Transfusion-transmissible infections and transfusion-related immunomodulation

Buddeberg, F; Beck Schimmer, B; Spahn, D R

Transfusion-transmissible infections and transfusion-related immunomodulation

Abstract

The risk of acquiring a transfusion-transmitted infection has declined in recent years. However, after human immunodeficiency virus and hepatitis B and C virus transmission were successfully reduced, new pathogens are threatening the safety of the blood supply, especially in the face of rising numbers of immunocompromised transfusion recipients. Despite new standards in the manufacture and storage of blood products, bacterial contamination still remains a considerable cause of transfusion-related morbidity and mortality. Better allograft survival in kidney transplant patients and higher cancer recurrence rate in surgical oncology patients after allogeneic blood transfusions highlighted a previously underestimated side-effect: transfusion-related immunomodulation (TRIM). The precise pathomechanism still remains uncertain; however, its mostly deleterious effects--such as a higher incidence of postoperative or nosocomial infections--is increasingly accepted. Although transfusion-related immunomodulation is thought to be mediated mainly by donor white blood cells, the benefit of leukoreduction on overall mortality and on infectious complications is highly debatable.

Transfusion-transmissible infections and

Transfusion-related immunomodulation

Felix Buddeberg, Beatrice Beck Schimmer, Donat R. Spahn

Corresponding author: Felix Buddeberg

Institute of Anaesthesiology
University Hospital Zurich
Raemistrasse 100
CH-8091 Zurich
Switzerland

felix.buddeberg@usz.ch
beatrice.beck@usz.ch
donat.spahn@usz.ch

Tel: +41 44 255 26 95
Fax: +41 44 255 44 09

Transfusion-transmissible infections and Transfusion-related immunomodulation

Abstract

The risk of acquiring a transfusion-transmitted infection has declined in recent years. However, after human immunodeficiency virus and hepatitis B and C virus transmission were successfully reduced, new pathogens are threatening the safety of blood supply, especially in the face of a rising number of immunocompromised transfusion recipients. Despite new standards in blood product manufacturing and storage, bacterial contamination still remains a considerable cause of transfusion-related morbidity and mortality.

Better allograft survival in kidney transplant patients and higher cancer recurrence rate in surgical oncology patients after allogeneic blood transfusions highlighted a previously underestimated side effect: transfusion-related immunomodulation. The precise pathomechanism still remaining uncertain, however, it's mostly deleterious effects such as a higher incidence of postoperative or nosocomial infections is increasingly accepted. Although transfusion-related immunomodulation is thought to be mainly mediated by donor white blood cells, the benefit of leukoreduction on overall mortality and on infectious complications is highly debated.

Word count: 149

Key words: Transfusion, infection, immunomodulation.

Transfusion-transmissible infections and Transfusion-related immunomodulation

For decades blood components were transfused in order to maintain an arbitrary haemoglobin threshold of 10 g/dL [1, 2], a thrombocyte count superior to 20'000 per μL [3] or to correct any coagulopathy [4]. Despite little medical evidence documenting the efficacy of prophylactic transfusions, they are associated with significant adverse effects [5]. The potential harm of a liberal transfusion strategy has been largely demonstrated [6], [7]. Nowadays there are three main risks for transfusion-related morbidity and mortality: a) incorrect blood component transfusions (70%), b) transfusion-transmissible infections (2%), and c) immunologic risks (28%) [8]. Incorrect blood component transfusions, although entirely preventable, remain the most frequent reported transfusion hazard with a 1'000 to 10'000 times higher frequency than the risk of viral infection from blood [9]. Standardized surveillance systems have been implemented to reduce the risk of administrative errors (leading to ABO-incompatible blood transfusion) [10]. Transfusion-transmissible infections, immunologic risks and nosocomial infections associated with blood transfusions will be reviewed in this article.

I. Transfusion-transmitted infections

Transfusion-transmitted infections due to a variety of agents, although rare, still demonstrate a serious risk in allogeneic blood transfusions [11]. In Western Countries the implicated pathogens are mainly viruses and bacteria, rarely parasites, and prion transmission has very likely occurred in a few documented cases.

1. Viruses

Virus transmission may be related to leukocytes contained in blood products such as cytomegalovirus, Epstein-Barr virus, human herpes virus 6-8, human T-cell leukaemia / lymphoma virus [12]. Conversely, other viruses are not exclusively cell bound such as HIV, hepatitis B and C viruses, Parvovirus B19, West Nile virus [13], TT virus [14], SEN virus [15].

Of all human herpes viruses, **cytomegalovirus** [CMV] is the most significant cause of transfusion-associated morbidity and mortality. The problem of transfusion-associated CMV infection differs from that of other transfusion-transmitted infections in that only certain groups of patients require CMV-free blood or blood components, i.e. seronegative pregnant women and their newborn babies, seronegative recipients of allogeneic bone marrow transplants from seronegative donors, individuals with AIDS, and other profoundly immunocompromised patients. CMV prevalence in the population ranges from 40% to 100%, determined by geographic and socioeconomic factors. The virus is confined to leukocytes in latently infected blood donors and can be reactivated after transfusion, resulting in an infection rate of 1%-12% among adults, of 20% among neonates, and as high as 57% among bone marrow transplant recipients [16]. Infection can result in retinitis, pneumonitis, hepatitis, gastroenteritis, and death. CMV infection in risk groups can be minimized by selection of CMV-seronegative donors. Since transmission of CMV from seropositive donors by blood components containing fewer than 5x 10^6 leukocytes per unit is unlikely, leukodepletion is an effective alternative to the use of seronegative blood products [17]. In countries, where only leukocyte-filtered blood products are available today, the incidence of CMV transfusion-related infections has significantly fallen after white blood cell reduction has been implemented [18].

