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REVIEW

Leucoreduction of blood components: an effective way to increase blood safety?

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Abstract

Over the past 30 years, it has been demonstrated that removal of white blood cells from blood components is effective in preventing some adverse reactions such as febrile non-haemolytic transfusion reactions, immunisation against human leucocyte antigens and human platelet antigens, and transmission of cytomegalovirus. In this review we discuss indications for leucoreduction and classify them into three categories: evidence-based indications for which the clinical efficacy is proven, indications based on the analysis of observational clinical studies with very consistent results and indications for which the clinical efficacy is partial or unproven.

Keywords: blood component transfusion, leucocyte reduction procedures, leucocyte reduction filtration, cost effectiveness, transfusion reactions.

Introduction

Leucocytes and leucocyte-derived cytokines are associated with several adverse outcomes of transfusion therapy[1]. Leucoreduction (LR) is the reduction of white blood cell (WBC) concentration in blood components, namely red blood cell concentrates (RBC), platelet concentrates (PC) and plasma obtained from the fractionation of whole blood or apheresis. There are many methods of LR but, currently, this process may be performed using selective LR filters, which enable less than 1×10^6 residual WBC to be obtained in a RBC or PC unit.

Over the past 30 years, it has been demonstrated that LR can reduce some adverse reactions due to blood component transfusion such as febrile non-haemolytic transfusion reactions (FNHTR), immunisation against human leucocyte antigens (HLA) and human platelet antigens (HPA), which may cause refractoriness to platelet transfusion, and transmission of cytomegalovirus (CMV)[2,3]. Furthermore, LR improves the clinical outcome, in terms of reducing mortality and post-operative infections, in patients undergoing cardiac surgery[2,3].

As far as concerns transfusion-related immunomodulation, risk of multi-organ failure and mortality in surgical patients, as well as the prevention of transmission of some viruses and prions, the possible benefits associated with LR are supported by less strong (or only experimental) evidence[4,5].

In this article, which deals with the role and cost-effectiveness of LR in contributing to the safety of blood transfusion, we review the indications for LR and arbitrarily classify them into three categories: evidence-based indications for which the clinical efficacy has been proven (based on results from randomised clinical trials [RCT] or meta-analyses), indications based on the analysis of observational clinical studies with very consistent results[6] and indications for which the clinical efficacy is partial or unproven (based on observational studies with less consistent results, cohort studies or case series).

Evidence-based indications for which the clinical efficacy has been proven

Prevention of immunisation against human leucocyte antigens and human platelet antigens, and platelet refractoriness

Platelet refractoriness may be due to several mechanisms: non-immune conditions such as splenomegaly, fever and/or sepsis, antibiotics, disseminated intravascular coagulation and complement-mediated destruction, as well as immune reactions such as alloimmunisation caused by previous pregnancies, transfusion or organ transplantation[7].

HLA immunisation is a complication of transfusion therapy which can lead to refractoriness to platelet transfusion, especially in onco-haematological patients. From 20 to 60% of these patients may develop this condition due to multiple transfusions[8]. Alloimmunisation occurs most commonly against HLA class I antigens, although HPA may also be involved[9].

The risk of alloimmunisation against HLA antigens and the consequent risk of platelet refractoriness were first assessed in the 1990s[10-16]. In 1988, an early study

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by Brand and co-authors showed that leucocyte-depleted RBC and multiple random donor platelet transfusions were very unlikely to induce primary immunisation to HLA and non-HLA platelet-reactive antigens[10]. Three years later, in a randomised trial, Oksanen *et al.* administered WBC-reduced PC and RBC to adult patients with acute leukaemia and non WBC-reduced blood components to control patients[11]. Patients in the former group did not develop platelet refractoriness whereas one patient in the control group became refractory and two developed transient HLA antibodies. Van Marwijk Kooy *et al.* conducted a prospective RCT to assess the role of LR in preventing HLA immunisation and platelet refractoriness[12]. Adult patients with acute leukaemia were transfused with PC prepared either by centrifugation (control group) or filtration (study group). Both groups received RBC that had been filtered, after buffy coat removal. Refractoriness occurred in 46% of the evaluable control patients and in only 11% of the study subjects ($p<0.005$). *De novo* anti-HLA antibodies were detected in 42% of the control patients and in only 7% of the patients who received filtered PC. In 1994, Williamson *et al.* carried out a RCT comparing patients who received either non-leucoreduced or bedside-filtered blood components[13]. Interestingly, both groups showed similar rates of alloimmunisation (37% among patients who received non-filtered blood components and 21% among patients transfused with bedside-filtered blood components; $p=0.07$). According to the authors, the efficacy of bedside filtration could have been hampered by intrinsic biological limitations, namely the possible immunogenic potential of stored blood component supernatants; in addition, the removal of WBC upon storage just before transfusion might not be the most effective way to prevent transfusion of WBC-derived cytokines.

