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REVIEW

Clinical effects of leucoreduction of blood transfusions

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ABSTRACT

For many years filtration for removal of leucocytes from red blood cell (RBC) and platelet transfusions was applied for selected patients to prevent cytomegalovirus (CMV) (re)activation, HLA immunisation and recurrent febrile nonhaemolytic transfusion reactions (FNHTR). Since the 1980s, there was also growing concern about cancer recurrence and postoperative infections. In this review we discuss the studies on possible benefits of leucoreduction. In 2001 the Dutch Health Council decided that all blood products should undergo leucoreduction by filtration, as a precautionary measure to reduce possible transmission of variant Creutzfeld-Jacob disease (vCJD). The incidences of transfusion-transmitted CMV infection, HLA immunisation and FNHTR are decreased by universal leucoreduction. However, transfusion-related immunomodulation with presumed negative effects on cancer immunosurveillance, postoperative infections or aggravating organ failure, investigated in randomised controlled trials, revealed no support for extended indications for leucoreduction. An exception was seen in cardiac surgery where leucoreduction reduced short-term mortality by approximately 50%. The exact mechanism(s) for this effect is (are) not known. Pro-inflammatory cytokines induced by leucocyte-containing RBC transfusions in combination with the inflammatory response after cardiac surgery may aggravate morbidity and could lead to mortality.

In this review we discuss the evidence for the benefits of universal leucoreduction. Based on the available evidence, reversal to the use of buffy-coat depleted RBCs and restricted indications for leucoreduction by filtration (extended with open-heart surgery) is a safe option.

KEYWORDS

Blood transfusion, leucoreduction, clinical effects

INTRODUCTION

In the 1970s awareness grew that leucocytes present in blood components intended for transfusion could transmit cytomegalovirus (CMV), evoke human leucocyte antigen (HLA) antibodies and induce immunosuppression. Leucocyte antibodies can cause febrile transfusion reactions, platelet transfusion refractoriness and for dialysis patients prolong the waiting time for a suitable, cross-match negative, renal transplant. Seemingly in contrast, patients receiving a kidney allograft showed improved graft outcome after pretransplant blood transfusions.[1] This observation caused concern for possible impairment of immune surveillance against cancer and susceptibility for postoperative infections.[2] This initiated research on the role of allogeneic leucocytes in blood components to remove these leucocytes by centrifugation and later by filtration, with gradually increasing efficiency from 1-log up to 4-log reduction. The presumed adverse and beneficial effects of passenger leucocytes in blood transfusions are referred to as transfusion-related immunomodulation (TRIM).

Besides leucoreduction of platelet transfusions, indications for leucoreduced (LR) red blood cells (RBCs) were initially restricted to patients with a high risk for the sequels of HLA antibodies and CMV transmission, e.g. intrauterine transfusions, prematurely born infants, patients needing platelet transfusions and patients with or awaiting an organ transplant. In addition, patients who suffered twice from a febrile non-haemolytic transfusion reaction (FNHTR) further received LR blood components. In 2001, as a precautionary measure to reduce possible transmission of variant Creutzfeldt-Jakob disease (vCJD), the Dutch Minister of Health ordered that all blood products should be leucocyte depleted. Transmission of vCJD is, a decade later, no longer considered to be a serious transfusion risk to justify universal leucoreduction (ULR). In this review we discuss studies on other possible clinical benefits of leucoreduction.

STUDIES ON CYTOMEGALOVIRUS TRANSMISSION

After infection, CMV is latently present in mononuclear cells lifelong. When T lymphocyte mediated control is lost due to immune suppressive treatment, endogenous CMV replication can lead to CMV disease. CMV disease in immunocompromised patients is life-threatening. Foetuses and prematurely born infants and CMV-negative recipients of solid organs or haematopoietic stem cells of CMV-negative donors are at risk to acquire primary transfusion transmitted (TT)-CMV infection. Besides TT-CMV infection, another concern was that immunomodulation by allogeneic leucocytes in blood components would stimulate endogenous virus replication in CMV-seropositive recipients. To prevent TT-CMV infection, seronegative donors (approximately half of the donor population) can be selected. Leucoreduction, removing leucocytes harbouring latent CMV, is another option. A systematic review of studies in newborns on leucoreduction reported a clinically relevant but not significant (OR: 0.19; 95% CI: 0.01 to 3.41) possible reduction of TT-CMV infection.[3] However, studies not conducted in the Western world often find no benefit of leucoreduction, attributed to a high level of community-acquired CMV infections in areas where CMV is more endemic.[4]