Epstein-Barr virus [EBV] is the causative agent of infectious mononucleosis, Burkitt lymphoma, nasopharyngeal carcinoma, and associated with other lymphoproliferative diseases (i.e. Hodgkin lymphoma, oral hairy leukoplakia). The incidence of latent EBV infection in the general population is approximately 90%. EBV-seronegative blood transfusion recipients who are immunocompromised are at risk of acquiring EBV infection. Several cases of transfusion-associated EBV disease, including lymphoproliferative disease, have been reported [19]. However, the relatively rare rate of transmission via transfusion does not justify screening for virus-free donor blood. Besides that, research showed that leukoreduction eliminated the PCR signal in 15 of 16 units of EBV-contaminated blood with none of the 15 EBV-negative patients who received the transfusions that had undergone leukoreduction experienced seroconversion [20].

Other viral agents are not exclusively cell bound and transmitted with any contaminated blood product (**HIV, hepatitis B and C viruses**). Besides the well known transfusion-transmitted viral agents, new viruses have recently been identified in transfusion-transmitted infections (**West Nile virus** [13], **TT virus** [14], **SEN virus** [15]).

Improvements in donor screening and testing and viral inactivation of plasma derivatives (by methylene blue or solvent-detergent treatment [21]) have resulted in substantial declines in transfusion-transmitted infections over the last two decades. Most recently, nucleic acid testing techniques have been developed to screen blood and plasma donations for evidence of very recent viral infections that could be missed by conventional serologic tests [22]. The risk of viral disease transmission has nowadays become so small that mathematical models are used to assess it. For example, the residual risk of a transfusion-transmitted **HIV** infection is 1 per 7.8 million donations, and for **hepatitis C virus** infection 1 per 2.3 million donations.

Since nucleic acid testing is not available for hepatitis B virus, the residual risk of a transfusion-transmitted infection is still 1 per 153,000 donations [23].

Parvovirus B19 is a common human pathogen that is more and more seen as a new threat to the safety of blood supply. The main route of transmission is through respiratory vesicles, with a majority of infections occurring during childhood and manifesting as erythema infectiosum. Parvovirus B19 can also be transmitted via blood transfusion and organ transplantation. The majority of adult populations show immunological evidence of a previous exposure to parvovirus B19. Although the immune response is able to clear infection and provide life-long protection against parvovirus B19, recent data suggest that in some, if not the majority, of individuals the acute phase of infection is followed by viral persistence in the blood or other tissues regardless of the host's immunocompetence. Transmission of parvovirus B19 by blood products and its resistance to common viral inactivation methods raises several blood safety questions, still unanswered. The diversity of parvovirus B19 strains and the ability of the virus to persist in the presence of specific antibodies raise the issue of transmissibility by transfusion not so much to immunocompetent recipients but rather to the large proportion of recipients in whom there is some degree of immunodeficiency [24].

2. Bacteria

Transfusion-transmitted infections through **bacteria** are mainly the result of bacterial contamination of blood products. In the past the safety of blood transfusions could be enhanced through modern phlebotomy practices (arm cleansing using an iodine / alcohol mix, and diverting the first 50ml of each blood donation), refrigeration of red cells, freezing of plasma and improved materials for transfusion product collection and storage [25]. However,

one in every 3000 units of donated blood is still contaminated with bacteria. Clinically significant sepsis results from 1 of every 250'000 red blood cell units transfused. Platelet concentrates are particularly vulnerable to bacterial contamination because their storage temperature (20-24°C) readily facilitates growth. Approximately 1 in every 2000 platelet concentrates is contaminated and 1 in every 25'000 platelet concentrates has been reported to cause sepsis [26]. Obviously, the risk of transfusion-transmitted bacterial infection is significantly higher than for transfusion-transmitted viral infections [27]. Implicated pathogens are mainly skin-commensal bacteria like Staphylococcus spp., Streptococcus spp. and Serratia spp. [28]. Quality control testing for bacterial contamination of platelet products by culturing samples under aerobic conditions until the end of the product shelf life has been effective in identifying and preventing the transfusion of bacterially contaminated platelet units [29].

Several studies have shown a higher incidence of nosocomial infections in transfused versus untransfused patients. Since the implicated pathomechanism is rather due to transfusion-related immunomodulation and secondary bacterial infection than to direct inoculation through a bacteria containing blood product, nosocomial infection after transfusion will be discussed in the chapter of transfusion-related immunomodulation.

3. Parasites

In Western Countries **protozoal** transfusion-transmitted infections are rather rare. However, on a global scale **malaria** remains one of the most common transfusion-transmitted infections [30]. With more and more people travelling to endemic areas, the number of donors who have a potential 'malaria risk' is increasing, and there is corresponding pressure on the collection

teams to correctly and effectively identify all such ‘malaria-risk’ individuals, and pressure on the recruitment staff to replace the donations lost, even if deferral is only temporary. Although the examination of blood films is still the basis for diagnosing acute malaria, it is not sufficiently sensitive for blood bank screening. In non-endemic countries, donor deferral in combination with screening for specific antimalarial immunoglobulin provides an effective means of minimizing the risk of transmission. In endemic countries, more specific donor questioning, consideration of seasonal variation and geographical distribution may help to identify the population of donors who are most likely to be infected. In addition, the administration of antimalarials to transfusion recipients may help to prevent transmission. Although rare and prevented by donor deferral and immunoglobulin testing, it is likely that cases of transfusion-transmitted malaria may still occur in non-endemic areas. Therefore malaria must always be considered in any patient with a post-transfusion febrile illness.

Primary **toxoplasmosis** has been reported to be transmitted from seropositive donors to immunocompromised recipients [31]. Other transfusion-transmitted protozoal pathogens include **Leishmania** and **Trypanosoma**. With a very low prevalence in Western Countries there is currently no donor screening for both of them. However, transfusion-transmitted infection by Leishmania or Trypanosoma seems to be successfully prevented by leukoreduction [32], [33].

4. Prions

Besides transfusion-transmitted infections through viral, bacterial or protozoal pathogens, there is growing concern about the risk of blood-borne transmission of **variant Creutzfeldt-Jakob disease (vCJD)**. Four cases of packed red blood cell transfusion-transmitted vCJD

have been described [34], [35], [36], [37]. No case of prion transmission by plasma products has been observed yet. Measures implemented in various countries and several technical factors may contribute to the prevention of prion transmission. Those measures include (a) the epidemiological surveillance of population in countries with cases of vCJD and/or bovine spongiform encephalopathy (BSE), (b) the deferral of blood donors who travelled or resided to countries with BSE, or who received transfusion or tissue transplant, (c) leukocyte reduction, and (d) the removal of the prion agents during the complex industrial fractionation process used to prepare plasma products [38].