In 1997, the multicentre Trial to Reduce Alloimmunization to Platelets (TRAP) clearly showed significant differences between patients transfused with filtered PC (F-PC), ultraviolet B-irradiated pooled PC (UVB-PC) or filtered apheresis platelets (F-AP) and controls who received unmodified, pooled PC[14]. Out of 530 patients, 13% of those in the control group became refractory to platelet transfusions, as compared with 3% in the F-PC group, 5% in the UVB-PC group, and 4% in the F-AP group ($p<0.03$ for each treated group as compared with controls).

Later, Vamvakas published a meta-analysis[15], which included the TRAP study data, and demonstrated a significant reduction of the cumulative relative risk of alloimmunisation against HLA antigens (−68%, 95% confidence interval [CI]: 0.18-0.56) resulting from the use of leucoreduced blood components. Both these studies also showed that naïve patients transfused with leucoreduced blood components had a lower risk of developing refractoriness to PC transfusion in comparison with patients with a high risk of previous immunisation (i.e. previous pregnancies)[14,15].

In 2005, Slichter *et al.* analysed the TRAP database to evaluate patient- and product-related characteristics that could influence post-transfusion platelet response in thrombocytopenic patients[16]. After evaluating factors affecting post-transfusion platelet increments, platelet refractoriness, and platelet transfusion intervals, they clearly showed that increasing the dose of platelets transfused or transfusing filtered apheresis platelets had a key role in reducing platelet refractoriness.

Recently, in 2014, Jackman *et al.* published an interesting study on the role of LR and UV treatment of PC in the prevention of immunisation frequency, duration, and magnitude (i.e. "qualitative HLA determination followed by evaluation of normalised background ratios for each of eight multi-antigen beads; significance was assessed for a normalised background ratio >10.8 for class I HLA antibodies and 6.9 for class II") of HLA antibody responses in transfusion recipients[17]. After selecting 321 patients from four different studies[14,18-21] (namely, 190 patients from TRAP[14], 72 from a microchimerism study in trauma patients[18], 37 patients from the Transfusion-Transmitted Viruses Study [TTVS][19,20], and 20 from the Transfusion-Related Infections Prospective Study [TRIPS])[21], they showed different immunisation behaviours. Interestingly, subjects who received leucoreduced or UV-treated blood products were less likely to generate class I HLA antibodies and patients who received leucoreduced blood were also less likely to generate class II HLA antibodies. Among those who received non-leucoreduced PC, 55% developed class I HLA antibodies and 51% developed class II HLA antibodies in comparison with 28% (class I) and 15% (class II) among those who received leucoreduced blood and 36% (class I) and 54% (class II) among those who received UV-treated blood, respectively. In addition, among alloimmunised subjects, LR resulted in a significant 2-fold reduction of the magnitude of class I HLA antibodies, and UV treatment resulted in a significant 3-fold reduction of the magnitude of class II HLA antibodies. The persistence of class I HLA antibodies was shorter with both treatments. Therefore, LR and UV treatment of blood products not only reduced the incidence of HLA antibody production, but also caused lower and more transient HLA antibody levels among sensitised transfusion recipients.

According to the review of the algorithms to recognise and manage HPA or combined HPA and HLA antibodies performed by Vassallo in 2009[22], after a poor response to platelet transfusions in thrombocytopenic patients (defined as a corrected count increment <5,000

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or inadequate platelet count increment), the possible strategies included transfusion of "fresh" (less than 48 hours old) ABO-compatible PC, HLA- and/or HPA-selected single donor platelets or pre-transfusion cross-matching. Furthermore, a recent guidance document on platelet transfusion for patients with hypoproliferative thrombocytopenia by Nahirniak and the members of the International Collaboration for Transfusion Medicine Guidelines recommends that "patients with hypoproliferative thrombocytopenia who are refractory to platelet transfusions and have class I HLA antibodies should probably receive class I HLA-selected or crossmatch-selected platelet transfusion to increase the platelet count" while "patients with hypoproliferative thrombocytopenia who are refractory to platelet transfusions and have HPA antibodies should probably receive HPA-selected or crossmatch-selected platelet transfusion to increase the platelet count"[23].

In conclusion, LR is effective (especially in high-risk patients) in preventing platelet alloimmune refractoriness, namely the persistent suboptimal platelet count increment following a platelet transfusion, arising from exposure to contaminating WBC in PC (class I HLA antigens) or less commonly, to platelet-specific antigens. This implies that, in patients with hypoproliferative thrombocytopenia, when leucoreduced platelet products are available, WBC-depleted platelets should be used as equivalent products to apheresis platelets[23].