The question also arose whether leucoreduction and CMV-seronegative donor selection are equally safe. A systematic review of all available studies until 2005, mainly conducted in bone marrow transplant (BMT) recipients, showed that compared with non-CMV screened and non-LR transfusions both approaches showed a huge and significant 92 to 93% reduction of TT-CMV infection. After transfusion of blood from CMV-seronegative donors an incidence of TT-CMV infection was observed in 1.45% of 829 recipients (11 studies) and after LR transfusions in 2.73% of 878 recipients (12 studies).[5] Three controlled studies compared selection of CMV-seronegative donors with leucoreduction by filtration in BMT patients.[6-8] A meta-analysis of the three studies revealed that CMV-seronegative transfusions compared with LR transfusions was associated with a 58% reduction in CMV risk (OR: 0.42; 95% CI: 0.22 to 0.79).[5] Recent prospective studies, using CMV-PCR, even observed a higher CMV conversion rate. Of 46 haemato-oncological patents, three (6.5%) became CMV-PCR positive. They had received 1316 blood products of which 460 derived from CMV-seropositive donors, suggesting a transmission rate of 0.65%/product. Because community-acquired CMV could have occurred during the study period spanning two years, the incidence of CMV transmission may be less, but this study illustrates that leucoreduction does not provide complete elimination of CMV transmission.[9] Besides technical failures, non-cell bound CMV in plasma that can be detected in donors up to a year after primo-infection might be a cause.[10] The VATS (Viral Activation Transfusion Study), a randomised controlled trial in human immunodeficiency virus (HIV) positive anaemic patients, showed that the viral replication rate of CMV and HIV was not enhanced after standard, non-LR RBC compared with LR-RBC transfusions, offering no support that allogeneic leucocytes lead to stimulation of endogenous CMV activation.[11] A recent survey of current practice in the United States on the prevention of TT-CMV infection showed that 65% of responding institutions considered both methods to be equally safe.[12] In the Netherlands, a coupled transfusion strategy (seronegative donor selection and leucoreduction) is advised for very high-risk patients, such as patients needing intrauterine transfusions and very low birth weight newborns, while transplant patients receive only LR blood components.[13] Although leucoreduction significantly reduces TT-CMV infection the question whether it is as safe as selection of CMV-seronegative donors has not been solved.

STUDIES ON FEBRILE NON-HAEMOLYTIC TRANSFUSION REACTIONS

Febrile non-haemolytic transfusion reactions (FNHTR) are the most common transfusion reactions.[14] FNHTR results from leucocytes in transfused blood destroyed by antibodies in the recipient, generating pyrogens *in vivo* or by pyrogenic cytokines such as IL-6, IL-8, TNF-α, IL-1β and CD40L, which are released during storage by contaminating leucocytes and platelets.[15,16] In a multivariate analysis the storage duration of RBCs before transfusion was identified as a more significant factor associated with FNHTR than leucocyte contamination.[17] In a randomised controlled trial (RCT) the supernatants of stored non-LR platelets and not the platelets themselves caused febrile reactions.[18] RCTs on the incidence of FNHTR after LR components as compared with standard products are scarce. In an RCT using FNHTR after platelet transfusions as primary endpoint a modest absolute decrease of 11.7% by leucoreduction was found.[19] Two large RCTs in multi-transfused patients evaluating transfusion reactions as secondary endpoint, the VATS[11] and the TRAP study (Trial to Reduce Alloimmunization to Platelets)[20] did not find a decrease of FNHTR, either for leucoreduction of red cells[11] or for leucoreduction of platelets.[20]

Because FNHTR in incidentally transfused patients is a rarer event, comparative studies would require large patient numbers. Such studies were mainly conducted as 'before-and-after ULR' retrospective studies. In the John Hopkins Hospital (Baltimore, Maryland, USA) a study was conducted comparing the year 1994 (before) with 2001 (after) evaluating more than 35,000 RBC transfusions. This study reports a reduction of FNHTR from 0.37 to 0.19% (p=0.0008).[21] A similar retrospective analysis from Canada comprising over 140,000 RBC and over 57,000 platelet units, reported a reduction of 0.33 to 0.11% in favour