Thanks to strict exclusion criteria for blood donors, improved transfusion product collection and storage practices, immunoglobulin and nucleic acid testing, and viral inactivation, the transfusion-transmitted infectious risk has declined significantly in the last decade. The implementation of leukoreduction in many countries has further improved blood transfusion safety.

Practice Points:

- New standards in blood product manufacturing and storage reduce the risk of transfusion-transmitted infections.
- Mainly in immunocompromised patients transfusion-transmitted viral infections still remain a considerable cause of transfusion-related morbidity and mortality.
- Bacterial contamination of a blood product, mainly concentrates of thrombocytes stored at 20-24°C, is the single most often encountered source of a transfusion-related infection.
- Leukoreduction reduces primary transmission of CMV and other infections due to WBC-associated pathogens.

Research Agenda:

- Further research is warranted to develop new manufacturing techniques to inactivate pathogens contained in the donated blood and to prevent secondary bacterial contamination of processed blood products

II. Transfusion-related immunomodulation (TRIM)

In 1973 Opelz et al. suspected an immunomodulating effect of red blood cell (RBC) transfusions to explain the better outcome of patients that had received blood transfusions prior to kidney transplant [39]. This immunosuppressive effect of RBC transfusions was confirmed by animal data and observational clinical studies, and in 1981 Gantt et al. suggested the possibility of an association between RBC transfusions and increased cancer recurrence [40]. Since then, more than a hundred clinical studies examined the possible adverse effect of RBC transfusions on cancer recurrence. Although it is now accepted that transfusions are associated with poorer prognosis in surgical cancer patients, the exact mechanism remains unclear [41]. Besides a higher recurrence rate of resected malignancies, transfused patients also show an increased incidence of nosocomial infections [42]. The incriminated pathomechanism of these mostly adverse effects of blood transfusions is called transfusion-related immunomodulation (TRIM).

1. Alloimmunization, tolerance, or immunosuppression

Allogeneic blood transfusions (ABTs) may either cause **alloimmunization, tolerance, or immunosuppression** in recipients. The specific immunomodulatory constituents are still unknown, though several are highly suspected: allogeneic mononuclear cells, HLA peptides, and soluble biologic response modifiers.

Alloimmunization

Allogeneic blood transfusions introduce a multitude of foreign antigens into the recipient, including **allogeneic mononuclear cells with cell-bound HLA antigens**. In general, fully HLA-mismatched transfusions lead to alloimmunization, while allogeneic transfusions sharing at least one HLA antigen with the recipient induce tolerance and to some degree immunosuppression [43].

In the patient population of acute myeloid leukemia **alloimmunization** with development of alloantibodies and of refractoriness to platelet transfusions was shown to be dependent on the white blood cell count and / or the functional status of transfused white blood cells [44]. The patients received either platelets obtained by apheresis from single random donors, pooled concentrates from random donors that had been leukocyte-reduced by filtration, or pooled concentrates from random donors whose leukocytes had been inactivated by ultraviolet B irradiation. There were no significant differences among the three treated groups in any study end point. However, all three types of treated platelets significantly reduced the development of lymphocytotoxic antibodies and alloimmune refractoriness to platelet transfusions, as compared with untreated, pooled platelet concentrates from random donors. This is consistent with the result of a meta-analysis showing that the rates of lymphocytotoxic antibodies in

patients who had received transfusions were lower in patients given leukocyte-reduced platelets and red cells than in controls who received standard blood components [45].

Tolerance

Transfused white blood cells (WBCs) with a partially matched HLA-pattern are protected from immunologic clearing through host defence mechanisms (**tolerance**) since the host immune system fails to recognize them as foreign. Besides that, it is also believed that allogeneic plasma containing soluble HLA antigens may enter the recipient's thymic circulation, producing clonal deletion of the recipient's T cells that are directed against allogeneic donor antigens [46]. In the clinical setting of recurrent spontaneous abortions, successful outcome of the next pregnancy was significantly more common in women injected with purified lymphocytes prepared from their husbands' blood than in those injected with their own lymphocytes. It is postulated that through the HLA-alloimmunization by the father's lymphocytes the embryos were no longer recognized as foreign by the mother's immune system and less frequently aborted [47].

In addition to the degree of HLA compatibility between donor and recipient, the immunogenicity of cellular or soluble HLA antigens depends on the viability of the donor antigen-presenting cells and the presence of co-stimulatory signals for the presentation of the donor antigens to the recipient's T-cells. Non-viable antigen-presenting cells and / or the absence of the requisite co-stimulatory signals result in T-cell unresponsiveness (**anergy**) and immune tolerance [48], [49], [50], [51].

Immunosuppression

HLA compatibility between donor and recipient may result in the persistence of allogeneic donor WBCs in the recipient (**microchimerism**) [52]. Microchimerism leads to the release of interleukins (IL-4, IL-10, and TGF-β) that inhibit the T-helper cell type 1 (Th-1)-mediated immune response. Impairment of Th-1 cytokine secretion (IL-2, IL-12, and interferon-γ) results in **impairment of various functions of cellular immunity** (including antigen processing, macrophage activation, T-cell cytotoxic function through CD 8 positive T-cells, and the neutrophils and monocyte cytocidal activity) [53]. The result is a shift toward a T-helper cell type-2 (Th-2) immune response favouring humoral immunity. This partially suppressed Th-1-mediated cytotoxic immune response is thought to be the underlying mechanism of the beneficial effect of blood transfusions in Crohn's disease patients with a statistically significant reduction in disease recurrence and a longer disease free interval [54].