Indications based on the analysis of observational clinical studies with very consistent results

Prevention of cytomegalovirus infection

CMV is one of the herpes viruses responsible for a common and harmless infection in immunocompetent subjects with a seroprevalence in adults ranging from 40 to 100%[24].

CMV infection in immunocompetent individuals is often asymptomatic (40-90%) or has a mild mononucleosis-like course, but it may be a life-threatening condition in some categories of subjects such as foetuses, premature infants, patients infected by human immunodeficiency virus, onco-haematological patients and CMV-seronegative candidates for solid organ or haematopoietic stem cell transplantation[25]. Infection in immunocompromised individuals may be associated with considerable mortality and morbidity such as pneumonitis, gastroenteritis and retinitis.

Transfusion-transmitted CMV infections have traditionally been explained by transmission of latently infected WBC. The issue of CMV transmission by blood products was first addressed in 1984, well before the introduction of LR[26].

Some early studies demonstrated that both policies, namely the use of CMV-seronegative blood components and LR, may be able to reduce CMV infection significantly in high-risk patients. However, controversies concerning "the gold standard" practice (only LR, only CMV-donor status, and LR plus CMV-donor status) are still ongoing[27-29].

In 2001, a panel of experts from the CMV Consensus Conference held in Canada, although agreeing that CMV infection could be prevented both by selecting CMV-negative blood donors and by transfusing leucoreduced blood components and that quality control of LR was excellent, issued recommendations for the use of CMV-negative blood products for high-risk populations as no consensus was reached on the question of whether serological CMV testing should be abandoned or not, given that universal LR had been recently introduced in that country[30].

In 2005, Vamvakas published a meta-analysis demonstrating that CMV-seronegative blood components are more efficacious than LR in preventing transfusion-transmitted infection[31]. This meta-analysis evaluated 829 patients (in 11 studies) transfused with CMV-negative blood components and 878 patients (in 12 studies) transfused with leucoreduced blood components. CMV infection was detected in 1.45% of the patients transfused with CMV-negative blood components compared to 2.73% of the patients transfused with leucoreduced blood components.

On the other hand, several retrospective studies demonstrated the efficacy of LR in preventing CMV transmission in transplanted patients. In 2005, Narvios *et al.* evaluated CMV infection in 72 CMV-negative allogeneic haematopoietic transplant patients who received bedside leucoreduced blood components[32]: 11 patients seroconverted; one patient was positive for CMV antigenaemia 4 months after transplantation, but did not have CMV infection; two out of 61 patients who did not seroconvert were CMV antigen-positive and did not have CMV infection.

In 2012, Nash *et al.* reported their experience with 100 CMV-negative patients who underwent allogeneic hematopoietic stem cell transplantation and received leucoreduced but mainly CMV-untested blood components[33]. In total, 6,465 units were transfused without any case of clinical CMV disease or positive CMV nucleic acid testing (NAT) after transplantation. Only two cases of seroconversion were reported and were attributed to passive antibody transmission. A recent retrospective analysis carried out in haematopoietic transplant patients confirmed the efficacy of universal LR in preventing transfusion-transmitted CMV infection[34]. In this study, recipients of leucoreduced or CMV-negative blood components did

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not show a significant difference in terms of infection (p=0.6244).

Although there is no general agreement, it is acknowledged that the use of either seronegative or leucodepleted blood components consistently reduces (93.1% and 92.3%, respectively)[31] the risk of transmitting CMV[35,36]. Nevertheless, persistence of CMV DNA after WBC removal explains the uncommon CMV infection in recipients of universally leucoreduced blood components[37] and fuels the ongoing debate on the relative safety of these two approaches as prevention strategies for transfusion-transmitted CMV.

In this scenario, a third, different strategy, namely transfusion of leucodepleted blood components from seropositive donors with seroconversion more than 1 year previously, in whom CMV DNA is rarely detectable or absent[38,39], has been proposed based on the rationale that this would prevent the transfusion of blood from donors with acute infection and high viral loads[39,40]. On this basis, in addition to LR, provision of seronegative blood products, provision of long-term seropositive blood donors, and provision of CMV DNA-negative blood products were even proposed as alternative strategies[41].

