Effects, Net-Costs and Cost-Effectiveness of Leukodepletion from the Healthcare Perspective

Survival and Health Effects (DE)		
PC	LD	ΔE
(life-years)	(life-years)	(LYG*) [95% CI]
10.6	11.4	0.8 [-0.27 to 1.84]
Net-costs (DC) and costs for PC and LD		
PC	LD	ΔC
(US$)	(US$)	(US$) [95% CI]
10163	9949	-214 [-1964 to 1536]
Cost-effectiveness LD incremental to PC (ΔC/ΔE)		
	Cost-saving†	

*LYG = life-years gained, †Confidence interval not defined

Table 4 | Mean Costs per Patient

Cost component*	**PC (US$)**	**LD (US$)**
ICU-care	5725	5643
Standard care	2610	2585
Antibiotics	58	70
Blood products (baseline; 20 US$ excess LD)	1770	1651
Blood products (Healthcare selective; 36 US$ excess LD)	1770	1745

From the empirical sample distribution of the ICER depicted in Figure 1 an acceptability curves is constructed, see Figure 2. Figure 2 demonstrates that leukodepletion has a probability of 59% to be cost-saving (no costs are accepted therefore the willingness to pay = 0, intercept of curve with y-axis). Furthermore, regardless of the costs of leukodepletion, i.e. the willingness to pay approaches infinity, the probability is solely governed by the clinical outcome in the clinical trial, and therefore the probability is tending towards 92.7%. The acceptability curve doesn't cross the 97.5% level of acceptability, therefore the health gains and net costs of LD are not significant different from PC for any given willingness to pay.

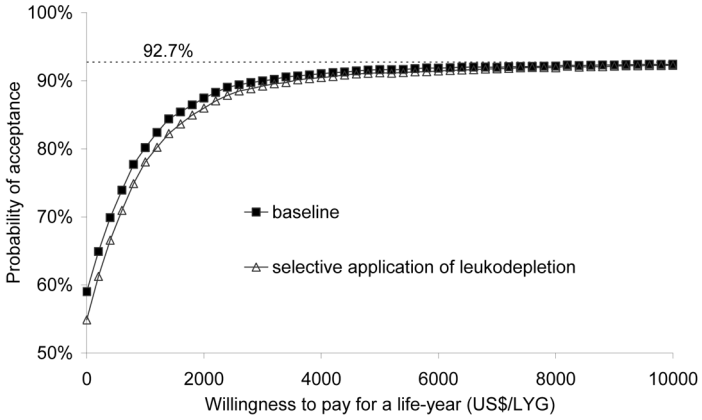

Figure 2 | Acceptability curve of leukodepletion constructed from 5000 bootstrap replicates. The 92.7% probability line represents the probability of acceptance when the willingness to pay for a life-year approaches infinity.

Sensitivity Analysis

Randomisation should ensure that there are no differences of patient characteristics in both treatment groups, however, non-significant differences in age and gender could have an effect on the longevity point estimation from gender and age specific life expectancies. Though no differences between age and gender are apparent in both groups (Table 2), adjusting for age and gender reduces the health gains observed for leukodepletion considerably, see table 5. The health gains are reduced from 0.8 life-years gained in baseline to 0.4 life-years gained after adjustment for age and gender differences. Still, the point estimate for the ICER remains cost-saving.

If leukodepletion is deployed selectively in cardiac surgery patients, the unit cost of leukodepletion rise compared to universal leukodepletion in our baseline (see above). Both the cost savings (Table 5) and therefore the probability of acceptance at a willingness to pay of 0 US$ (Figure 2) are less. LD remains a dominant intervention if the discount rate is raised to 5%, see Table 5.

Table 5 | Sensitivity Analysis: Health Effects, Net-Costs and Cost-Effectiveness of Leukodepletion for Different Scenarios

Scenario*	**Survival and Health Effects (ΔE) (3% discount rate)**		
	PC (life-years)	**LD (life-years)**	**ΔE (LYG†) [95% CI]**
Age and gender baseline adjusted	10.6	11.4	0.4 [-0.67 to 1.44]
[trial related mortality excluded]	[11.3]	[11.7]	
Healthcare perspective 0% discount rate	13.6	15.0	1.4 [-0.29 to 3.15]
Healthcare perspective 5% discount rate	9.0	9.6	0.5 [-0.25 to 1.35]
0 to 3 transfusions	12.8	13.2	0.5 [-1.01 to 1.95]
4 or more transfusions	8.9	10.2	1.4 [-0.02 to 2.75]
	Net-costs (ΔC) and costs for PC and LD (3% discount rate)		
	PC (US$)	**LD (US$)**	**ΔC (US$) [95% CI]**
Age and gender baseline adjustment	10163	9949	-214 [-1964 to 1536]
Healthcare, selective (36 US$ excess costs LD)	10163	10042	-121 [-1877 to 1636]
Patients with 0 to 3 transfusions	6000	5747	-253 [-960 to 455]
Patients with 4 or more transfusions	13532	12615	-917 [-3667 to 1834]
	Cost-effectiveness (US$/LYG†) LD incremental to PC		
Age and gender baseline adjustment	Cost-saving‡		
Healthcare 0% and 5% discount rate	Cost-saving‡		
Healthcare, selective	Cost-saving‡		
Patients with 0 to 3 transfusions	Cost-saving‡		
Patients with 4 or more transfusions	Cost-saving [cost-saving to 4300]		

*Discount rate 3% and excess costs of leukodepletion US$20, except where stated, †LYG = life-years gained,‡ Confidence interval not defined

Analysis of cost-effectiveness for different levels of blood transfusions received reveals that patients receiving 4 or more transfusions benefit more (increased cost savings and health gains) from leukodepletion. Still, in both scenarios LD is dominant to PC. Acceptability curves of LD for different levels of blood transfused received shows that only the curve of patients receiving 4 or more transfusion crosses the 97.5% level of significance, see Figure 3. The confidence interval for the cost-effectiveness for leukodepletion in patients receiving 4 or more transfusions stretches from dominant to 4300 US$ per life-year gained (97.5 percentile). Leukodepletion in patients receiving less than 4 transfusions seems to have a higher probability of acceptance compared to the baseline in a willingness to pay range of 0 to 1000 US$ per life-year gained. However, constructing acceptability curves from only the bootstrap replicates with a positive health gain, shows that the maximum probability of acceptance of leukodepletion in patients receiving 0 to 3 transfusions is reduced from 83% to 60%. Disregarding bootstrap replicates with health losses has little effect on the baseline acceptability curve and the acceptability curve of leukodepletion for patients receiving 4 or more transfusions [19].

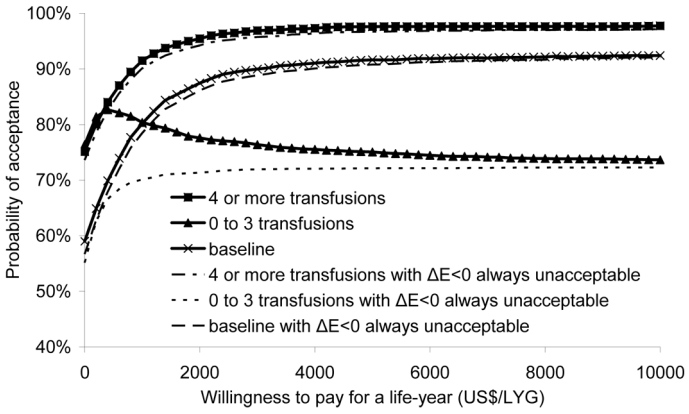

Figure 3 | Acceptability curves of leukodepletion for different levels of erythrocyte transfusions constructed from 5000 bootstrap replicates. Effect of regarding bootstrap replicates with loss of health ($\Delta E<0$) and cost savings ($\Delta C<0$) unacceptable, is shown.