Transfusion-associated microchimerism has been detected and studied in severely injured, transfused patients. Injury induces an immunosuppressive and inflammatory milieu in which replication-competent leukocytes of fresh blood products may persist in the recipient's blood. Transfusion-associated microchimerism is present in approximately 50% of transfused severely injured patients at hospital discharge and is not affected by leukoreduction [55]. It is postulated that, if the conditions are right for transfusion-associated microchimerism to develop, engraftment occurs with a very small number of pluripotent hematopoietic cells, regardless of the total number of donor WBCs present. It is possible that a relative paucity specifically of donor antigen-presenting cells (as might occur with leukoreduction) might limit the stimulation of the recipient's cellular immune response, in turn leading to a greater likelihood of developing microchimerism than would be expected based purely on the total number of donor WBCs transfused [56]. In approximately 10% of patients, the chimerism

from a single blood donor may increase in magnitude over months to years, reaching as much as 2% to 5% of all circulating leukocytes [57].

In a most negative way, in transfusion-related immunosuppressed recipients, microchimerism may result in the development of **transfusion-associated graft-versus-host disease (TAGVHD)**. It is a rare (approximately 1:1 million units transfused) but potentially lethal complication with a mortality rate > 90%, in which immunocompetent donor cells proliferate and attack host hemopoietic cells, skin, liver, and bile duct epithelial cells. Although more common in immunocompromised patients, the risk factors for the development of graft-versus-host disease include transfusions from donors with a similar HLA-pattern, the use of relatives as donors, male recipients, and fresh blood containing viable lymphocytes [58].

Biologic response modifiers accumulating in blood components during storage have been implicated in the pathogenesis of TRIM. These mediators are contained in intracellular WBC granules, and are released in a time-dependent manner as the WBCs deteriorate during storage [59]. It has been shown that at least some of these biologic response modifiers (histamine, eosinophil cationic protein, eosinophil protein X, TGF-β) may contribute to the development of **immunosuppression** and tissue damage by inhibition of neutrophils, cytotoxic T-cells and natural killer cells [60], [61], [62], [63].

2. Pro-inflammatory state after blood transfusion

Research on **transfusion-related acute lung injury (TRALI)** raised the suspicion that ABTs in general and WBC-containing ABTs in particular mediate their deleterious effect through a pro-inflammatory mechanism. The findings of several observational clinical studies, showing

a higher incidence of multiple organ failure in transfused versus untransfused patients, seem to support this hypothesis. The precise mechanism of this pro-inflammatory state after ABTs is unclear, but most evidence suggests that tissue injury with consequent pulmonary oedema is mediated by reactive oxygen species and proteolytic enzymes released from activated neutrophils [64]. In the context of TRALI, bioactive lipids and cytokines that accumulate during storage of blood products are supposed to prime recipient's neutrophils and activate pulmonary endothelial cells – a mechanism called "non-immune TRALI" [65].
In what is called "immune TRALI", donor's alloantibodies directed against Human Leukocyte Antigens (HLA) or Human Neutrophil Antigens (HNA) bind to recipient's neutrophils and activate them. After pooling in the pulmonary vasculature, the activated neutrophils release inflammatory mediators that lead to vascular leakage and pulmonary oedema [66].

Another relatively common pro-inflammatory state after blood transfusion is **febrile non-hemolytic transfusion reaction (FNHTR)** that is either mediated by pro-inflammatory cytokines that accumulate during storage or by previously formed leukocyte antibodies in the patient or antibodies contained in the donor's blood units. The overall incidence of febrile non-hemolytic transfusion reactions has decreased significantly since leukoreduction has been implemented [67].

3. Clinical studies on transfusion-related immunomodulation

Particular to transfusion medicine is that results from **clinical studies** are quite difficult to analyse and interpret according to the mostly heterogeneous study populations, the lack of a universally applied transfusion trigger and blood product manufacturing that differs from one

country to the other. More specifically, in North America a WBC-containing ABT means unmodified (or "buffy-coat-rich") RBCs, whereas in Europe patients normally receive buffy-coat-reduced RBCs.

By buffy-coat reduction approximately 75% of the donor's WBCs are removed by centrifugation and extraction of the whitish layer in between the plasma and red blood cell layers. Only leukocyte reduction by filtration reduces the number of transfused leukocytes per RBC unit from approximately 10^9 to less than 5 x 10^6. Through leukocyte filtration there is a loss of less than 10% of RBCs and an additional cost of about $35 per unit.

TRIM is known to occur unequivocally, but its clinical significance remains controversial. The contribution of WBCs as a causal agent of TRIM is heavily debated. Assuming that mononuclear cells with cell-bound HLA-antigens are the main responsible constituents of immunomodulation we would expect a better outcome in patients that receive WBC-depleted blood products or autologous transfusions. A TRIM effect by transfusion of soluble HLA peptides would only be prevented by autologous blood, whereas soluble biologic response modifiers could be quantitatively reduced by pre-storage WBC reduction or transfusion of fresh autologous blood. Any differences in outcomes reported following the implementation of pre-storage WBC reduction in "before-and-after" studies can therefore be attributed to allogeneic mononuclear cells and / or WBC-derived soluble mediators, because the patients transfused before or after implementation of WBC reduction differed only in their exposure to WBCs.

Vamvakas et al. [68] categorize in their review the twenty-two available **randomized controlled trials** based on the inference that they permit about possible mediators of TRIM, and examine the strength of evidence available for relying on WBC reduction or autologous

transfusion to prevent TRIM effects (i.e. higher overall mortality risk, higher rate of infectious complications, higher cancer recurrence rate).

Through all randomized controlled trials only in cardiac surgery the findings were conclusive for a deleterious TRIM effect of WBC-containing RBCs with a higher short-term (up to three month post transfusion) mortality rate [69], [70], [71]. Though, even in this setting, the reasons for the excess deaths attributed to WBC-containing ABTs remain elusive. The initial hypothesis was that WBC-containing ABTs may predispose more than leukoreduced ABT to multiple organ failure which, in turn, may predispose to mortality. However, up to now, no randomized controlled trial has demonstrated a higher incidence of multiple organ failure or any particular cause of death in WBC-containing compared to leukoreduced ABTs. The available randomized controlled trials did not show any increased risk of postoperative infection rate or cancer recurrence in WBC-containing ABTs, either.