At present, there is a significant variability in local policies both at the hospital level and the blood operator level, nationally and internationally. While many centres use CMV-safe products (i.e. containing less than $<1,5\times10^6$ residual leucocytes per unit) for high-risk adult transplant patients, many continue to order CMV-seronegative leucoreduced blood components for high-risk neonates, intrauterine transfusions and pregnant patients[36]. In some countries, such as Canada, the national guidelines allow the combined policy only for high-risk patients, such as candidates for transplants[42]. In the USA, a recent survey of current practices found that 65% of the interviewed institutions evaluated selection of CMV-negative blood donors and leucoreduced blood components similarly safe[43]. On the other hand, the 1999 Swiss clinical practice recommendations considered seronegative units to be the gold standard for the prevention of transfusion-transmitted CMV, and concluded that leucoreduced units had not yet been proven equivalent[44]. According to the 2015 Italian recommendations for transfusion therapy in neonatology and to the 2009 guidance document for the transfusion of red blood cells[45,46], these two strategies are both able to effectively prevent transfusion-transmitted CMV. In fact, both leucoreduced and CMV-seronegative units have an equal and very low residual risk of transmitting CMV. CMV-seronegative products are able to transmit CMV, if donated during the 6- to 8-week serological window period after the infection while leucoreduced units can transmit this virus because of the partial failure to remove WBC from a very limited number of units (0-2%). Naturally, as window-phase seroconversion is associated with cell-free CMV in the plasma, which is not removed by LR, these two strategies are not additive[47,48]. Interestingly, despite the evidence that the efficacy of these two methods in preventing transfusion-transmitted CMV may not be additive, an ongoing prospective birth cohort study, aimed at estimating the incidence of transfusion-transmitted CMV infection in low-birth weight neonates (≤1,500 g) who receive CMV-seronegative plus leucoreduced blood components (NCT00907686, available at www.clinicaltrials.gov), is being carried out[49]. The primary outcome of this study is to evaluate the incidence of CMV infection in patients who receive a combination of CMV-seronegative and leucoreduced blood components. The secondary outcome is to detect CMV-DNA and/or elevated residual WBC counts (due to WBC filter failures) in transfused blood components and to correlate these results with episodes of breakthrough CMV infection in the study population.

At present, internationally shared guidelines on the prevention of transfusion-transmitted CMV in high-risk patients do not exist and a recent survey "highlights that while many centres are becoming more comfortable using CMV-safe products for high-risk adult transplant patients, many continue to order CMV-seronegative leucoreduced products for high-risk neonates, intrauterine transfusions and pregnant patients"[36]. In addition, most centres continue to have a dual inventory and provide both leucoreduced and CMV sero-negative products to selected high-risk populations.

There is, therefore, a clear need for international collaborative studies comparing rates of transfusion-transmitted CMV infection following use of CMV-safe and/or CMV-negative products, which will help to collect the data necessary to develop the aforementioned international guidelines on CMV risk management.

However, as these studies would be impractical due to the large numbers of patients needed to produce convincing results, the improved performance of WBC filters and the currently available literature provide adequate evidence regarding the equivalence of using leucoreduced products alone. In addition, a recent estimate of the residual risk associated with leucoreduced-only blood components carried out in Australia showed that, notably, this risk is below the threshold of 1 in 1 million, generally considered negligible[50].

Febrile non-haemolytic transfusion reactions

FNHTR are defined as rises in temperature greater than or equal to 1 °C that cannot be explained by the patients' clinical picture. Such reactions are often

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associated with other inflammatory symptoms such as chills, cold, rigors, and discomfort. Nausea, vomiting, and headache may also be present. Generally, FNHTR occur during a transfusion or within 4 to 6 hours after its conclusion[51].

Several mechanisms have been proposed to explain the role of donor leucocytes in the pathogenesis of fever in transfusion recipients. In particular, three of the above-mentioned mechanisms are worth mentioning: infusion of passenger lymphocytes into recipients alloimmunised against leucocytes and platelets; infusion of pyrogenic cytokines (interleukin-6 [IL-6], interleukin-8 [IL-8], tumour necrosis factor-alpha [TNF-α], interleukin-1beta [IL-1β], and CD40L) and other inflammatory mediators (activated complement proteins and neutrophil-priming lipids) that accumulate during storage; and infusion of blood components contaminated with bacteria or bacterial products[1,52,53]. All these three mechanisms cause fever through the release of inflammatory cytokines.

In 2004, three retrospective studies demonstrated the efficacy of LR in significantly lowering the incidence of FNHTR after the transfusion of pre-storage leucoreduced PC and RBC[54-56]. Paglino *et al.* assessed a total of 145,369 RBC and 137,982 PC transfused in 8 years and found a significant decrease of the relative FNHTR rate: 47.1% for RBC (from 0.34 to 0.18%; p<0.0001) and 93.1% for PC (from 2.18 to 0.15%; p<0.0001)[54]. King *et al.* reported a reduction of FNHTR events from 0.37% (n=60) to 0.19% (n=37; p=0.0008) after 16,246 and 19,916 RBC transfusions, respectively[55]. Yazer *et al.* confirmed the significant reduction of FNHTR (from 0.37% to 0.19%, p=0.001) after 72,949 RBC transfusions and 50,555 PC transfusions, respectively[56].

