Transfusion associated complications in cardiac surgery: the swan song of the allogeneic leukocytes?
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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 indispensable 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>
<thead>
<tr>
<th>Type of transfusion reactions</th>
<th>Estimated incidences</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-immune-mediated</strong></td>
<td></td>
</tr>
<tr>
<td>Transfusion-transmitted infections</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>RBC: 1:1.000; platelets: 1:2.000</td>
</tr>
<tr>
<td>Transfusion-associated sepsis</td>
<td>RBC: 1:250.000; platelets: 1:25.000</td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>1:7.800.000</td>
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<tr>
<td>Hepatitis B</td>
<td>1:800.000</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>1:3.000.000</td>
</tr>
<tr>
<td>Parvo B19</td>
<td>&lt;1:1.000.000</td>
</tr>
<tr>
<td>Parasites</td>
<td>&lt;1:1.000.000</td>
</tr>
<tr>
<td>Prions</td>
<td>&lt;1:1.000.000</td>
</tr>
<tr>
<td>Transfusion-associated circulatory overload (TACO)</td>
<td>2-8:100</td>
</tr>
<tr>
<td>Iron-deposition; hemosiderosis</td>
<td>Starts after &gt;20 units RBCs</td>
</tr>
<tr>
<td><strong>Immune-mediated</strong></td>
<td></td>
</tr>
<tr>
<td>Acute hemolytic transfusion reactions</td>
<td>1:18.000</td>
</tr>
<tr>
<td>Delayed hemolytic transfusion reactions</td>
<td>1:2.500-4.000</td>
</tr>
<tr>
<td>Febrile non-hemolytic transfusion reactions</td>
<td>1-7:100</td>
</tr>
<tr>
<td>Allergic transfusion reactions</td>
<td>1-3:100</td>
</tr>
<tr>
<td>Anaphylactic reactions</td>
<td>1:20.000-47.000</td>
</tr>
<tr>
<td>Transfusion-associated graft versus host disease (TA-GVHD)</td>
<td>&lt;1:1.000.000</td>
</tr>
<tr>
<td>Post-transfusion purpura (PTP)</td>
<td>&lt;1:1.000.000</td>
</tr>
<tr>
<td>Transfusion-related acute lung injury (TRALI)</td>
<td>1:1.000-5.000</td>
</tr>
</tbody>
</table>

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 Creutzfeldt-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-haemolytic 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-incompatibility 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 (DHTTR) 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, DHTTRs occur in 1 per 2,500-4,000 RBC transfusions and mostly these reactions have a mild course; however fatal DHTTRs can occur [24]. In the Netherlands 8 cases of DHTTRs 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 preventative 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 allogenic 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)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>TRALI</th>
<th>TACO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of symptoms</td>
<td>&lt;6 hours</td>
<td>Mainly &lt;6 hours</td>
</tr>
<tr>
<td>Respiratory symptoms</td>
<td>Dyspnea</td>
<td>Dyspnea</td>
</tr>
<tr>
<td>Central venous pressure</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Pulmonary wedge pressure</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Fluid balance</td>
<td>Positive or negative</td>
<td>Positive</td>
</tr>
<tr>
<td>X-ray thorax</td>
<td>Bilateral infiltrates</td>
<td>Bilateral infiltrates with signs of fluid overload</td>
</tr>
<tr>
<td>Echocardiography</td>
<td>Normal ejection fraction</td>
<td>Decreased ejection fraction</td>
</tr>
<tr>
<td>B-type natriuretic peptide</td>
<td>Low or normal</td>
<td>High</td>
</tr>
</tbody>
</table>

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 perioperative 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 1x10⁶/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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