of LR-RBC and 0.45 to 0.19% for LR platelets (p<0.001).[22] The introduction of ULR more than halved FNHTR in these studies, but it should be noted that in both of these large surveys the non-LR transfusions were not buffy-coat reduced (removing approximately 60% of the leucocytes and 90% of the platelets) as was standing practice in most European countries, including the Netherlands. Residual FNHTR to LR blood components may arise from small amounts of residual leucocytes in case of heavily immunised patients with strong antibodies or from soluble factors (IL-8, sCD40-ligand) released by leucocytes and platelets in the hours prior to filtration or during storage of platelets.[16,23,24] In case of platelet transfusions, which are leucoreduced to prevent alloimmunisation, reduction of storage interval or even washing before transfusion may limit FNHTR and other adverse events.

STUDIES ON TRANSFUSION-RELATED LUNG INJURY

Transfusion-related lung injury (TRALI) is a life-threatening transfusion reaction with an estimated incidence of 1:1000 to 5000 plasma-containing blood transfusions. TRALI is defined as non-cardiogenic lung oedema presenting within six hours after completion of transfusion. Although strong leucocyte-reactive antibodies in donor plasma can cause TRALI, the syndrome is more often the result of two or multi-hit events. Endogenous neutrophil priming associated with the patient's underlying illness, combined with biological response modifiers (BRMs) in blood products, result in neutrophil-induced pulmonary endothelial damage leading to capillary leakage. Besides leucocyte-reactive antibodies present in donor plasma, soluble factors accumulating during storage of red cells and platelet products have been associated with TRALI.[24-27] Although the presence of leucocytes contributes to the generation of BRMs during storage enhancing accumulation of lipid-priming agents and lysophosphatidylcholines (lyso-PC) as neutrophil-priming factors, sCD40L and other cytokines released by platelets may play a key role in endothelial activation causing TRALI.[25,28-30] To further reduce FNHTR and TRALI, removal of plasma or washing of blood components before transfusion has been proposed.[26,31]

STUDIES ON PLATELET TRANSFUSION REFRACTORINESS

Patients who receive platelet transfusions can develop refractoriness, defined as lack of post-transfusion increment and mostly resulting from clinical causes increasing platelet turn-over. Anti-HLA class I antibodies are the major cause of immunological platelet transfusion refractoriness. Platelets strongly express HLA class I antigens and depending on the strength of allo-antibodies, transfused incompatible platelets are immediately or more slowly destroyed. The immune response to foreign HLA antigens differs from an immune response against all other antigens, which is dependent on the indirect pathway. In the indirect pathway, foreign antigen is processed to peptides and presented by self HLA class II antigens on antigen presenting cells (APCs) and activates self CD4+ T-cells. Through the direct pathway, foreign donor HLA class II expressing APCs directly stimulate recipient CD4+ cells approximately 100 times more efficiently than by the indirect pathway.[32] In peripheral blood, dendritic cells, monocytes and B-cells but not platelets constitutionally express HLA class II antigens. Removal of class II bearing white cells virtually abolishes HLA class I immunisation by platelets. The mechanism has not been completely unravelled, but an active process is presumed requiring even a low number of donor leucocytes.[33]
Besides foreign HLA antigens (signal 1) also co-stimulatory molecules are necessary (signal 2) for an effective APC-CD4+ T-cell interaction. After approximately two weeks of storage, leucocytes lose expression of co-stimulatory molecules associated with impaired immunogenicity.[34,35] Experiments in the mouse confirmed that, dose-dependently, addition of viable leucocytes but not non-viable leucocyte (fragments) to a platelet suspension, strongly enhanced antibodies against HLA class I antigens.[36]

After an initial observational study,[37] from 1983 to 1995, six (five small European) RCTs with a sample size of seven to 46 patients and in total comprising 295 patients (140 after prior exposure to pregnancy and/or transfusions and 155 naive patients) compared leucocyte-reduced with standard platelet transfusions. A meta-analysis of these early studies concluded on a 68% reduction of risk for platelet refractoriness (95% CI: 0.18 to 0.56) by LR platelet transfusions.[38] In 1997 the results of a larger study from the US, the TRAP study,[20] comprising 400 patients, came to a similar outcome of a highly significant 74% reduction in platelet refractoriness. In both the combined European studies and the US TRAP study immunological naive patients benefited most from leucoreduction (>85% reduction in refractoriness) as compared with patients with prior pregnancies (circa 50% reduction).[38] Since leucoreduction of platelet transfusions, refractoriness towards random platelet transfusions due to HLA antibodies affects less than 5% of the patients.[39]