In 2012, Wang *et al*. in a retrospective analysis of 70,015 platelet doses assessed the incidence of FNHTR associated with transfusions of prestorage-leucoreduced pooled platelets, post-storage-leucoreduced pooled platelets, non-leucoreduced pooled platelets, and apheresis single-donor platelets[57]. There were 152 (0.22%) FNHTR: the rate of reactions to apheresis single-donor platelets, prestorage-leucoreduced pooled platelets, post-storage-leucoreduced pooled platelets, and non-leucoreduced pooled platelets were 0.07%, 0.16%, 0.30%, and 0.20%, respectively. The difference in the percentage of FHNTR between non-leucoreduced pooled platelets and other types of leucoreduced platelets was statistically significant only in comparison with apheresis single-donor platelets (p=0.008) and post-storage-leucoreduced pooled platelets (p=0.045). This study, therefore, confirmed previous data showing that the timing of LR during storage probably influences the amount of accumulated cytokines in the transfused PC and has a key role in the pathogenesis of FNHTR.

In 2002, a multicentre RCT was carried out by Heddle *et al.* in adults with haematological malignancies randomised to receive one of three types of platelet products[58]: (i) PC from which the plasma supernatant had been removed and a platelet storage solution had been added; (ii) whole blood-derived PC leucoreduced prestorage by filtration; and (iii) prestorage leucoreduced apheresis PC. This study was designed to determine whether prestorage LR was more effective than plasma removal in preventing platelet-associated transfusion reactions and to compare the effectiveness of two methods of prestorage LR for preventing reactions. Unfortunately, due to the low power of the study, a significant difference in the frequency of reactions associated with plasma-depleted (21.3%) and prestorage leucoreduced (12.3%) PC was not shown. In addition, no difference in the frequency of reactions to the two types of prestorage leucoreduced PC was detected and the frequency of severe reactions to these PC was limited to only 1 to 2% of transfusions.

Although it is the most frequent adverse effect following the transfusion of RBC and PC, FNHTR are not typically life-threatening and LR effectively reduces the frequency of FNHTR during PC and RBC transfusions. However, despite its relative high frequency, FNHTR is definitively quite rare and a RCT aimed at comparing the incidence of this adverse outcome in study subjects (transfused with leucoreduced blood components) and controls (transfused with non-leucoreduced blood components) would require a huge number of patients[5]. For this reason, the currently available evidence of clinical benefits of LR stems mainly from "before-and-after" universal LR retrospective observational studies rather than RCT. Although subjects included in observational studies more closely resemble those patients we come across in daily clinical practice, results from such studies should be confirmed by controlled clinical trials in order to further strengthen their very consistent results.

Indications for which the clinical efficacy is partial or unproven

Transfusion immunomodulation

Experimental evidence suggests that RBC transfusion may have an effect on the immune system causing a dysregulation of innate immunity and inflammatory processes with a consequent increased susceptibility to infections, as well as down-regulation of cellular immunity (T cells and NK cells). This may also have effects on immune status in the case of neoplasia and may cause hyper-activation of B cells during alloimmunisation phenomena[59-63]. In addition to cellular and cytokine production, other mechanisms may still modulate immune response in recipients. RBC units also contain non-polar lipids and a mixture of pro-inflammatory

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lysophosphatidylcholines, which modulate the activity of NK and T cells, act as an NK-cell chemoattractant, induce dendritic cell maturation and stimulate the production of pro-inflammatory cytokines; eicosanoids (prostaglandins and thromboxanes) can also accumulate in RBC[64-68]. All these elements are now widely recognised as key players in transfusion-related immunomodulation, which is strongly associated with an increased risk of bacterial infection, promotion of cancer growth, multi-organ failure, and increased mortality in oncological patients.

In the last 10 to 15 years, the results of several observational studies and RCT dealing with the role of LR in preventing post-operative infections have been published. In 2002, Vamvakas performed a meta-analysis of eight heterogeneous RCT that investigated the relationship between the use of non-leucoreduced blood components and post-operative infections[69]. All these trials included patients randomised to receive leucoreduced or non-leucoreduced red cell concentrates or whole blood. Unfortunately, information about platelet or plasma transfusion was not reported. This analysis led to the conclusion that LR did not provide a significant benefit to the patients, in terms of infectious complications and mortality.

In contrast, two other meta-analyses showed different results. Fergusson *et al.*[2] and Blumberg *et al.*[3] integrated the results of all RCT published or reported up to 2002 and showed a decrease in the risk of post-operative infections attributable to LR. Fergusson *et al.* showed a summary risk ratio (RR) of 0.60 (95% CI: 0.38-0.93) in transfused patients[2]; the reduction of post-operative infections was more consistent in cardiac surgery (RR: 0.77; 95% CI: 0.61-0.97). Blumberg *et al.*[3] confirmed the statistically significant reduction of post-operative infections (odds ratio, 0.522; 95% CI: 0.332-0.821; p=0.005) and mortality in cardiac surgery whereas LR was devoid of effects in other surgical settings[70-72].