DISCUSSION

Leukodepleted erythrocytes over standard buffy-coat-depleted packed cells is a cost-saving intervention in patients undergoing valve surgery with or without CABG from the healthcare perspective. However, the average cost-saving of US$214 per patient failed to show significance in the acceptability analysis. It was expected that the cost of care would be less in the LD group, since there is a significant difference between the PC and LD group in the risk of post-operative infections. All costs are lower in the LD group, except for the costs of antibiotics due to the prophylaxis in both groups. Health gains of LD incremental to PC are not significant and are further reduced when adjusted for age and gender differences. However, the achieved health gains are in the range of the increase in life expectancy by CABG compared to medical therapy over 10-year follow up in patients with three vessel disease (5.7 months) [20,21]. Moreover, the estimated health gains greatly surpass the health gains estimated for implemented procedures in blood banking such as HIV NAT screening which yielded on average 16 hours per patient [22]. The threshold for pharmaco-economic acceptability has been published at US$50,000 per QALY gained in the USA [23]. However, the pharmaco-economic acceptance criteria and thresholds differ between health-care settings, societies and interventions. For instance, cost-effectiveness ratios in health care of approximately US$100,000 to US$1,000,000 per life-year gained are readily accepted for transplantation and intensive care settings in the developed world [24-26]. Most of the procedures to minimize allogeneic transfusion risks did not show cost-utility ratios below the current US$50,000 per QALY gained threshold for economic acceptability and were up to several million US$ per QALY or life-year gained for solvent detergent treatment of plasma, nucleic acid amplification testing and HIV p24 antigen testing [27]. In the available meta-analyses cardiac surgery is the only clinical setting where leukodepletion has shown health benefits. The result of this analysis is only applicable for valve surgery patients (with or without CABG) and can't be extended to other clinical settings. Pending additional randomised clinical trials, leukodepletion may be less cost-effective in other clinical settings. The higher excess costs for selective leukodepletion only in cardiac surgery patients reduces only the probability of being cost-saving compared to universal leukodepletion. Leukodepletion in patients receiving 4 or more transfusion is more likely to be accepted than in baseline (all patients) or patients receiving less than 4 transfusions. This outcome is also supported by the conclusion of the Canadian Co-ordinating Office for Health Technology Assessment that leukodepletion could be cost-effective for patients receiving many transfusions [10]. In patients receiving less than 4 transfusions bootstrap replicates with cost savings and health

losses are important drivers for a high probability of acceptance, though being less preferable from the societal and political perspective. Disregarding bootstrap replicates with negative health effects had little effect on the baseline acceptability curve and the acceptability of leukodepletion in patients receiving 4 or more transfusions but decreased the probability of acceptance substantially for patients receiving less than 4 transfusions.

Performing cost-effectiveness analysis alongside a clinical trial is a relatively young method under development in pharmaco-economics [17,18]. As with this clinical trial, clinical trials are unfortunately designed solely to determine clinical effects and not specifically designed for evaluating cost-effectiveness. Therefore, in the design of this trial, variability in cost-data was not taken in to account in the power determination. This economic analysis was performed from the healthcare perspective, therefore only direct costs were considered. The preferred societal perspective includes indirect as well as direct costs. However, the impact of in-direct costs, such as productivity and leisure losses, would be limited in this analysis because of the relatively advanced age (mean 65 years) of the included patients and the severity of the underlying disease.

Governed by society's perception of blood transfusion risks [28], governments seem to be willing to allocate significant budgets to improve transfusion safety. However, we have to keep in mind that if health budgets are fixed, allocation of budgets to less favourable cost-effective strategies in healthcare, neglects potential other more cost-effective strategies with higher health gains. Leukodepleted erythrocytes applied in cardiac valve surgery with or without CABG compared to standard buffy-coat-depleted erythrocytes showed a (non-significant) moderate favourable economic profile. More randomised clinical trials powered for health economics are needed to underpin or reject the suggested favourable economic profile.

ACKNOWLEDGEMENTS

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Chapter 8

Postoperative Complications Associated with Transfusion of Platelets and Plasma in Cardiac Surgery

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ABSTRACT

Background: Studies in cardiac surgery have reported increased postoperative morbidity and mortality after allogeneic red blood cell (RBC) transfusions. Whether platelet and/or plasma transfusions are a marker for more concomitant RBC transfusions or are independently associated with complications after cardiac surgery is unknown.

Study design and methods: Data from two randomized controlled studies were combined to analyze the effects of platelet and/or plasma transfusions on postoperative infections, length of stay in the intensive care unit (ICU), all-cause mortality and mortality in the presence or absence of infections in the postoperative period.

Results: After adjusting for confounding factors, plasma units and not RBC transfusions were associated with all-cause mortality. Leukocyte-containing RBC transfusions and platelet transfusions were associated with mortality occurring in the presence of or after infections. Number of (leukocyte-containing) RBC transfusions was also significantly associated with postoperative infections and with ICU-stay for 4 or more days.

Conclusion: Although it is difficult to separate the effects of blood components, we found that in cardiac surgery peroperative plasma transfusions are independently associated with all-cause mortality. Leukocyte-containing RBC transfusions and platelet transfusions are independently associated with mortality in the presence of infections in the postoperative period. Future transfusion studies in cardiac surgery should concomitantly consider the possible adverse effects of all the various transfused blood components.

INTRODUCTION

Patients undergoing cardiac surgery are at increased risk for bleeding, because of thrombocytopenia secondary to hemodilution, platelet dysfunction and consumption of platelets in the extracorporal circuit. In addition, intra-operatively anticoagulant medication is administered to these patients. To improve hemostasis, platelets and fresh-frozen plasma (FFP) are often transfused in the peri-operative and postoperative periods. However, neither the efficacy nor the safety of platelet and plasma transfusions has been demonstrated.

Retrospective studies in cardiac surgery have shown that allogeneic red blood cell (RBC) transfusions are associated with an increased risk of postoperative infections and mortality in a dose-dependent manner [1-4]. In two randomized controlled trials (RCTs) of patients undergoing cardiac surgery [5,6], we found an increase in postoperative infections and mortality related to the number of transfused RBCs. Postoperative infection and mortality were significantly higher in patients receiving buffy-coat-depleted (BCD) RBCs than in patients receiving leukocyte-reduced (LR; filtered) RBCs, which suggested a role of allogeneic leukocytes in provoking postoperative infections and mortality. However, patients receiving multiple RBC transfusions often receive platelets and plasma transfusions as well. Whether platelet and plasma transfusions contribute to such postoperative complications or are just a surrogate marker for the need for a higher number of RBC transfusions is unknown. Previous retrospective studies yielded conflicting results concerning the association between platelet and plasma transfusions and outcome in cardiac surgery [7-13]. The purpose of this current analysis of the combined data from our two RCTs [5,6], is to investigate whether transfusion of platelet concentrates and FFP are independently associated with postoperative infection and mortality in cardiac surgery, after adjusting for the effect of the number of RBC transfusions and other confounders.

MATERIAL AND METHODS

Our two double-blinded RCTs had been conducted at two university hospitals in the Netherlands. The ethical review boards of the hospitals approved the trial protocols and informed consent was obtained from the patients. The design of these studies have been reported elsewhere in detail [5,6]. In summary, the first study [5] was a single-center study conducted between 1992 and 1994. Patients undergoing coronary-artery-bypass-graft (CABG) or cardiac valve surgery or a combination of both, were randomised to receive,

when transfusion was necessary, BCD RBCs or LR RBCs (randomly assigned to receive either before storage from freshly drawn RBCs or filtered after storage before transfusion RBCs). For the current analysis we included only the patients who had received prestorage LR RBCs (n=305) and those who had received BCD RBCs (n=306), because these were the randomization arms also transfused in the second study. The second study [6] was conducted between 1999 and 2001 at two hospitals and included patients undergoing valve surgery with or without CABG. In this study BCD RBCs (n=237) were compared with pre-storage LR RBCs (n=237). In both studies oral anticoagulants and aspirin were stopped at least 5 days before surgery. Heparin (3 mg/kg) was administered before initiation of bypass with a target activated clotting time of 400 seconds. The priming of the cardiopulmonary bypass circuit was comparable in both studies. After termination of bypass, heparin was antagonized by protamine sulphate at a 1:1 ratio. In both studies, all patients received prophylactic antibiotics postoperatively for 24 hours (for subjects undergoing CABG) or 48 hours (for subjects undergoing valve surgery). In the second study, only patients considered to be at high risk for bleeding received aprotinin at one hospital. Postoperatively, all patients were admitted to the intensive care unit (ICU) until they had been extubated and had no longer needed positive inotropes. In one hospital, in the second study, patients without complications were transferred to a medium care unit before they were transferred to the department of cardiothoracic surgery.