In the large meta-analysis of Vamvakas et al. no benefit from autologous transfusion in preventing adverse TRIM effects could be found. This is probably due to the long storage time (4 to 6 weeks) autologous blood is usually subjected to. Storage lesions and the accumulation of biologic response modifiers through time seem to abolish the expected advantage of autologous over allogeneic blood.

One reason for the lack of significant differences in outcome between recipients of leukoreduced and WBC-containing blood products may be the fact that randomisation was mostly performed at hospital entry and not only prior to blood component transfusion. From this follows that, in both groups, patients that did not receive any blood transfusion, may have diluted a potential clinical benefit of leukoreduction.

Limiting meta-analyses to patients who actually received transfusions, Blumberg et al. [72] could demonstrate that leukoreduced transfusions significantly reduced the summary odds ratio of postoperative infection (odds ratio 0.52; 95% confidence interval: 0.33-0.82). In a

study by Fergusson et al. [73] the pooled relative risk ratio for transfused patients in regard of postoperative infection was 0.60 (95% confidence interval: 0.38-0.93), although the overall mortality rate remained unchanged by leukoreduction.

Retrospective cohort studies that were performed with data from before and after implementation of universal leukoreduction show controversial results. No clear statement can be made whether universal leukoreduction was indeed associated with any clinical advantage with regard of overall mortality rate, infection rate, or cancer recurrence [74], [75], [76], [77], [78].

To summarize, we conclude that, despite all the published data available today, it still remains unproven that leukoreduction improves morbidity or mortality. Future trials should only randomize patients after a decision is made to transfuse. Since in Western Europe and North America the majority of blood transfusions are now leukoreduced, the undertaking of further randomized controlled trials comparing recipients of WBC-containing ABTs with recipients of leukoreduced ABTs is yet unlikely.

However, several reasons support the implementation of universal leukoreduction without statistical proof: 1) There are some data suggesting benefit; 2) Risks associated with leukoreduction are extremely low; 3) There is no apparent benefit of transfusing the white cells that are filtered; 4) Leukoreduction reduces febrile non-hemolytic transfusion reactions, primary alloimmunization to human leukocyte antigens and refractoriness to random-donor platelet transfusions, and last but not least reduces primary transmission of CMV and other infections due to WBC-associated pathogens.

4. Nosocomial infections

Transfusion of RBCs, independent of WBC count, has emerged as a potential risk factor for nosocomial infection in intensive care patients, trauma patients and cardiovascular surgery patients. Although the exact pathomechanism remains unclear, some sort of TRIM effect seems to be the underlying precondition.

Taylor et al. [42] assessed 1717 patients admitted to a 40-bed medical-surgical-trauma ICU. Nosocomial infection rate was 15.4% in the transfusion group versus 2.9% in the non-transfusion group. A dose-response pattern was apparent, the risk of infection being greater, the more blood units a patient received. For each unit of blood received the odds ratio of developing a nosocomial infection increased by a factor of 1.5. Transfusion was also associated with both increased length of stay in the ICU and in the hospital and higher mortality rates.

In a consecutive study with 2085 patients enrolled, Taylor et al. [79] confirmed their previous findings, the incidence of nosocomial infections being significantly higher in transfused (14.3%) than in non-transfused (5.8%) patients, independent of their predicted survival probability.

Claridge et al. [80] investigated 1593 consecutive adult patients admitted to a level I trauma centre. The infection rate in patients who received at least one unit of RBCs was 33.0% versus 7.6% in patients who did not receive any transfusion. They also reported a linear correlation between the number of transfused blood units and the incidence of infection.

As the two studies mentioned before, Banbury et al. [81] and Sreeram et al. [82] found an increased rate of infections in cardiac surgical patients receiving RBC transfusions.

Shorr et al. [83] looked at the relationship between RBC transfusion and ICU-acquired bloodstream infection in 4892 patients in 284 adult ICUs across the United States. A total of

3.3% of the study population developed an ICU-acquired bloodstream infection. Using a multivariate analysis adjusting for severity of illness, primary diagnosis, use of mechanical ventilation, placement of central venous catheters, and ICU length of stay, three variables were independently associated with diagnosis of a new bloodstream infection: a) baseline treatment with cephalosporins, b) higher organ failure assessment score, and c) RBC transfusion. In a secondary analysis of this same cohort, they noted that transfusion independently increased the risk for ventilator-associated pneumonia (odds ratio 2.16; 95% confidence interval: 1.27-3.66) as well in a positive dose-response relationship.

Hill et al. [84] evaluated in a **meta-analysis of twenty peer-reviewed articles** the association between blood transfusion and postoperative infection. In line with the above mentioned data, they found a direct correlation between postoperative infection rate and blood transfusion.

A restrictive strategy for transfusion of RBCs has been shown to be associated with equal or superior outcome to a liberal transfusion strategy [6]. A lower thrombocyte transfusion threshold has been shown to be safe in terms of bleeding complications and even superior in order to prevent alloimmunization and refractoriness to platelet transfusion [85]. Most clinical uses of fresh frozen plasma, even those currently recommended by practice guidelines, are not supported by evidence from randomized clinical trials [86].

A clear benefit of a liberal transfusion strategy missing, a risk of transfusion-transmitted infection still existing (by primarily infected or secondarily contaminated blood products), and evidence ever growing of transfusion-related immunomodulation with a higher incidence of nosocomial infections in transfused patients, we really need very good arguments to still deliberately transfuse our patients.