On the other hand, in cardiac surgery, LR had no effects on the total amount of blood lost, the total number of RBC transfused[73], or alloimmunisation reactions[74,75]. Moreover, LR has no impact on other outcomes such as 30-day mortality and post-operative comorbidity, as well as incidence of pneumonia and need for mechanical ventilation[76].

In 2007, Vamvakas addressed the reasons why meta-analyses of RCT on the association between non-leucoreduced allogeneic blood transfusion and post-operative infection produced discordant results[77]. He pointed out that the reasons for the aforementioned discordant results were probably due to researchers who did or did not investigate medical sources of heterogeneity and did or did not include the most recent RCT. Intention-to-treat and as-treated comparisons produced concordant results.

In a similar way, transfusion of leucoreduced blood components in oncological patients resulted in contrasting data. An early Danish trial was carried out between 1992 and 1995 on 589 colorectal cancer patients, including 142 who received buffy-coat-depleted RBC, 118 who received leucoreduced units, and 329 who did not receive any type of blood components. Although it showed that patients transfused with leucodepleted RBC had fewer post-operative infectious complications than patients transfused with buffy-coat-depleted units, long-term outcome data were lacking[78]. In 2005, Jensen *et al.* published the results of a 7-year follow-up of the above-mentioned patients and showed that recipients of buffy-coat-depleted or leucoreduced RBC had worse outcome (7-year survival) than non-transfused patients[79]. In 2011, the cause-specific mortality in the aforementioned patients was analysed by Mortesen *et al.* in a post-trial 15-year follow-up study. This confirmed that patients who were not transfused during surgery had a better prognosis than transfused patients[80]. In fact, according to the authors, both recipients of leucoreduced RBC and recipients of buffy-coat-depleted RBC had decreased long-term survival due to cardiovascular disease, although results were not statistically significant different in the latter group.

In 2007, Skanberg *et al.* randomised 642 patients with colon cancer to receive leucoreduced or buffy-coat-depleted RBC and showed that the study patients had better outcomes (in terms of length of hospital stay [<20 days] and need for respiratory support), but no improvement in overall survival due to leucocyte depletion of blood products after 6 years of follow-up[81]. In the same way, hospital stay was shorter and survival significantly increased in non-transfused patients compared to transfused patients. Koch *et al.* also showed that LR is associated with a poor post-operative course[82].

These studies confirmed that patients do not benefit from liberal transfusion practices and, similar to recent data, suggest that a more conservative approach (haemoglobin transfusion trigger <7 g/dL) can lead to reduced mortality and morbidity also in critically ill or bleeding patients[83,84].

In conclusion, the available evidence from clinical trials is not uniform and does not confirm the experimental evidence suggesting an immunosuppressive effect of blood transfusion. Statistically significant reductions of post-operative infections and mortality were shown in cardiac surgery. As far as other settings are concerned, the clinical effect of LR is probably smaller than detectable by trials and future studies might be needed to address the issue properly.

Prevention of other infections

Currently, various approaches, such as strict donor selection criteria, as well as serological and molecular

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screening tests, are used to avoid transfusion-transmitted infections. Notwithstanding this multi-step approach, infections still remain a challenge in transfusion medicine. Bacterial contamination of blood components occurs mainly at the time of venepuncture, due to inadequate skin preparation or asymptomatic bacteraemia in the donor. Although a consistent reduction of the risk of bacterial contamination of blood components is obtained through the diversion of the first part of the donation of blood and blood components[85], LR may be an additional effective tool in preventing transmission of bacteria through blood components[86]. Andreu *et al.* confirmed this by analysing bacterial infection rates before and after the universal implementation of LR in France[87]: bacterial sepsis and FNHTR were the only events that were significantly reduced ($p<0.001$) from 3.8% to 1.7% and from 32.9% to 25.8%, respectively. Data about the prevention of viral infections, such as those caused by Epstein-Barr virus (EBV) and human T-lymphotropic virus (HTLV/1-2), have also been reported. LR may eliminate the need to screen blood for EBV for at-risk patients by reducing the viral load[88,89]. As far as concerns HTLV/1-2, three policies may be implemented[90]: (i) in developed non-endemic countries that have started universal control of donated blood and universal leucodepletion, the current very low observed incidence and prevalence among blood donors (reflecting a very low estimated risk of an HTLV-1 positive donation entering the blood supply) should prompt further review of the transmission risk and a possible change of the prevention strategy. The systematic screening of all donations should be questioned (and possibly interrupted, if already in use) after accurate evaluation of the residual HTLV transfusion risk, while leucodepletion of cellular blood products should be maintained or implemented if not in place. (ii) In developed countries with areas of high endemicity, the suppression of anti-HTLV screening is not recommended but testing should be combined with leucodepletion until the efficiency of the latter procedure in preventing HTLV transmission is unequivocally proven. (iii) In developing countries in which HTLV is endemic and the residual risk of transfusion-transmitted infection is greater, given the high cost of universal testing and leucodepletion, more cost-effective strategies for blood donation screening need to be defined and assessed such as look-back studies aimed at infected-donor deferral and continued haemovigilance monitoring.