Blood Products and Transfusions

The blood products in both studies were similar and fulfilled the requirements and the specifications of the Dutch standards for Blood Banks. Platelet concentrates were prepared from five pooled buffy coats and were prestorage LR by filtration. BCD RBCs were prepared by removal of the buffy coat and plasma, followed by reconstitution with 100 mL saline-adenine-glucose-mannitol. Prestorage filtration of RBC units was performed within 24 hours after whole-blood collection, by passage through a leuko-reduction filter (Cellselect-Optima, NPBI International-Fresenius HemoCare, the Netherlands). FFP was prepared by separation from whole donor-blood by hard spin and freezing within 6 hours at < -23°C. Women with a history of pregnancy were excluded from plasma donations, except in one hospital in the second study. In both studies the precise number of transfused RBCs, plasma units and platelet concentrates was recorded. At the time of both studies no documented guidelines for blood transfusions were present. The decision to transfuse blood products was based on the hemoglobin level (less than 8-8.5 gr/dl), platelet count (less than 100 x 10^9/l), total amount of blood loss and/or presence of bleeding disorders.

Endpoints

In both studies, postoperative infections had been a secondary endpoint based on the criteria of Centers for Disease Control and Prevention [14] and were scored during the hospital stay of the patients. In both studies, the length of stay in the ICU had been recorded in days. Because respiratory failure was not documented in the first study, length of ICU-stay for at least 4 days was considered to indicate need for prolonged mechanical ventilation. In the first study, mortality was a secondary endpoint and it was registered until day 60 postoperatively. In the second study mortality at 90 days postoperatively was the primary endpoint, although 60-days mortality was also recorded.

Statistical Analysis

Breslow-Day and Tarone's tests were used to examine whether the data from two RCTs were sufficient homogeneous to permit combined analyses of both studies. Data were expressed as mean ± SD, number or percentage as appropriate. For comparison of qualitative parameters, the Fisher's exact test or chi-square test was used and for the comparison of quantitative parameters, the t-test or Mann-Whitney *U* test. To estimate risk factors for postoperative infection, ICU-stay for 4 or more days, overall mortality and mortality occurring in the presence or in the absence of postoperative infections known variables associated with these postoperative complications were included in a univariate analysis. Multivariate analysis of the risk factors was performed using a logistic regression enter/backward stepwise model to estimate independent predictors for postoperative complications. The following variables were eligible for inclusion in this model: study (our 1992-1994 versus our 1999-2001 RCT), age, gender, type of surgery (CABG, valve or the combination of both), cardiopulmonary bypass time, number of transfused RBC's, randomization arm (BCD vs. LR RBCs) and number of transfused plasma units and number of transfused platelet units. Time on cardiopulmonary bypass was analyzed as a categorical variable (in hr). The number of transfused RBC units, transfused plasma units and transfused platelet units were forced into the multivariate analysis. The results of the univariate analysis are reported as p values and the results of the multivariate analysis as odds ratios (ORs) with 95% confidence intervals (CIs). All p values are two tailed. Analyses were performed using the SPSS 17.0 (SPSS Inc, Chicago IL, USA).

RESULTS

The data from the two studies comprised 1.085 patients; 611 from the first study and 474 from the second study. Testing for homogeneity indicated that it was legitimate to pool the data from both studies. Only ICU-stay was different between both studies which was due to the presence of a medium care in one hospital in the second study. In the combined population of 1.085 patients; 316 patients (29.1%) had developed postoperative infection and 80 patients (7.4%) had died. Of the patients who died, 41 patients (51.3%) died who had developed infections in the postoperative period, while 27 patients (33.8%) died from a cardiac cause without any postoperative infection. In total 12 patients (15.0%) died from other reasons (e.g., bleeding or multiple-organ-dysfunction-syndrome) without any postoperative infection.

In Table 1 the attributes of the patients are presented who developed postoperative infections and of the patients who died. Compared with patients who did not develop infections or patients who survived, patients who developed infections and patients who died were older, more often female; they had a longer duration of surgery and received more units RBCs and plasma and they had received platelet transfusion more often. The patients who received plasma and/or platelet transfusions were older, had a longer duration of surgery, and had received more RBC transfusions than patients who did not receive plasma or platelet transfusions. More infections and more deaths were observed in patients who received plasma or platelet transfusions (Table 2). As shown in Figures 1 and 2 patients who received more RBC units also received more plasma units (of 615 patients receiving 4 or more units RBCs, 403 patients [65.5%] had received 4 or more units plasma units as well) and patients who received more RBC units also received platelet transfusion more often (of 615 patients receiving 4 or more units RBCs, 233 patients [37.9%] had received also platelet transfusions).

Table 3 shows the results of univariate analyses concerning risk factors for the development of postoperative infection, length of ICU-stay at least 4 days, overall mortality, mortality in the presence (and absence) of infections in the postoperative period. Multivariate analysis showed that study, age, and number of RBC units transfused were associated with both postoperative infection and ICU-stay for at least 4 days. In addition to these factors, randomization arm and sex were associated with postoperative infections and the time on cardiopulmonary bypass with ICU-stay for at least 4 days (Table 4). Plasma or platelet transfusions were not associated with postoperative infections or ICU-stay for at least 4 days. All-cause mortality was associated with age, time on cardiopulmonary bypass,

Table 1 | Attributes of All Patients and Patients Developing Postoperative Infections and Patients who died in the Hospital

Attributes	Patients without infections N=769	Patients with infections N=316	p	Patients who survived N=1005	Patients who died N=80	p
First study (%)	421 (54.7)	190 (60.1)	0.10	578	35 (43.8)	0.01
Female (%)	246 (32.0)	134 (42.4)	0.001	338	42 (52.5)	0.001
Age (years)	63.8 ± 11.9	67.9 ± 10.1	<0.001	64.5 ± 11.6	70.8 ± 9.0	<0.001
Type of surgery (%)						
CABG	324 (42.1)	135 (42.7)	0.19	440	19 (23.7)	<0.001
Valve	313 (40.7)	114 (36.1)	0.20	396	31 (38.7)	0.55
CABG + valve	132 (17.2)	67 (21.2)	0.48	169	30 (37.5)	<0.001
Preop. Aspirin (%)	226 (29.4)	87 (27.5)	0.55	290	23 (28.7)	>0.90
Preop. Anticoagulans (%)	236 (30.7)	115 (36.4)	0.07	330	21 (26.2)	0.26
Preop. Heparin (%)	15 (2.0)	6 (1.9)	>0.90	21	0	0.39
Aprotinin use (%)	135 (17.6)	39 (12.3)	0.11	159	15 (18.8)	0.74
Cardiopulmonary bypass time (min)	128 ± 50	143 ± 62	<0.001	129 ± 52	173 ± 70	<0.001
Aortic clamping time (min)	78 ± 39	86 ± 44	0.003	79 ± 40	100 ± 46	<0.001
No. RBC transfusions						
Mean ± SD	4.5 ± 4.1	8.5 ± 8.1	<0.001	5.1 ± 4.8	13.2 ± 10.7	<0.001
Units of RBCs (%)						
0	69 (9.0)	5 (1.6)		73	1 (1.2)	
1-3	333 (43.3)	67 (21.2)	<0.001	390	10 (12.5)	<0.001
≥4	367 (47.7)	244 (77.2)	<0.001	542	69 (86.2)	<0.001
Randomization arm (%)						
BCD	357 (46.4)	186 (58.9)	<0.001	492	51 (63.7)	<0.001
LR	412 (53.6)	130 (41.1)		513	29 (36.2)	
Plasma transfusions (%)	654	301 (95.2)	<0.001	877	78 (97.5)	0.004