Practice Points:

- Transfusion-related immunomodulation is known to occur unequivocally, but its clinical significance remains controversial.
- The precise pathomechanism of transfusion-related immunomodulation is not yet understood: There is evidence that allogeneic blood transfusions may either cause alloimmunization, tolerance, or immunosuppression. However, research on transfusion-related acute lung injury raised the suspicion that allogeneic blood transfusions may rather induce a pro-inflammatory state.
- The specific immunomodulatory constituents are still unknown, though several are highly suspected: allogeneic mononuclear cells, HLA peptides, and soluble biologic response modifiers.
- The contribution of white blood cells as a causal agent of transfusion-related immunomodulation was subject of recently published meta-analysis. Though the results were rather confounding and permitted no clear statement on a possible benefit of leukoreduction on overall mortality, infectious complications, or cancer recurrence rate.
- There is clear evidence that leukoreduction reduces febrile non-hemolytic transfusion reactions and primary alloimmunization to human leukocyte antigens with refractoriness to random-donor platelet transfusions. However, leukoreduction failed to reduce the incidence of transfusion-related microchimerism and transfusion-associated graft-versus-host disease.
- Transfusion of RBCs, independent of WBC count, has emerged as a potential risk factor for nosocomial infection

Research Agenda:

- Further research is warranted to understand the exact pathomechanism of transfusion-related immunomodulation
- Further randomized controlled trials are needed to elucidate the role of donor white blood cells in the pathogenesis of transfusion-related immunomodulation

Summary

In the last decades the risk of acquiring a transfusion-transmitted infection has declined substantially. After human immunodeficiency virus and hepatitis B and C virus transmission were successfully reduced, due to a rising number of immunocompromised transfusion recipients, cytomegalovirus has become an important issue in transfusion medicine. Besides that new pathogens are threatening the safety of blood supply (i.e. West Nile virus, TT virus, SEN virus). Although relatively rare in Western Countries, on a global scale, malaria is one of the most common transfusion-transmitted infections. Despite new standards in blood product manufacturing and storage, bacterial contamination still remains a considerable cause of transfusion-related morbidity and mortality.

Transfusion-related immunomodulation is known to occur unequivocally, but its clinical significance remains controversial and its pathomechanism is not yet precisely understood. There is evidence that allogeneic blood transfusions may either cause alloimmunization, tolerance, or immunosuppression. However, research on transfusion-related acute lung injury indicates that allogeneic blood transfusions may induce a pro-inflammatory state. The specific immunomodulatory constituents are still unknown, though allogeneic mononuclear cells, HLA peptides, and soluble biologic response modifiers are highly suspected. The contribution of white blood cells as a causal agent of transfusion-related immunomodulation remains uncertain and no clear statement on a possible benefit of leukoreduction on overall mortality, infectious complications, or cancer recurrence rate is possible today. However, nosocomial infections are more frequent in transfused than in non-transfused patients.

Transfusing blood products remains potentially harmful and thus should be reduced to a very minimum, also in the light of transfusion-related infectious complications.

Word count: 250

Conflict of interest:

There is no conflict of interest for any of the authors.

References

1. Cane R. Hemoglobin: how much is enough? Crit Care Med 1990;**18**:1046-1047.
2. Madjdpour C, Spahn DR, Weiskopf RB. Anemia and perioperative red blood cell transfusion: a matter of tolerance. Crit Care Med 2006;**34**(5 (Suppl.)):102-108.
3. Consensus conference. Platelet transfusion therapy. JAMA 1987;**257**(13):1777-1780.
4. Consensus conference. Fresh-frozen plasma. Indications and risks. JAMA 1985;**253**(4):551-553.
5. Madjdpour C, Spahn DR. Allogeneic red blood cell transfusions: efficacy, risks, alternatives and indications. Br J Anaesth 2005;**95**(1):33-42.
6. Hébert PC, Wells G, Blajchman MA, et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. N Engl J Med 1999;**340**(6):409-417.
7. Gould S, Cimino MJ, Gerber DR. Packed red blood cell transfusion in the intensive care unit: limitations and consequences. Am J Crit Care 2007;**16**(1):39-49.
8. Group SHoTS. Serious hazards of transfusion. [cited; Available from: www.shotuk.org
9. Davies A, Staves J, Kay J, et al. End-to-end electronic control of the hospital transfusion process to increase the safety of blood transfusion: strengths and weaknesses. Transfusion 2006;**46**(3):352-364.
10. Dzik WH. New technology for transfusion safety. Br J Haematol 2007;**136**(2):181-190.
11. Busch M, Kleinman S, Nemo G. Current and emerging infectious risks of blood transfusions. JAMA 2003;**289**(8):959-962.
12. Cervia J, Wenz B, Ortolano G. Leukocyte reduction's role in the attenuation of infection risks among transfusion recipients. Clin Infect Dis 2007;**45**(8):1008-1013.

13. Pealer L, Marfin A, Petersen L, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 2003;**349**(13):1236-1245.
14. Wang J, Lee C, Kao J, et al. Incidence and clinical presentation of posttransfusion TT virus infection in prospectively followed transfusion recipients: emphasis on its relevance to hepatitis. Transfusion 2000;**40**(5):596-601.
15. Umemura T, Yeo A, Sottini A, et al. SEN virus infection and its relationship to transfusion-associated hepatitis. Hepatology 2001;**33**(5):1303-1311.
16. Kühn J. Transfusion-Associated Infections with Cytomegalovirus and Other Human Herpesviruses. Infusionsther Transfusionsmed 2000;**27**(3):138-143.
17. Ronghe M, Foot AB, Cornish J, et al. The impact of transfusion of leucodepleted platelet concentrates on cytomegalovirus disease after allogeneic stem cell transplantation. Br J Haematol 2002;**118**(4):1124-1127.
18. Bowden R, Slichter SJ, Sayers M, et al. A comparison of filtered leukocyte-reduced and cytomegalovirus (CMV) seronegative blood products for the prevention of transfusion-associated CMV infection after marrow transplant. Blood 1995;**86**(9):3598-3603.
19. Alfieri C, Tanner J, Carpentier L, et al. Epstein-Barr virus transmission from a blood donor to an organ transplant recipient with recovery of the same virus strain from the recipient's blood and oropharynx. Blood 1996;**87**(2):812-817.
20. Wagner H, Klüter H, Kruse A, et al. Relevance of transmission of Epstein-Barr virus through blood transfusion. Beitr Infusionsther Transfusionsmed 1994;**32**:138-141.
21. MacLennan S, Williamson LM. Risks of fresh frozen plasma and platelets. J Trauma 2006;**60**(6 Suppl):S46-50.
22. Chamberland M, Alter H, Busch M, et al. Emerging infectious disease issues in blood safety. Emerg Infect Dis 2001;**7**(3 Suppl):552-553.
23. O'Brien S, Yi Q, Fan W, et al. Current incidence and estimated residual risk of transfusion-transmitted infections in donations made to Canadian Blood Services. Transfusion 2007;**47**(2):316-325.
24. Parsyan A, Candotti D. Human erythrovirus B19 and blood transfusion - an update. Transfus Med 2007;**17**(4):263-278.
25. Hillyer C, Josephson C, Blajchman M, et al. Bacterial contamination of blood components: risks, strategies, and regulation: joint ASH and AABB educational session in transfusion medicine. Hematology Am Soc Hematol Educ Program 2003:575-589.