STUDIES ON TRANSFUSION-INDUCED ALLOIMMUNISATION

Few studies investigated HLA immunisation after LR RBC transfusions. An observational before-and-after ULR study in RBC-transfused dialysis patients found no reduction in

HLA antibodies.[40] An RCT comparing buffy-coat depleted versus LR RBC in cardiac surgery patients observed not only a similar incidence of HLA antibodies in both groups, but also of RBC antibodies.[41]

Red blood cells carry very few HLA class I antigens, but maybe their longer survival time creates a better opportunity for indirect presentation compared with short-living platelets. The presence of allogeneic leucocytes in RBC transfusion was also presumed to enhance antibodies against RBC antigens. Data in animal studies suggested that a concomitant inflammatory reaction as danger signal, as could be given by transfusion of allogeneic leucocytes, would enhance RBC alloimmunisation.[42] Observational studies on this subject are not equivocal.[43] A large Dutch before-and-after ULR study comparing buffy-coat depleted RBC and LR RBC found no difference in RBC alloimmunisation.[44]

STUDIES ON PRETRANSPLANTATION BLOOD TRANSFUSIONS

Pretransplantation third-party blood transfusion reducing kidney graft rejection has been investigated in only three RCTs of different designs.[45-47] One study in 52 patients compared the effect of standard unmodified RBCs with buffy-coat-poor or washed RBC on the development of HLA antibodies and graft survival. No difference in outcome was observed, but the leucoreduced products did not meet the standards ($<10^6$ leucocytes/unit) and all products may have been equally effective.[45] In a multicentre randomised study in 423 prospective cadaver kidney transplantation patients, a better one-year (90 *vs* 82%; p=0.02) and five-year (79 *vs* 70%; p=0.025) graft survival was observed after three random pretransplantation transfusions of unmodified RBCs compared with no transfusions.[46] Also severe rejections were significantly reduced in patients receiving RBCs. In a third multicentre 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 for less than 72 hours. There was no difference in graft survival at one year (90, 92 and 92%) or at five years (79, 84 and 80%) respectively. The incidence of acute rejections in patients who had received an HLA-DR shared transfusion was lower than observed in the other two groups (19 *vs* 33%), but this was not statistically significant in this small study.[47] The three studies do not allow a combined analysis because of heterogeneity in design and the different immunosuppressive protocols and blood products used. Although the largest study found a protective effect of blood transfusion on renal graft survival,[46] the smaller study designed on the presumed mechanism of induction of allograft tolerance by HLA-DR sharing blood transfusions was not supportive.[47] Lacking more confirmatory studies an evidence-based conclusion on graft-tolerising effect by pretransplant allogeneic leucocytes in blood products is as yet not possible.

STUDIES ON CANCER RECURRENCE

After the finding that blood transfusions could improve renal transplant outcome,[1] concern was expressed of a deleterious effect of (leucocyte-containing) transfusions on cancer immune surveillance.[2] This initiated over 100 retrospective and observational clinical studies, not resulting in conclusive data.[40] In contrast few RCTs in colorectal cancer surgery with curative intent have been conducted to compare leucocyte-containing (buffy-coat depleted) with LR RBCs. Local and distant cancer recurrence was similar in both groups at short-term and at five-year follow-up.[49-52] It may be noted that colorectal cancer is a weakly immunogenic tumour and that the malignant cells can downregulate HLA expression and co-stimulatory molecules allowing tumour cells to escape from immune attack, whether or not the immune response is suppressed by transfusions.[53] An immunosuppressive effect of leucocyte-containing RBC transfusions on cancer immunosurveillance has been shown to be absent for colorectal cancer; it has never been shown in other malignancies either, although because of lack of studies it can still not be excluded.

STUDIES ON POST-TRANSFUSION INFECTIONS

Another concern of the immunosuppressive effect of leucocyte-containing RBC transfusions was susceptibility for infections, in particular in the postoperative period. Sixteen RCTs, conducted in various clinical settings, evaluated post-transfusion infections as primary or secondary endpoint after LR RBC transfusions; six in colorectal surgery,[49,50,54-57] six in open heart surgery[58-63] and four in miscellaneous conditions.[11,64-66] These studies varied as to single or multiple centre design, clinical diagnosis, methods to document infections and proportion of transfused patients ranging from 14 to 95% and revealed different outcomes (*table 1*).