Attributes	Patients without infections N=769	Patients with infections N=316	p	Patients who survived N=1005	Patients who died N=80	p
No. plasma transfusions						
Mean ± SD	3.4 ± 3.2	5.9 ± 6.3	<0.001	3.6 ± 3.3	10.5 ± 9.8	<0.001
Units of plasma (%)						
0	115 (15.0)	15 (4.7)		128	2 (2.5)	
1-3	340 (44.2)	98 (31.0)	<0.001	422	16 (20.0)	<0.001
≥4	314 (40.8)	203 (64.2)	<0.001	455	62 (77.5)	<0.001
Platelet transfusions (%)	177 (23.0)	120 (38.0)	<0.001	242	55 (68.8)	<0.001
Mean ± SD	0.4 ± 0.9	0.9 ± 1.9	<0.001	0.4 ± 0.9	2.3 ± 3.5	<0.001
0	592 (77.0)	196 (62.0)	<0.001	763	25 (31.3)	<0.001
1	118 (15.3)	59 (18.7)	0.18	156	21 (26.2)	0.02
≥2	59 (7.7)	61 (19.3)	<0.001	86	34 (42.5)	<0.001

*For patients with or without infections or who died and survived. RBC=Red blood cells, BCD=Buffy-coat depleted, LR=Leukocyte-depleted

Table 2 | Attributes of Patients Transfused (Versus not Transfused) with Platelets or Plasma

Attributes	Patients with platelet transfusions N=297	Patients without platelet transfusions N=788	p	Patients with plasma transfusions N=955	Patients without plasma transfusions N=130	p
First study (%)	57 (19.2)	554 (69.0)	<0.001	608 (63.6)	3 (2.3)	<0.001
Female (%)	133 (44.8)	247 (31.3)	<0.001	324 (33.9)	56 (43.1)	0.05
Age Mean ± SD (yrs)	68.0 ± 11.7	63.9 ± 11.3	<0.001	65.5 ± 11.1	61.5 ± 14.3	< 0.001
Type of surgery (%)						
CABG	34 (11.4)	425 (53.9)	<0.001	457 (47.8)	2 (1.5)	<0.001
Valve	152 (51.2)	275 (34.9)	<0.001	325 (34.0)	102 (78.5)	<0.001
CABG + valve	111 (37.4)	88 (11.2)	<0.001	173 (18.1)	26 (20.0)	<0.001
Preop. Aspirin (%)	80 (26.9)	233 (29.6)	0.41	286 (29.9)	27 (20.8)	0.03
Preop. Anticoagulans (%)	88 (29.9)	263 (33.4)	0.25	319 (33.4)	32 (24.6)	0.04
Preop. Heparin (%)	7 (2.3)	14 (1.8)	0.62	17 (14.6)	4 (3.1)	0.30
Aprotonin use (%)	82 (27.6)	92 (11.7)	0.22	124 (13.0)	50 (38.5)	0.52
Cardiopulmonary bypass time (min)	166 ± 66	120 ± 43	<0.001	135 ± 56	112 ± 38	<0.001
Aortic clamping time (min)	107 ± 46	70 ± 33	<0.001	80 ± 34	80 ± 42	0.84
No. RBC transfusions, Mean ± SD	9.9 ± 8.5	4.1 ± 3.3	<0.001	6.1 ± 6.0	2.3 ± 2.2	<0.001
Units of RBC (%)						
0	3 (1.0)	71 (9.0)	<0.001	44 (4.6)	30 (23.1)	<0.001
1-3	59 (19.9)	341 (43.3)	<0.001	332 (34.8)	68 (52.3)	<0.001
≥4	235 (79.1)	376 (47.7)	<0.001	579 (60.6)	32 (24.6)	<0.001
Randomization arm (%)						
BCD	152 (51.2)	391 (49.6)	0.68	486 (50.9)	57 (43.8)	0.14
LR	145 (48.8)	397 (50.4)		469 (49.1)	73 (56.2)	

Attributes	Patients with platelet transfusions N=297	Patients without platelet transfusions N=788	p	Patients with plasma transfusions N=955	Patients without plasma transfusions N=130	p
Infections (%)	120 (40.4)	196 (24.9)	<0.001	301 (31.5)	15 (11.5)	<0.001
Respiratory tract infections (%)	65 (21.9)	89 (11.3)	<0.001	144 (15.1)	10 (7.7)	0.02
ICU-stay ≥4 days (%)	169 (56.9)	183 (23.2)	<0.001	323 (33.8)	29 (22.3)	0.009
Mortality (%)	55 (18.5)	25 (3.2)	<0.001	78 (8.1)	2 (1.5)	0.004
Mortality with infections(%)	33 (11.1)	9 (1.1)	<0.001	41 (4.3)	1 (0.8)	0.05
Mortality without infections (%)	22 (7.4)	16 (2.0)	<0.001	37 (3.9)	1 (0.8)	0.07

RBC=Red blood cells, BCD=Buffy-coat depleted, LR=Leukocyte-depleted

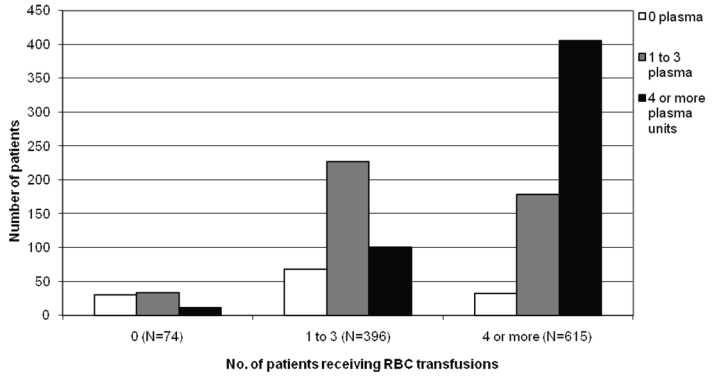

Figure 1 | Distribution of number of patients receiving red blood cell (RBCs) transfusions (0,1-3 or ≥4 units) with plasma transfusions (0,1-3 or ≥4 units).

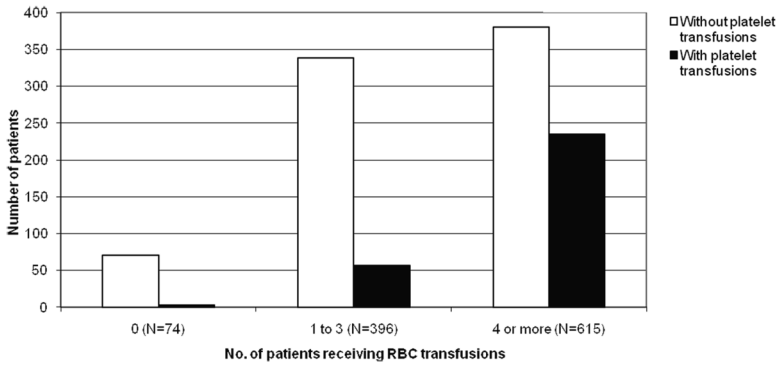

Figure 2 | Distribution of number of patients receiving red blood cell (RBCs) transfusions RBCs (0,1-3 or ≥ 4 units) with (or without) platelet transfusions.

Table 3 | Risk Factors for Postoperative Infections, ICU-stay ≥4 days, Overall Mortality and Deaths occurring in the Presence (or Absence) of Infections Based on Univariate Analyses

Risk factors	Infections	ICU-stay ≥4 days	Mortality	Mortality with infection	Mortality without infection
Study (first/second)	<0.001	<0.001	<0.001	<0.001	<0.001
Age	<0.001	<0.001	<0.001	<0.001	0.06
Gender (male/female)	0.001	<0.001	0.003	0.04	0.03
Type of surgery (CABG/valve/both)	0.39	<0.001	<0.001	<0.001	0.02
Cardiopulmonary bypass	<0.001	<0.001	<0.001	<0.001	<0.001
Number of RBC transfusions	<0.001	<0.001	<0.001	<0.001	0.003
Randomization (BCD/LR)	<0.001	0.41	0.004	0.005	0.24
Platelet transfusions	<0.001	<0.001	<0.001	<0.001	<0.001
Number of plasma transfusions	<0.001	<0.001	<0.001	<0.001	<0.001

RBC=Red blood cells, BCD=Buffy-coat depleted, LR=Leukocyte-depleted

Table 4 | Results of Multivariate Analyses for Postoperative Infections and ICU-stay for ≥4 days.