26. Blajchman M, Beckers E, Dickmeiss E, et al. Bacterial detection of platelets: current problems and possible resolutions. Transfus Med Rev 2005;**19**(4):259-272.
27. Kuehnert M, Roth V, Haley N, et al. Transfusion-transmitted bacterial infection in the United States, 1998 through 2000. Transfusion 2001;**41**(12):1493-1499.
28. Rao PL, Strausbaugh LJ, Liedtke LA, et al. Bacterial infections associated with blood transfusion: experience and perspective of infectious diseases consultants. Transfusion 2007;**47**(7):1206-1211.
29. Fang C, Chambers LA, Kennedy J, et al. Detection of bacterial contamination in apheresis platelet products: American Red Cross experience, 2004. Transfusion 2005;**45**(12):1845-1852.
30. Kitchen A, Chiodini P. Malaria and blood transfusion. Vox Sanguinis 2006;**90**(2):77-84.
31. Nelson J, Kauffmann D, Ciavarella D, et al. Acquired toxoplasmic retinochoroiditis after platelet transfusions. Ann Ophthalmol 1989;**21**(7):253-254.
32. Cardo L, Salata J, Harman R, et al. Leukodepletion filters reduce Leishmania in blood products when used at collection or at the bedside. Transfusion 2006;**46**(6):896-902.
33. Cardo L, Asher L. Transfusion medicine illustrated. Electron micrographic study of the removal of Trypanosoma cruzi from blood products by leukodepletion filters. Transfusion 2006;**46**(7):1067-1068.
34. Llewelyn C, Hewitt P, Knight R, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 2004;**363**(9407):417-421.
35. Peden A, Head M, Ritchie D, et al. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 2004;**364**(9433):527-529.
36. Wroe S, Pal S, Siddique D, et al. Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. Lancet 2006;**368**(9552):2061-2067.
37. team E. Fourth case of transfusion-associated vCJD infection in the United Kingdom. Euro Surveill 2007;**12**(1):E070118.4.
38. Burnouf T, Padilla A. Current strategies to prevent transmission of prions by human plasma derivatives. Transfus Clin Biol 2006;**13**(5):320-328.
39. Opelz G, Sengar D, Mickey M, et al. Effect of blood transfusions on subsequent kidney transplants. Transplant Proc 1973;**5**(1):253-259.
40. Gantt C. Red blood cells for cancer patients. Lancet 1981;**2**(8242):363.

41. Busch O, Hop W, Hoynck van Papendrecht M, et al. Blood transfusions and prognosis in colorectal cancer. N Engl J Med 1993;**328**(19):1372-1376.
42. Taylor RW, Manganaro L, O'Brien J, et al. Impact of allogenic packed red blood cell transfusion on nosocomial infection rates in the critically ill patient. Crit Care Med 2002;**30**(10):2249-2254.
43. Roelen D, van Rood J, Brand A, et al. Immunomodulation by blood transfusions. Vox Sanguinis 2000;**78**(2 Suppl):273-275.
44. (TRAP) TtRAtPSG. Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions. The Trial to Reduce Alloimmunization to Platelets Study Group. N Engl J Med 1997;**337**(26):1861-1869.
45. Andreu G, Dewailly J. Prevention of HLA alloimmunization by using leukocyte-depleted components. Curr Stud Hematol Blood Transfus 1994;**60**:29-40.
46. Naji A. Induction of tolerance by intrathymic inoculation of alloantigen. Curr Opin Immunol 1996;**8**(5):704-709.
47. Mowbray J, Gibbings C, Liddell H, et al. Controlled trial of treatment of recurrent spontaneous abortion by immunisation with paternal cells. Lancet 1985;**1**(8435):941-943.
48. Rothstein D, Sayegh M. T-cell costimulatory pathways in allograft rejection and tolerance. Immunol Rev 2003;**196**:85-108.
49. Mincheff M, Meryman H. Costimulatory signals necessary for induction of T cell proliferation. Transplantation 1990;**49**(4):768-772.
50. Brand A. Immunological aspects of blood transfusions. Blood Rev 2000;**14**(3):130-144.
51. Sho M, Sandner S, Najafian N, et al. New insights into the interactions between T-cell costimulatory blockade and conventional immunosuppressive drugs. Ann Surg 2002;**236**(5):667-675.
52. Dzik WH. Mononuclear cell microchimerism and the immunomodulatory effect of transfusion. Transfusion 1994;**34**(11):1007-1012.
53. Gafter U, Kalechman Y, Sredni B. Blood transfusion enhances production of T-helper-2 cytokines and transforming growth factor beta in humans. Clin Sci (Lond) 1996;**91**(4):519-523.
54. Peters W, Fry R, Fleshman J, et al. Multiple blood transfusions reduce the recurrence rate of Crohn's disease. Dis Colon Rectum 1989;**32**(9):749-753.