Several meta-analyses were performed but these came to different conclusions. Meta-analyses using intention-to-treat analyses seldom found associations between LR transfusions and postoperative infections.[67] The other meta-analyses, restricted to transfused patients only, thereby excluding 36% of the study population, reported up to almost 60% reduction in postoperative infection after transfusion of LR RBC.[68] A role of leucocyte-containing RBCs on the increase

Table 1. *Characteristics of patients participating in RCTs comparing leucodepleted versus leucocyte-containing RBCs*

Author; year	No. patients/ No. transfused (%)	Clinical setting	Transfused patients	No. RBCs mean±SD or median (range)	Transfused patients with >4 RBC (%)	Main endpoints	Results (LD vs BCD)
Jensen *et al.*; 1992 [54]	197/ 104 (53)	Colorectal surgery	LD 48 WB 56	LD 2 (1-4) WB 2 (1-5)	ND	Infections	0.2 vs 23%[b]
Houbiers *et al.*; 1994 [49]	697/446 (64)	Colorectal cancer surgery	LD 216 BCD 230	LD 3 (1-10) BCD 3 (2-11)	LD 104 (31) BCD 94 (26)	Cancer recurrence Infections	30 vs 32% 36 vs 32%
Jensen *et al.*; 1996 [55]	586/ 260 (44)	Colorectal surgery	LD 118 BCD 142	LD 2 (1-5) BCD 2 (1-6)	ND	Infections Mortality	3.0 vs 23%[b] 3.4 vs 2.8%
Tartter *et al.*; 1998 [56]	221/ 59 (27)	Colorectal surgery	LD 25 BCC 34	ND	ND	Infections	15 vs 44%[b]
Titlestad *et al.*; 2001 [57]	279/ 112 (45)	Colorectal surgery	LD 48 BCD 64	LD 3 (2-4.3) BCD 3 (2-6)	ND	Infections	45 vs 37%
van Hilten *et al.*; 2004 [65]	1051/ 545 (52)	Colorectal cancer surgery and aortic aneursm	LD 267 BCD 278	LD 3.5 BCD 3.5	LD 62 (23) BCD 58 (21)	Infections Hospital stay MODS Mortality	23 vs 23% –2.4 days[b] 14 vs 17%[b] 10.3 vs 8.4%
Skanberg *et al.*; 2007 [50]	642/298 (46)	Colorectal cancer	LD 137 BCD 161	LD 3.6 ± 0.3 BCD 3.6 ± 0.3	ND	Respiratory support Hospital stay Mortality	3.6 vs 8.1% 15.5 vs 15.5 days 52.5 vs 49.7%
Nathens *et al.*; 2006 [66]	1864/ 268 (14)	Trauma patients	LD 136 BCC 132	LD 9.2 ± 9.6 PC 8.6 ± 9.9	ND	Infections MODS Mortality ALI	30 vs 36% 5.9 vs 6.6% 22 vs 19% 42 vs 43%
van de Watering *et al.*; 1998 [58]	914/ 866 (95)	CABG ± valve surgery	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)	Infections Mortality	17 vs 18 vs 23% 3.6 vs 3.3 vs 7.8%[b]
Bracey *et al.*; 2002 [59]	357/ 295 (83)	CAGB ± valve surgery	LD 136 BCC 159	LD 3 PC 3	ND	Infections Mortality ICU/Hospital stay	ns; data ND 5.9 vs 7.5% ns; data ND
Wallis *et al.*; 2002 [60]	597/ 409 (69)	CABG ± valve surgery	LD 176 BCC 175 PR 158	WBF 3.9 ± 3.9 BCD 3.5 ± 2.6 PC 2.9 ± 1.8	ND	Infections Mortality	49 vs 38 vs 35% 0.5 vs 2.9 vs 2.5%[b]
Bilgin *et al.*; 2004 [61]	474/ 432 (91)	Valve surgery ± CABG	LD 216 BCD 216	LD 6.2 ± 7.1 BCD 5.9 ± 6.1	LD 145 (67) BCD 131 (61)	Infections MODS Mortality	23 vs 32%[b] 20 vs 21% 8.4 vs 12.7%
Connery *et al.*; 2005 [62c]	98/ 69 (70)	Primary CABG	LD 38 BCC 31	LD(SF) 5.6± 13 PC 5.6±10	LD 16 (42) PC 15 (48)	Infections Mortality	13 vs 26% (PTI: 0 vs 13%[b]) 2.6 vs 3.2%
Boshkov *et al.*; 2006 [63]	1227/ 562 (46)	CABG ± valve surgery	LD 304 BCC 258	ND	ND	Mortality	4.9 vs 9.7%[b]
Dzik *et al.*; 2002 [64]	2780 (100)	All patients	LD 1355 BCC 1425	LD 2 (1-9) PC 2 (1-9)	LD 498 (35) PC 474 (35)	Mortality Hospital stay Antibiotics	9 vs 8.5% 8.8 vs 8.9 days 31.5 vs 34%
Collier *et al.*; 2001 [11]	531/524 (99)	HIV-positive	LD 259 BCC 262	Mean 7.3	ND	Mortality HIV RNA level	58% vs 53% Similar