Risk factors	Infections			ICU-stay ≥4 Days		
	MVA OR	MVA 95% CI	p	MVA OR	MVA 95% CI	p
Study (first/second)	0.62	0.41-0.93	0.02	1.81	1.20-2.73	0.01
Age (years)	1.03	1.01-1.04	<0.001	1.02	1.01-1.03	0.01
Gender (male/female)	1.43	1.06-1.93	0.02	1.18	0.86-1.60	0.30
Type of surgery	0.97	0.75-1.25	0.80	1.16	0.89-1.52	0.27
Cardiopulmonary bypass time (hours)	1.08	0.91-1.28	0.38	1.21	1.01-1.44	0.04
Number of RBC transfusions	1.12	1.07-1.17	<0.001	1.21	1.15-1.28	<0.001
Randomization arm (BCD/LD)	1.67	1.25-2.23	<0.001	0.86	0.64-1.15	0.30
Number of platelet transfusions	1.05	0.89-1.24	0.54	1.16	0.76-1.76	0.50
Number of plasma transfusions	1.01	0.96-1.07	0.61	0.99	0.94-1.06	0.94

RBC=Red blood cells, BCD=Buffy-coat depleted, LR=Leukocyte-depleted

plasma transfusions, platelet transfusions and randomization arm, but not with the number of RBC units transfused (Table 5). Randomization arm, age, platelet transfusions and plasma transfusions were associated with deaths occurring in the presence of infections in the postoperative period. Time on cardiopulmonary bypass and number of transfused plasma units were associated with deaths without postoperative infections (Table 5).

Table 5 | Results of Multivariate Analyses for Overall Mortality and Deaths occurring in the Presence (or Absence) of Infections

	Mortality			Mortality with infection			Mortality without infection		
	MVA OR	MVA 95% CI	p	MVA OR	MVA 95% C I	p	MVA OR	MVA 95% CI	p
Study (first/second)	0.61	0.31-1.22	0.16	0.66	0.26-1.64	0.37	0.60	0.24-1.49	0.27
Age (Years)	1.05	1.02-1.08	0.003	1.07	1.02-1.11	0.01	1.02	0.99-1.06	0.18
Gender (male/female)	1.55	0.90-2.66	0.12	1.41	0.67-2.94	0.37	1.87	0.90-3.88	0.10
Type of surgery (CABG/Valve/Both)	1.30	0.86-1.98	0.22	1.31	0.75-2.29	0.34	1.26	0.73-2.19	0.41
Cardiopulmonary bypass time (hours)	1.39	1.08-1.78	0.01	1.15	0.82-1.61	0.42	1.80	1.34-2.43	<0.001
Number of RBC transfusions	0.99	0.94-1.06	0.84	1.05	0.98-1.12	0.15	0.93	0.84-1.02	0.11
Randomization arm (BCD/LD)	1.80	1.04-3.13	0.04	2.12	1.01-4.58	0.05	1.36	0.70-2.77	0.40
Number of platelet transfusions	1.37	1.12-1.68	0.002	1.29	1.03-1.61	0.03	1.14	0.93-1.41	0.22
Number of plasma transfusions	1.14	1.06-1.23	<0.001	1.11	1.01-1.21	0.02	1.10	1.01-1.21	0.04

RBC=Red blood cells, BCD=Buffy-coat depleted, LR=Leukocyte-depleted

DISCUSSION

In this retrospective analysis of data from two randomized controlled trials [5,6], the number of transfused plasma units was independently associated with all-cause mortality. Although leukocyte-containing RBCs were associated with mortality, the number of transfused RBC units was not. The number of transfused RBC units, but not the number of transfused plasma units or the receipt of platelet transfusion, was associated with the development of postoperative infections and with the stay in the ICU for at least 4 days. Transfusion of platelet units was associated with mortality with postoperative infections developed during the hospital-stay. Because patients who receive RBC transfusions, receive also plasma and platelet transfusions, it is difficult to determine whether plasma and platelet transfusions could be independently associated with postoperative complications.

Our results are consistent with those of several previous studies which reported that infections after cardiac surgery are associated (in a dose-dependent manner) with the number of RBC transfusions [1-4]. We previously reported that the excess mortality secondary to leukocyte-containing RBC transfusions was due to higher mortality associated with postoperative infections [15]. To identify other risk factors (or confounders) than RBCs, in the current analysis we distinguished between deaths occurring in the presence or absence of infections in the postoperative period. Because in our first RCT [5] organ failure was not recorded, in this analysis we used ICU-stay for at least 4 days as parameter for prolonged mechanical ventilation (and thus for respiratory failure). In practice, after extubation almost all patients were transferred from the ICU to the ward within 24 hours. In agreement with other studies [16,17], we found in the multivariate analysis that the most important predictors of longer ICU-stay were the number of transfused RBCs and the patient's age. However, in contrast to others [18-21], we found no association between prolonged mechanical ventilation and plasma or platelet transfusions.

The finding that plasma transfusions are associated with all-cause mortality, while leukocyte-containing RBC transfusions and platelet transfusions are associated only with deaths with postoperative infections in the postoperative period, were unanticipated. A predominant role of plasma transfusions on outcome after cardiac surgery is consistent with the results of Ranucci et al [22]. Other studies that focused on plasma transfusions reported contradictory findings [7-9]. Similarly platelet transfusions were reported not to be associated with mortality [10,11], except in two studies that found an association with platelet transfusions and mortality in cardiac surgery; however, these studies applied no corrections for concomitant RBC or plasma transfusions [12,13].

Plasma-containing blood products have been implicated in the pathogenesis of transfusion-related acute lung injury (TRALI). Patients undergoing cardiac surgery are at higher risk to develop TRALI, even if leukocyte-reactive antibodies in transfused plasma are absent [23,24]. However, TRALI seems unlikely as a cause of enhanced mortality because parameter for respiratory failure (prolonged ICU-stay) was not associated with plasma transfusion in our study. On the other hand, in a laboratory analysis we found in patients with low mannose-binding lectin (MBL) levels is a risk factor in the development of multiple-organ-dysfunction-syndrome (MODS), which may contribute to mortality [25]. Besides allogeneic leukocytes in RBC products inducing higher pro-inflammatory cytokine levels after cardiac surgery associated with more postoperative infections and mortality [26], platelet units also contain bioactive mediators. Increased CD40 ligand (CD40L or CD154) present in platelet units can induce production and release of proinflammatory markers [27,28]. Both leukocyte-containing RBCs and platelet transfusions could thus aggravate an existing inflammatory reaction impairing the outcome after cardiac surgery. More investigations are needed on the possible causal roles of transfusion of different blood components.

This study has several limitations. First, this is an observational analysis, despite the fact that the data were extracted from two RCTs. Second, the combined studies had not been designed to investigate the effects of plasma and platelet transfusions. Evidence-based transfusion triggers for platelet and plasma transfusions had not been used in either of our RCTs. Instead, plasma and platelet transfusions were administered based on institutional habits and the preferences of the clinicians. In both studies, also information on blood loss was not adequately documented. Aprotinin to reduce the risk of bleeding was administered only in the second study and only for selected patients (16% of the current population). In an earlier study we found no effect of aprotinin in the development of postoperative complications [26]. Therefore, the effects of aprotinin were not considered in this analysis. Finally, our two RCTs had been performed 7 years apart. Between the time periods that the two studies were conducted, surgical procedures and transfusion practices has changed, as reflected in the difference in length of ICU-stay between the two studies.

Nowadays, more restrictive criteria for RBC transfusion are used. In the periods when our studies were performed no clear consensus on the indications for plasma and platelet transfusions in these patient populations was present. Nevertheless despite guidelines for blood transfusions, the patients that receive perioperative blood transfusions in cardiac surgery is still high [29]. As the use of antiplatelet agents increases, we expect that more patients will receive blood transfusions in the future. In conclusion, we found that the number of RBC transfusions was not associated with postoperative mortality, whereas plasma transfusions

were dose-dependently associated with all-cause mortality. The transfusion of platelets was associated with mortality in the presence of (leukocyte-containing) RBCs. However, only few retrospective studies have considered the effects of plasma and platelet transfusions, which predominantly are transfused to patients who also received RBC transfusions. Our findings underscore the need for further studies to investigate the aggregate effects of all the various blood components transfused in cardiac surgery.