55. Lee T, Paglieroni T, Utter G, et al. High-level long-term white blood cell microchimerism after transfusion of leukoreduced blood components to patients resuscitated after severe traumatic injury. Transfusion 2005;**45**(8):1280-1290.
56. Utter GH, Nathens AB, Lee T-H, et al. Leukoreduction of blood transfusions does not diminish transfusion-associated microchimerism in trauma patients. Transfusion 2006;**46**(11):1863-1869.
57. Reed W, Lee T, Norris P, et al. Transfusion-associated microchimerism: a new complication of blood transfusions in severely injured patients. Semin Hematol 2007;**44**(1):24-33.
58. Brand A. Immunological aspects of blood transfusions. Transpl Immunol 2002;**10**(2-3):183-190.
59. Nielsen H, Reimert C, Pedersen A, et al. Time-dependent, spontaneous release of white cell- and platelet-derived bioactive substances from stored human blood. Transfusion 1996;**36**(11-12):960-965.
60. Bury T, Corhay J, Radermecker M. Histamine-induced inhibition of neutrophil chemotaxis and T-lymphocyte proliferation in man. Allergy 1992;**47**(6):624-629.
61. Peterson C, Skoog V, Venge P. Human eosinophil cationic proteins (ECP and EPX) and their suppressive effects on lymphocyte proliferation. Immunobiology 1986;**171**(1-2):1-13.
62. Ghio M, Contini P, Mazzei C, et al. Soluble HLA class I, HLA class II, and Fas ligand in blood components: a possible key to explain the immunomodulatory effects of allogeneic blood transfusions. Blood 1999;**93**(5):1770-1777.
63. Ghio M, Ottonello L, Contini P, et al. Transforming growth factor-beta1 in supernatants from stored red blood cells inhibits neutrophil locomotion. Blood 2003;**102**(3):1100-1107.
64. Anderson B, Harken A. Multiple organ failure: inflammatory priming and activation sequences promote autologous tissue injury. J Trauma 1990;**30**(12 Suppl):S44-49.
65. Silliman CC, Boshkov L, Mehdizadehkashi Z, et al. Transfusion-related acute lung injury: epidemiology and a prospective analysis of etiologic factors. Blood 2003;**101**(2):454-462.
66. Popovsky M, Moore S. Diagnostic and pathogenetic considerations in transfusion-related acute lung injury. Transfusion 1985;**25**(6):573-577.
67. King K, Shirey R, Thoman S, et al. Universal leukoreduction decreases the incidence of febrile nonhemolytic transfusion reactions to RBCs. Transfusion 2004;**44**(1):1-4.

68. Vamvakas E, Blajchman M. Transfusion-related immunomodulation (TRIM): An update. Blood Rev 2007;**21**(6):327-348.
69. Van de Watering L, Hermans J, Houbiers J, et al. Beneficial effects of leukocyte depletion of transfused blood on postoperative complications in patients undergoing cardiac surgery: a randomized clinical trial. Circulation 1998;**97**(6):562-568.
70. Bilgin Y, Van de Watering L, Eijsman L, et al. Double-blind, randomized controlled trial on the effect of leukocyte-depleted erythrocyte transfusions in cardiac valve surgery. Circulation 2004;**109**(22):2755-2760.
71. Wallis J, Chapman C, Orr K, et al. Effect of WBC reduction of transfused RBCs on postoperative infection rates in cardiac surgery. Transfusion 2002;**42**(9):1127-1134.
72. Blumberg N, Zhao H, Wang H, et al. The intention-to-treat principle in clinical trials and meta-analyses of leukoreduced blood transfusions in surgical patients. Transfusion 2007;**47**(4):573-581.
73. Fergusson D, Khanna MP, Tinmouth A, et al. Transfusion of leukoreduced red blood cells may decrease postoperative infections: two meta-analyses of randomized controlled trials. Can J Anaesth 2004;**51**(5):417-424.
74. Baron J, Gourdin M, Bertrand M, et al. The effect of universal leukodepletion of packed red blood cells on postoperative infections in high-risk patients undergoing abdominal aortic surgery. Anesth Analg 2002;**94**(3):529-537.
75. Hébert PC, Fergusson D, Blajchman M, et al. Clinical outcomes following institution of the Canadian universal leukoreduction program for red blood cell transfusions. JAMA 2003;**289**(15):1941-1949.
76. Fergusson D, Hébert PC, Lee S, et al. Clinical outcomes following institution of universal leukoreduction of blood transfusions for premature infants. JAMA 2003;**289**(15):1950-1956.
77. Llewelyn C, Taylor R, Todd A, et al. The effect of universal leukoreduction on postoperative infections and length of hospital stay in elective orthopedic and cardiac surgery. Transfusion 2004;**44**(4):489-500.
78. Phelan H, Sperry J, Friese R. Leukoreduction before red blood cell transfusion has no impact on mortality in trauma patients. J Surg Res 2007;**138**(1):32-36.
79. *Taylor* RW, *O'Brien* J, *Trottier* SJ, et al. Red blood cell transfusions and nosocomial infections in critically ill patients. Crit Care Med 2006;**34**(9):2302-2308.
80. Claridge J, Sawyer R, Schulman A, et al. Blood transfusions correlate with infections in trauma patients in a dose-dependent manner. Am Surg 2002;**68**(7):566-572.

81. Banbury M, Brizzio M, Rajeswaran J, et al. Transfusion increases the risk of postoperative infection after cardiovascular surgery. J Am Coll Surg 2006;**202**(1):131-138.
82. Sreeram G, Welsby I, Sharma A, et al. Infectious complications after cardiac surgery: lack of association with fresh frozen plasma or platelet transfusions. J Cardiothorac Vasc Anesth 2005;**19**(4):430-434.
83. Shorr AF, Jackson WL, Kelly KM, et al. Transfusion practice and blood stream infections in critically ill patients. Chest 2005;**127**(5):1722-1728.
84. Hill G, Frawley W, Griffith K, et al. Allogeneic blood transfusion increases the risk of postoperative bacterial infection: a meta-analysis. J Trauma 2003;**54**(5):908-914.
85. Stroncek DF, Rebulla P. Platelet transfusions. Lancet 2007;**370**(9585):427-438.
86. Stanworth S, Brunskill S, Hyde C, et al. Is fresh frozen plasma clinically effective? A systematic review of randomized controlled trials. Br J Haematol 2004;**126**(1):139-152.

Word count: 7'095 (including abstract, main text, practical points, research agenda, summary, and reference list)