[a]Data on ALI were reanalysed and presented in another publication[33] than the initial publication.[18] [b]Statistically significant (p<0.05) between BCD and LD (SF+FF). [c]This RCT was interrupted early. **ALI = acute long injury; BCC = buffy-coat-containing RBCs; BCD = buffy-coat depleted RBCs; FF = fresh filtered RBCs; LD = leucodepleted RBCs; MODS = multi-organ dysfunction syndrome; ND = not documented; ns = not significant; PR = plasma-reduced RBCs; PTI = pulmonary tract infections; SF= stored filtered RBCs; WB = whole blood; WBF = white blood cell filtered.**

of postoperative bacterial infections is not proven beyond reasonable doubt. However, only a few studies adjusted for the number of administered RBC, which may be an important factor as observed in cardiac surgery patients.

STUDIES ON MORTALITY AFTER SURGERY

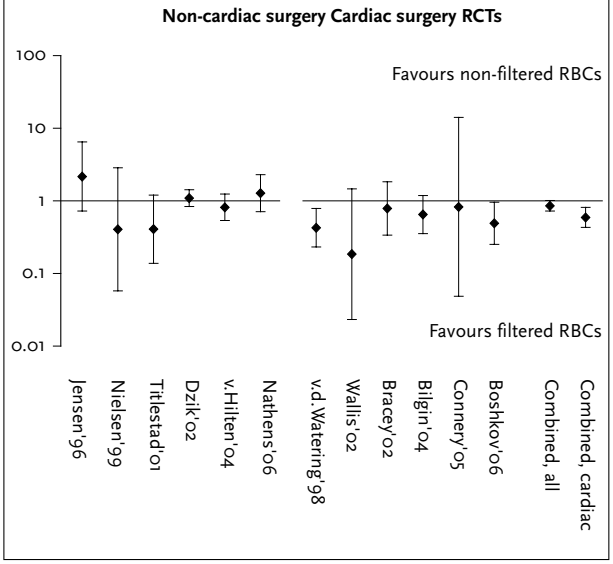
A Dutch randomised controlled trial, aimed to investigate development of HLA-antibodies and postoperative infections after RBC transfusions in cardiac surgery, found surprisingly a higher mortality rate in patients receiving leucocyte-containing RBC transfusions.[58] Mortality due to multi-organ dysfunction syndrome (MODS) was the major cause of excess deaths after non-LR transfusions. In this study patients were randomised to three different blood products; buffy-coat-depleted (BCD) RBCs were compared with two filtered RBCs: either fresh filtered RBCs before storage (FF) or stored filtered RBCs (SF). Between the two types of filtered RBCs the mortality rate was not different. This suggests that soluble mediators, still present in the SF products, caused no more adverse effects than FF RBCs, lacking leucocyte-derived soluble

factors. A subsequent Dutch RCT conducted in high-risk cardiac surgery (anticipating higher transfusion needs) investigated the effect of leucoreduction on the incidence of MODS, but found no difference in incidence (circa 20%) after leucocyte-containing RBCs or LR RBCs.[61] 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 three RBC units suffered higher mortality in the group receiving BCD RBC.