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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 pure 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

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.

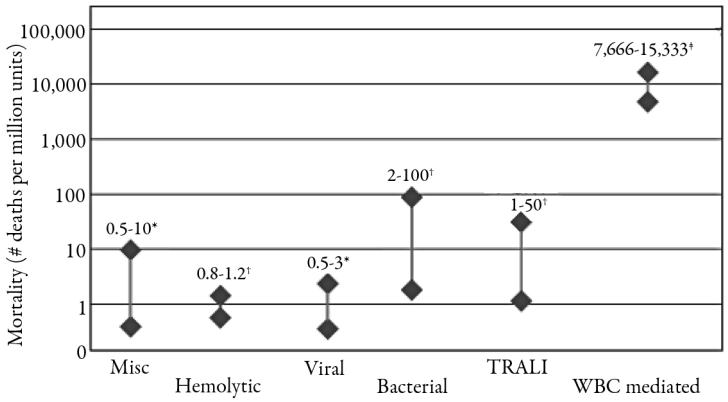

Figure 1 | Estimates of risk of death per unit transfused RBCs for several transfusion-related complications in patients undergoing cardiac surgery. (with permission, from Despotis et al. Transfusion 2008; 48:2S-30S)

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.

Author; year	No. patients (% transfused)	No. RBCs mean ± SD or median	Main endpoints	Results	p values[b]
van de Watering et al; 1998 [9]	914/ 866 (95)	FF 5.3 ± 4.1	1) Infections	1) 16.9 vs 17.9 vs 23.0%	1) 0.13
		SF 5.5 ± 5.6	2) 60-day mortality	2) 3.6 vs 3.3 vs 7.8%	2) 0.01
		BCD 5.4 ± 5.1			
Bracey et al; 2002 [11][a]	357/ 295 (83)	LD 3	1) Infections	1) ns; data ND	1) ns; data ND
		BCD 3	2) Mortality	2) 5.9 vs 7.5%	2) ns; data ND
			3) ICU-/Hospital-stay	3) ns; data ND.	3) ns; data ND
Wallis et al; 2002 [12]	597/ 409 (69)	WBF 3.9 ± 3.9	1) Infections	1) 11.3 vs 10.8 vs 17.7%	1) 0.1
		BCD 3.5 ± 2.6	2) 90-day mortality	2) 0.5 vs 2.9 vs 2.5%	2) 0.2
		PR 2.9 ± 1.8			
Bilgin et al; 2004 [Chapter 2]	474/ 432 (91)	LD 6.2 ± 7.1	1) Infections	1) 22.6 vs 31.6%	1) 0.02
		BCD 5.9 ± 6.1	2) MODS	2) 20.4 vs 20.7%	2) 0.98
			3) Hospital mortality	3) 5.5 vs 10.1%	3) 0.05
			4) 90-day mortality	4) 8.4 vs 12.7%	4) 0.16
Connery et al; 2005 [13]	98/ 69 (70)	LD 5.6 ± 13	1) Infections	1) 13.2 vs 25.8%	1) 0.22
		BCD 5.6 ± 10	2) 30-day mortality	2) 2.6 vs 3.2%	2) 1.0
Boshkov et al; 2006 [14][a]	1227/ 562 (46)	ND	1) Serious infections	1) ns; data ND	1) ns; data ND
			2) 60-day mortality	2) 4.9 vs 9.7%	2) 0.36

[a]Available only as abstract, [b]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 extra-corporeal 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 4×10^9/l or above 12×10^9/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).

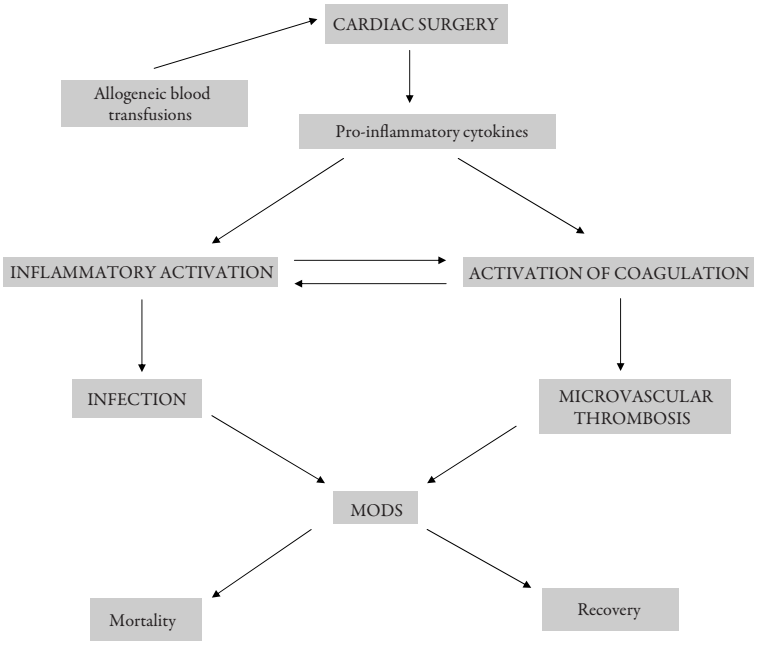

Figure 2 | Relation between allogeneic blood transfusions, inflammation and coagulation.

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

Possible factors	Relation with allogeneic leukocytes
Effects of RBC storage lesions	Not related
Investigated by the storage time of RBCs (Chapter 5)	
Effects of stored plasma-derived factors	Not related
Investigated by fresh-filtered and stored-filtered RBCs [9]	
Activation of the complement system by lectin pathway	Not related
Investigated substrate: MBL (Chapter 6)	
Activation of the inflammatory systems	Higher pro-inflammatory and lower anti-inflammatory cytokines
Investigated substrates: IL-6,IL10 and IL-12 (Chapter 5)	
Acute phase reaction	Not related
Investigated substrate: procalcitonin (Chapter 6)	
Activation of the coagulation system	???
Not investigated	

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.

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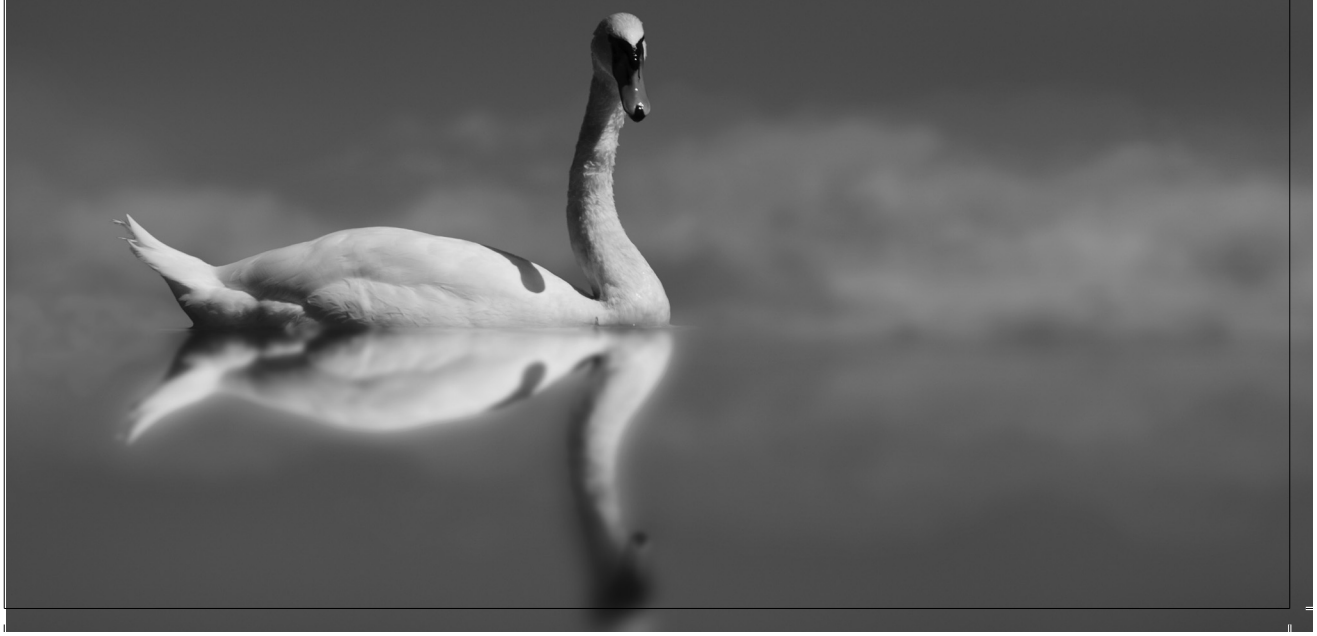
Chapter 10