The transmission of variant Creutzfeld-Jakob disease (vCJD) from blood components is possible, since prions may be transmitted through buffy coat[91]. It is also possible to reduce vCJD transmission through LR filters[92-94]. Two experimental studies performed in animal models evaluated filter performance in preventing prions transmission. In 2011, McCutcheon *et al.* demonstrated, in a sheep model of vCJD, that standard LR filters are not able to reduce the level of prion proteins[95]. In contrast, 1 year later, Lacroux *et al.* demonstrated that prion transmission through blood components might be avoided using last-generation filters developed for prion proteins[96]. This opened up new possibilities for LR in countries in which the risk of transmission of vCJD through blood components is intermediate-high.

However, as blood risk assessment in transmissible spongiform encephalopathy animal models suggests that infection is at least distributed among WBC and plasma[97], LR can only partially remove prion-associated blood infectivity[92]. Therefore, in the last few years, complementary methods to actively secure blood transfusion further reducing the blood-borne risk of vCJD transmission were developed[98-100]. Data obtained from the evaluation of filter performances are presented in Table I. Recently, Cardone *et al.* demonstrated that it is possible to obtain a 100-fold reduction in blood components inoculated with scrapie-infected hamster brain homogenates by using affinity prion reduction filters[101]. In addition, further very promising results were added to the available experimental evidence on the potential of LR in preventing blood-borne vCJD by an ongoing study (after 5 to 6 years of progress) on prion blood removal performances of the P-Capt filter in macaques, an extremely relevant model for human prion diseases[97]. This study evaluated resin affinity-based prion removal filters designed for human transfusion.

In conclusion, the only evidence, albeit very promising, on the effectiveness of LR in the prevention of the transmission of vCJD through transfusion of blood components is currently based on (robust) data from experimental studies carried out in animal models.

Transfusion-associated acute lung injury and transfusion-associated Graft-versus-Host disease

Transfusion-associated acute lung injury (TRALI) and transfusion-associated Graft-versus-Host disease (TA-GVHD) are life-threatening transfusion reactions that may involve leucocytes. However, the clinical effects are mainly due to more complex mechanisms than simple leucocyte contamination.

TRALI is a syndrome characterised by acute respiratory distress and its classical and fulminant presentation is indistinguishable from adult respiratory distress syndrome, secondary to other causes such as toxic inhalation, sepsis or aspiration. It is a severe transfusion reaction characterised by non-cardiogenic lung oedema, hypoxaemia and respiratory distress occurring about 6 hours after blood transfusion. The incidence of TRALI differs greatly in retrospective and prospective studies; in some countries there is also

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Table I - Efficacy of different filters in preventing prion infections.

Author, year of publication	Type of blood	Type of infection	Type of filter	Effect on prion transmission
Gregori, 2004[92]	Pooled hamster blood	Scrapie-infected	*Whole blood filter* Leucotrap WB collection set (Haemonetics/ Pall)	Reduced: −42% of the initial infectivity
Krailadsiri, 2006[94]	Human blood	PrPc	*Whole blood filters* Baxter RZ2000 § Macopharma LST1 §§ NPBI §§ Pall WBF2 §§	Reduced: LR-RBC: 2-15% of WB values LR-FFP: 3-25% of WB values
			RBC filters Baxter R2000 § Baxter R3000 § Macopharma LCR4 § NPBI §§ Pall RCM1 §§	Reduced for all filters. Significant for: Pall Day 0 ($p<0.001$) NPBI Day 0 ($p<0.05$) Baxter R2000 ($p<0.05$)
			Platelet filters# Asahi PLX5 Pall AutostopBC Terumo Imugard III	Not modified. Amicus gave significantly higher values than all other groups (ANOVA $p<0.0001$) and the Autostop significantly lower ($p=0.04$). The levels of supernatant PrPc were also significantly different (ANOVA $p<0.0001$), with the Autostop and the MCS showing lower values than all other groups ($p<0.0001$) and the Amicus higher values.
			Apheresis platelets# Haemonetics MCS Gambro LRS Baxter Amicus	
			Plasma filters Baxter Pall	 −69% −60%
Mc Cutcheon, 2011[95]	Sheep blood	PrPSc	*RBC filter* T3953 *PRP filter* Fresenius/NPBI CompoStop F730	Not modified*
LaCroux, 2012[96]	Sheep blood	PrPSc	*RBC filter* ASAHI KASEI combination filter Pall Medical Leucotrap Affinity Plus	Reduced** (still present in lymphoid tissue and central nervous system)
Cardone, 2014[101]	Human blood	Scrapie-infected hamster brain	*Whole blood/affinity prion reduction filter* WBF3/LAPRF1 WBF3/LAPRF2	Reduced*** 2.1 log >2.1 log
			RBC filter/affinity prion reduction filter BPF4/ LAPRF1 BPF4/ LAPRF2	<2.4 log 2.0 log
Lescoutra-Etchegaray, 2014[102]	Human blood	Scrapie-infected hamster brain	*RBC filter* P-Capt (PSE3080XB, Macopharma)	Reduced 3-log
Lescoutra-Etchegaray, 2015[97]	Brain homogenates	Macaque	*P-Capt* (prion removal after leucoreduction)	No sign of infection
			Prototype PMC#005 (simultaneous leucoreduction and prion removal)	Infection in one animal