In total 12 RCTs investigated mortality in different clinical settings: six in cardiac surgery[58-63] and six in other settings.[55,57,64-66,69] Overall, no adverse effect of leucocyte-containing transfusions on short-term mortality has been found (OR: 1.14; 95% CI: 0.89 to 1.45). This meta-analysis identified an exception for cardiac surgery (OR: 1.72; 95% CI: 1.05 to 2.81) (*figure 1.*)[67]

The observation that it is not the soluble mediators released by leucocytes during storage but rather the number of units transfused that entails the worse outcome,[58,61] suggests that more complex surgical patients requiring more RBC transfusions are more susceptible to TRIM. We analysed in more detail the causes of death in our two RCTs in cardiac surgery.[70] This revealed an excessive mortality rate in patients who received standard BCD RBCs, compared with before storage filtered LD RBCs; these patients died from a combination of MODS and the presence of infections in the postoperative period (OR: 2.92; 95% CI: 1.22 to 6.97; p=0.02). All other causes of short-term mortality, such as bleeding, cardiac causes, surgical complications, postoperative infections alone and MODS without infections, were equal in both transfusion arms.

Figure 1. *Short-term mortality in RCTs comparing filtered RBCs and non-filtered RBCs (OR, 95% CI)*



Comparison of long-term mortality after transfusions of BCD RBCs or LR RBCs has only been investigated after colorectal cancer surgery, which observed no difference in survival.[51] Although in cardiac surgery the long-term survival is negatively influenced after perioperative allogeneic blood transfusions as compared with nontransfused patients,[71] the long-term effect of allogeneic leucocytes in RBCs after cardiac surgery is not known.

COST-EFFECTIVENESS OF LEUCODEPLETION

Analyses on the cost-effectiveness of leucodepletion are scarce and are mainly based on observational data, mainly in selected patient cohorts. Leucoreduction of whole blood was associated with lower hospital costs than leucocyte-containing blood transfusions in colorectal surgery[72] and leucoreduction of platelets was cost-beneficial in the treatment of acute myeloid leukaemia and lymphoma.[73] The cost-effectiveness in cardiac surgery was analysed based on data derived from two studies performed in the Netherlands.[58,61] The results showed that RBC leucodepletion was cost-effective. The benefit of leucodepletion of RBCs was between $220 and $310 US per life-year gained in coronary artery bypass graft patients[74] and $214 US per cardiac valve surgery patient, on average.[75] Only one RCT is available that can be applicable to estimate the general costs of ULR. In this study all patients in the Massachusetts General Hospital (Boston, Massachusetts, USA) were randomised to standard RBC and LR-RBCs. No clinical benefit but also no increase of costs were associated with LR.[64]

POSSIBLE MECHANISMS OF TRANSFUSION-RELATED IMMUNOMODULATION

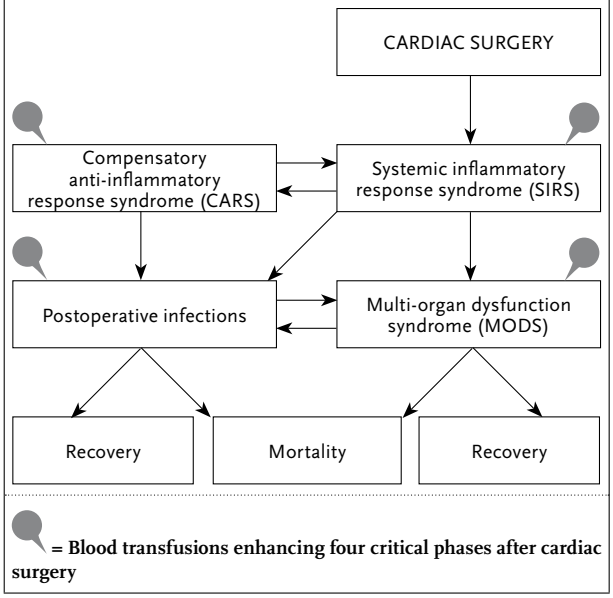
From the mentioned RCTs, a negative effect of allogeneic leucocytes in RBC transfusions was only found in open heart surgery showing (transfusion dose related) excess mortality from postoperative infections in patients who had developed MODS, indicative for a two- or multiple-hit event. This suggests a synergistic effect between transfusion-related immunomodulation and excessive tissue damage and/or with extracorporeal bypass. In cardiac surgery blood is exposed to the extracorporeal circuit, hypothermia and ischaemia/reperfusion injury. Tissue damage generates products and exposes structures of degraded tissue (e.g. heat-shock proteins, proteases) interacting with sensors (Toll-like receptors) on macrophages leading to immediate release of stress

hormones, inflammatory cytokines and chemokines.[76] Besides release of cortisol, serotonin, TNF-α, IL-1-α, IL-6 and IL-8, the coagulation and complement systems are activated.[77]