Summary/Samenvatting



SUMMARY

Worldwide more than 800.000 patients undergo cardiac surgery annually, in the Netherlands this regards 1 of 1.000 inhibitants. To compensate blood loss during and after cardiac surgery a lot of allogeneic blood is often transfused. Blood transfusions can cause unexpected adverse reactions. In **Chapter 1** we discuss immunologic and non-immunologic transfusions reactions. The association between blood transfusions and higher morbidity and mortality in patients who underwent cardiac surgery and in intensive-care-unit-(ICU)-patients is discussed in detail. Special attention is given to transfusion-related immunomodulation (TRIM). The exact mechanism of TRIM is not known, although it has been suggested that allogeneic leukocytes and leukocyte-derived mediators play a prominent role in the development of TRIM. By filtration the number of allogeneic leukocytes in donated blood can be reduced by more than 99.9%. In the past leukocyte-depleted (or leukocyte-reduced) red blood cells (RBCs) were transfused to selected patient populations. For the first time the beneficial effects of leukocyte-depleted red blood cells on postoperative mortality (due to multiple-organ-dysfunction-syndrome [MODS]) was shown in the Netherlands in a randomized controlled trial in patients who underwent cardiac surgery.

In **Chapter 2** the results of a new randomized controlled trial in 474 patients undergoing cardiac valve surgery is presented. Patients receiving buffy-coat depleted (which contain 20-30% donor leukocytes) allogeneic RBC transfusions had a (dose-dependent) higher hospital-mortality and postoperative infection rates than patients receiving leukocyte-depleted red blood cell transfusions. The incidences of 90-day mortality, multiple-organ-dysfunction-syndrome, ICU-stay and hospital-stay were not different between both groups.

To investigate the causes of these differences between leukocyte-containing allogeneic RBC transfusions and leukocyte-depleted RBC transfusions, data from two randomized controlled trials were pooled. In **Chapter 3** the results of this re-analysis of 1.085 patients are presented. The results revealed that mortality associated with postoperative infections was higher in the patients receiving leukocyte-containing allogeneic RBC transfusions than leukocyte-depleted RBC transfusions. Although mortality not associated with postoperative infections was similar in both groups. The only cause of death that differed significantly between both groups was the combination of multiple-organ-dysfunction-syndrome and infections. This re-analysis shows that allogeneic leukocytes transfused during cardiac surgery may be associated with more infections with fatal outcome.

Chapter 4 is a review of all randomized controlled trials investigating the effects of leukocyte-depleted RBC transfusions. The study conclusions are that in contrast to

conflicting outcomes in different clinical studies, in cardiac surgery there is evidence that leukocyte-depleted RBC transfusions have benefits by lowering postoperative complications. As possible explanation for these results in cardiac surgery, we suggest that leukocyte-containing blood transfusions act as a second insult to the development of surgery-induced systemic inflammatory response.

To investigate the role of allogeneic leukocytes on several mediators in relation with postoperative complications laboratory analyses were performed. Therefore blood samples were taken in patients participating in the randomized controlled trial described in Chapter 2. In **Chapter 5** the effects of RBC transfusions on inflammatory markers are presented. The results revealed that patients who received more than 3 red blood cell units had significantly higher cytokine IL-6 concentrations after leukocyte-containing, buffy-coat depleted RBC as compared to patients who had received similar numbers of leukocyte-depleted RBC units. Patients who developed postoperative infections and multiple-organ-dysfunction-syndrome showed, respectively, increased concentrations of cytokines IL-6 and IL-12 in the group that received leukocyte-containing RBC units. This analysis suggests that leukocyte-containing RBC transfusions may affect the development of postoperative complications by modulation of the postoperative proinflammatory response after cardiac surgery. In **Chapter 6** we present the effects of blood transfusions on mannose-binding lectin (MBL), a recognition molecule of the lectin pathway of the complement system, and the clinical role of MBL after cardiac surgery. Cardiac surgery was associated with MBL consumption, independent of transfusion of allogeneic leukocytes. Patients with MBL-deficiency developed no MODS, unless they have been transfused with plasma units. This suggests that plasma transfusions is associated with MBL reconstitution and can have a deleterious effect in cardiac surgery. In **Chapter 7** the cost-effectiveness of transfusion of leukodepleted RBCs has been estimated. This analysis revealed that leukodepletion of RBCs is cost effective in cardiac valve surgery.

In **Chapter 8** the clinical effects of plasma and platelet transfusions during cardiac surgery are shown. These additional analyses from data of the two randomized controlled trials revealed that plasma transfusions was associated with all-cause mortality, while (in particular leukocyte-containing) RBC and platelet transfusions are both independently associated with postoperative infections and longer ICU-stay.

In **Chapter 9** we discuss all results of the studies described in this thesis in their current scientific perspective. Finally possible direction for future studies are provided.

SAMENVATTING

Wereldwijd ondergaan jaarlijks meer dan 800.000 patiënten een open-hart operatie; in Nederland wordt 1 op de 1.000 inwoners geopereerd. Om bloedverlies te compenseren wordt tijdens en na een open-hart operatie vaak allogeen bloed getransfundeerd. Bloedtransfusies kunnen verschillende onverwachte bijwerkingen veroorzaken. In **Hoofdstuk 1** worden de meest klinisch relevante immunologische en non-immunologische transfusie reacties besproken. De associatie tussen bloedtransfusies en hogere morbiditeit en mortaliteit bij intensive-care (IC)-patiënten en patiënten die een hartoperatie ondergingen wordt uitvoerig bediscussieerd. Speciale aandacht wordt gegeven aan transfusie-gerelateerde immunomodulatie (TRIM). Het exacte mechanisme van TRIM is niet bekend, maar er wordt wel gesuggereerd dat allogene leukocyten en van leukocyten afgeleide mediatoren een prominente rol spelen in de ontwikkeling van TRIM. Door middel van filtratie kan het aantal leukocyten van het gedoneerde bloed met meer dan 99.9% gereduceerd worden. In het verleden werden leukocyten-gedepleteerde (leukocyten-gereduceerde) rode bloedcellen getransfundeerd aan geselecteerde patiëntenpopulaties. In een gerandomiseerde gecontroleerde trial uit Nederland werd voor het eerst de gunstige effecten van leukocyten-gedepleteerde rode bloedcellen op postoperatieve infecties en mortaliteit (ten gevolge van multi-orgaan-dysfunctie-syndroom [MODS]) bij patiënten die een hartchirurgie ondergingen aangetoond. Deze patiënten hadden minder postoperatieve infecties en ook de sterfte na operatie was gereduceerd.

In **Hoofdstuk 2** worden de resultaten van een nieuwe gerandomiseerde gecontroleerde trial bij patiënten die een hartklep-operatie ondergingen gepresenteerd. Patiënten die allogene buffy-coat gedepleteerde (bevatten 20-30% donor leukocyten) rode bloedceltransfusies ontvingen, hadden een (dosisafhankelijk) hogere ziekenhuissterfte en postoperatieve infecties dan bij patiënten die leukocyten-gedepleteerde rode bloed celtransfusies ontvingen. Terwijl de incidenties van 90-dagen mortaliteit, MODS, IC-verblijf en ziekenhuis-verblijf niet verschillend waren niet verschillend tussen de beide groepen.

Om de oorzaken van deze verschillen tussen allogene leukocyten-bevattende rode bloed celtransfusies en leukocyten-gedepleteerde rode bloed celtransfusies te onderzoeken werden de gegevens van twee gerandomiseerde gecontroleerde studies samengevoegd. In **Hoofdstuk 3** worden de resultaten van deze re-analyse van 1.085 patiënten gepresenteerd. Uit deze resultaten bleek dat de sterfte geassocieerd met postoperatieve infecties hoger was bij de patiënten die allogene leukocyten-bevattende rode bloed celtransfusies kregen dan bij patiënten die leukocyten-gedepleteerde rode bloed celtransfusies ontvingen; hoewel de

sterfte niet geassocieerd met postoperatieve infecties vergelijkbaar was in beide groepen. De enige doodsoorzaak die significant verschilde tussen beide groepen was de sterfte ten gevolge van de combinatie van MODS en infecties. Deze re-analyse toont aan dat allogene leukocyten die tijdens hartchirurgie getransfundeerd worden, geassocieerd kunnen zijn met meer infecties met een fatale afloop.

In **Hoofdstuk 4** wordt een overzicht van alle gerandomiseerde gecontroleerde studies die de effecten van leukocyt-gedepleteerde rode bloed celtransfusies hebben onderzocht weergegeven. We vonden dat in tegenstelling tot de tegenstrijdige resultaten in verschillende klinische studies, in hartchirurgie er weldegelijk bewijs is dat leukocyten-bevattende rode bloedceltransfusies nadelige effecten hebben op postoperatieve complicaties. Tevens bespreken we de mogelijke verklaringen voor deze resultaten in hartchirurgie. Wij suggereren dat leukocyten-bevattende rode bloedceltransfusies fungeren als een tweede insult voor de ontwikkeling van operatie-geïnduceerde systemische inflammatoire respons.

Om de rol van allogene leukocyten te onderzoeken in relatie met verschillende mediatoren met postoperatieve complicaties werden laboratorium analyses uitgevoerd. Hiervoor werden er bloedmonsters afgenomen bij patiënten die deelnamen aan de gerandomiseerde gecontroleerde studie beschreven in Hoofdstuk 2. In **Hoofstuk 5** worden de effecten van bloedtransfusies op inflammatoire markers gepresenteerd. De resultaten laten zien dat patiënten die 4 of meer eenheden rode bloedcellen ontvingen significant hogere cytokine IL-6 concentraties hadden in de leukocyten-bevattende, buffy-coat-gedepleteerde groep dan in de leukocyten-gedepleteerde groep. Patiënten die postoperatieve infecties en multiple-organ-dysfunctie-syndroom ontwikkelden hadden respectievelijk hogere concentraties cytokines IL-6 en IL-12 in de leukocyten-bevattende, buffy-coat-gedepleteerde groep dan in de leukocyten-gedepleteerde groep. Uit deze analyse bleek dat transfusie van leukocyt-bevattende rode bloedcellen van invloed is op de ontwikkeling van postoperatieve complicaties door modulatie van het pro-inflammatoire postoperatieve reactie na een hartoperatie. In **Hoofdstuk 6** worden de effecten van bloedtransfusies op mannose-binding lectine (MBL), een molecuul van de lectine-route van het complement systeem, en de klinische rol van MBL na hartchirurgie gepresenteerd. Hartchirurgie was geassocieerd met MBL consumptie, onafhankelijk van de transfusie van allogene leukocyten. Patiënten met een MBL-deficiëntie ontwikkelden geen MODS, tenzij zij getransfundeerd werden met plasma-eenheden. Dit suggereert dat transfusie van plasma-eenheden geassocieerd is met reconstitutie van MBL en schadelijke effecten kan hebben bij hartchirurgie. In **Hoofdstuk 7** wordt een schatting gemaakt van de kosten-effectiviteit van transfusie van

leukocyten-gedepleteerde rode bloedcellen in hartklepchirurgie. Deze analyse toonde aan dat leukodepletie van rode bloedcellen kosten besparend is in hartklepchirurgie.

In **Hoofdstuk 8** worden de klinische effecten van transfusies van plasma en bloedplaatjes tijdens hartchirurgie getoond. Bij deze aanvullende analyse van data van de twee gerandomiseerde gecontroleerde studies is gebleken dat transfusie van plasma geassocieerd was met sterfte, terwijl transfusies van (in het bijzonder leukocyten-bevattende) rode bloedcellen en bloedplaatjes significant geassocieerd waren met postoperatieve infecties en langer IC-verblijf.

In **Hoofdstuk 9** bespreken we alle resultaten van de studies beschreven in dit proefschrift binnen hun huidige wetenschappelijke perspectieven. Ten slotte, worden mogelijke richtingen voor toekomstig onderzoek gegeven.

DANKWOORD

De zwanenzang is een verwijzing naar de mythe dat een zwaan als hij zijn einde voelt aankomen, uit blijdschap voor de weldaden die hem te wachten staat na de dood, nog eenmaal wonderschoon zingt. Toen ik dit las, dacht ik aan de allogene leukocyt die aanleiding gaf voor vele onderzoeken die mijn illustere voorgangers verrichten. Na een proefschrift volgeschreven te hebben verdwijnt de allogene leukocyt als wetenschappelijk object uit ons leven. Echter in mijn dagelijkse leven zal deze cel met een diameter van 0,000001 meter op allerlei manieren een rol blijven spelen!

In dit proefschrift is veel over de witte bloedcel in transfusie-eenheden geschreven. Maar het onzichtbare is dat de veel analyses en schrijfwerk tijdens de nachtdiensten werd verricht. Daarom zou het toepasselijk geweest zijn als de verdediging ook 's nachts kon plaatsvinden!

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Mijn klinische loopbaan begon ooit in het Leyenburg-ziekenhuis. Daar ben ik door de bevlogenheid van de internisten aangestoken en heb ik besloten om internist te worden. Een speciale dank gaat naar Dr van der Vijver voor de vele leermomenten (van het nemen van een bloedkweek tot en met de betekenis van de zwanenzang). In Leiden kon ik verder mijn onderzoek uitwerken en verdiepen in de transfusie-geneeskunde op de afdeling Immunohematologie en Bloedtransfusie. Daarvoor dank ik Professor de Vries en alle medewerkers van het transfusie-lab, de hemaferese-afdeling en het stamcel-lab.

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CURRICULUM VITAE

De auteur van dit proefschrift werd op 5 maart 1970 geboren te Eindhoven. Hij behaalde in 1989 zijn VWO diploma aan de Gemeentelijke Scholengemeenschap Genderdal te Eindhoven. Nadien ging hij geneeskunde studeren aan de Erasmus Universiteit te Rotterdam (1989-1997). Na zijn studie werkte hij als arts-assistent niet in opleiding op de afdelingen Interne Geneeskunde en Cardiologie van het toenmalige Leyenburg-ziekenhuis (tegenwoordig Haga ziekenhuis) te Den Haag (1998-1999). Vanaf 2000 werkte hij als arts-onderzoeker op de afdeling Hematologie aan het AMC te Amsterdam (Onder supervisie van Prof. Dr. M.H.J. van Oers), waar een begin aan dit proefschrift werd gemaakt. Vanaf 2002 werkte hij als arts-assistent niet in opleiding bij de afdeling Immunohematologie en Bloedtransfusie van het Leids Universitair Medisch Centrum; dit proefschrift werd daar tevens verder uitgewerkt. In mei 2005 werd de opleiding tot internist gestart in het IJsselland ziekenhuis te Capelle aan den IJssel (Opleiders: Dr. H.R.A. Fischer en Dr. H.E. van der Wiel). De opleiding werd vanaf mei 2007 voortgezet in het Erasmus MC te Rotterdam (Opleider: Prof. Dr. J.L.C.M. van Saase). In september 2009 werd de opleiding tot hematoloog gestart eveneens in het Erasmus MC te Rotterdam (Opleiders: Prof. Dr. P. Sonneveld en Prof. Dr. J.J. Cornelissen). Vanaf augustus 2010 is de auteur geregistreerd als internist. Vanaf september 2011 werkt hij als internist-hematoloog op de afdeling Hematologie van het Erasmus MC. In 2001 werd de Jaap Steenbergen Stipendium tijdens de najaarsvergadering van de Nederlandse Vereniging voor Haematologie voor de beste presentatie ontvangen en in 2003 kreeg de auteur de Onderzoeksprijs van de Nederlandse Vereniging voor Bloedtransfusie.

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