Cardiopulmonary bypass surgery always leads to a systemic inflammatory response syndrome (SIRS). SIRS is characterised by two or more of the following criteria: hypothermia (temperature less than 36°C) or fever above 38°C, tachycardia more than 90 beats/min, tachypnoea (more than 20/min) or pCO_2 less than 4.4 kPa (32 mm Hg), leucopenia below 4 x 10^9/l or leucocytosis above 12 x 10^9/l. SIRS reflects a cytokine storm with an abnormal regulation of cytokines and is immediately counteracted by a compensatory anti-inflammatory response syndrome (CARS).[78] 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-α pathway, impairing natural defence against invading micro-organisms.[79] 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. Cytokine profiles have been extensively investigated during and after cardiac surgery. A study that evaluated the cytokine pattern up to 48 hours after CABG surgery in 24 patients recovering uneventfully from SIRS shows that cardiac surgery immediately evokes a biphasic cytokine response.[80]

In patients participating in an RCT comparing LR RBCs with BCD RBCs the pro- and anti-inflammatory cytokine profiles were investigated.[81] The analyses revealed that patients who would develop infections had higher IL-6 and patients who would develop MODS higher IL-12 concentrations in the group that received more than three units of leucocyte-containing RBCs. These findings support that leucocyte-containing blood transfusions amplify an inflammatory response in addition to an ongoing SIRS induced by cardiac surgery. This may lead to a more profound CARS associated with enhanced susceptibility for postoperative infections. Leucocyte-containing RBC transfusions to patients with an activated inflammatory response seems to imbalance the postoperative SIRS-CARS equilibrium by initially aggravating SIRS. The findings that fresh-filtered RBCs and after storage-filtered RBCs both reduced postoperative complications compared with leucocyte-containing RBCs suggests that not the soluble mediators accumulated in stored RBCs but the allogeneic leucocytes are the culprit in these clinical effects of transfusion-related immunomodulation.[82] The possible mechanisms leading to mortality in association with allogeneic (leucocyte-containing) RBCs after cardiac surgery are shown in *figure 2*.

Figure 2. *Possible mechanism between allogeneic blood transfusions and mortality in cardiac surgery*



= Blood transfusions enhancing four critical phases after cardiac surgery

In addition to the role of leucocyte-containing RBC transfusions to postoperative mortality after cardiac surgery, we found an independent role for platelet transfusions enhancing mortality.[83] Platelets expressing CD40L upon activation (in the extracorporeal bypass circuit as well as during storage of platelet products) are presumed to play a vital link between coagulation and inflammation and may enhance microthrombi and venous thromboembolism, in particular under changing rheological conditions.[84-92] Both thrombi and infection play a pivotal role in the development of MODS and mortality.[87,88]

CONCLUSIONS

We have reviewed the state of evidence for other benefits of universal leucoreduction. We conclude that: 1) For prevention of TT-CMV infection, in addition to leucoreduction, selection of CMV-seronegative donors is still applied for high-risk patients. 2) Leucoreduction of platelet transfusions is significantly associated with a large reduction of HLA-antibody formation and refractoriness to random donor platelet transfusions; however, ULR does not seem to prevent HLA antibodies after RBC transfusions and does not influence alloimmunisation against RBC antigens. 3) Universal leucoreduction halves the incidence of FNHTR, but cytokines and chemokines accumulating during storage of cellular blood products are responsible for residual FNHTR and TRALI. 4) Transfusion-related

immunomodulation with presumed negative effects on cancer immunosurveillance, postoperative infections or aggravating organ failure, investigated in randomised controlled trials, revealed no support for extended indications for leucoreduction except in cardiac surgery using extracorporeal bypass circulation where leucoreduction of RBCs reduced short-term mortality by approximately 50%, resulting from a combination of MODS and infections. This likely represents a multi-hit synergy between pro-inflammatory cytokines induced by leucocyte-containing transfusions, deepening of compensatory immunosuppression enhancing infections, in combination with activated platelets that may aggravate micro-thrombosis leading to MODS mortality.

Recently, abolishment of universal leucoreduction and going back to leucoreduction for the classical indications, adjusted with open heart surgery has been questioned.[93] Based on the available evidence, restriction of indications for leucoreduction and reversal to the use of buffy-coat depleted RBCs is a safe option.

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