Effect on prion transmission is expressed in percentage or logarithm. §Filtration was carried out under two conditions: (i) ambient temperature on the day of collection (day 0) and (ii) after holding at 4 °C overnight (day 1).[§§] Filters were suitable only for condition (ii). #Only the MCS+ required WBC filtration (LRF6H filter, Pall Biomedical); the Spectra LRS and Amicus used fluidised particle bed and elutriation, respectively, to achieve leucoreduction. The concentrations of total PrPc antigen, expressed as units of PrPc per mL, were measured in all samples. Reduction, if present, is expressed as a percentage. For platelets, because the manufacturing methods used did not allow a direct comparison of products before and after filtration, a grouped study design was used for whole blood-derived platelets to enable a direct comparison of different filtered products with unfiltered product from the same pool and an ungrouped comparison with apheresis platelets. *In this study, sheep blood components had the same specifications as those for human blood using similar processing methods and commercially available blood packs and leucoreduction filters. Whole blood has shown the highest rate of transmission (37.5% of recipients infected), followed by buffy coat (32.4%), platelets (24.3%), then red cells (18.9%) and plasma (13.2%). **In this study, leucoreduction filters and filters combining leucoreduction and the capture of non-leucocyte associated prion infectivity are evaluated. In this table, RBC filters (ASAHI KASEI and Pall) are both combined filters for leucoreduction and prion depletion. Systematic detection of abnormal PrP (using both immunohistochemistry and western-blot analysis) in lymphoid tissues and central nervous system was carried out in recipients. The reduction was evaluated 400 days after inoculation. *** Whole blood and RBC were leucoreduced by commercial filters (WBF3 for blood and BPF4 for RBC, Pall Corporation), divided in two identical aliquots, and finally filtered through either LAPRF1 orLAPRF2 filters (Leukotrap, Pall Corporation). Effect on prion transmission is expressed as removal factor (RF), calculated as log titer of prefiltered samples minus log titer after filtration.
WB: whole blood; PrP^{Sc}: prion protein; RBC: red blood cell; PRP: platelet-rich plasma; LR-RBC: leucoreduced-red blood cell; LR-FFP: leucoreduced-fresh frozen plasma.

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a tendency to under-diagnosis and underreporting of this syndrome[103]. Overall, its incidence is estimated to range between 0.08% and 15% of patients receiving a blood transfusion. According to the two-hit hypothesis, the capillary leak causing TRALI results from two succeeding events: the adhesion of primed neutrophils to pulmonary endothelial cells (first hit) and the subsequent activation of both cells by antibodies or inflammatory mediators present in transfused blood (second hit)[104-106]. The pathogenesis of TRALI involves both antibody-mediated and antibody-negative mechanisms and several transfusion-related and recipient-related risk factors variably interact in each case in triggering the syndrome. Massive transfusion, mechanical ventilation, sepsis, haematological malignancies, end-stage liver disease and cardiac surgery are all acknowledged important risk factors for TRALI. It is not, therefore, surprising that the patients mainly involved are those who are critically ill[104-114].

Universal LR has been associated, on the one hand[115], with a decreased incidence of acute respiratory distress syndrome in a cohort of critically ill patients and, on the other hand[116], with no reduction of lung injury. In 2010, Blumberg *et al.*[117] showed a statistically significant, 83% reduction of the rate of TRALI from 2.8 to 0.48 cases per 100,000 components transfused (p=0.01) before and after universal LR, respectively. Interestingly, LR is also associated with a reduction of accumulation of lysophosphatidylcholines, which have been implicated in the onset of TRALI[118]. Recently, Silliman *et al.* showed that prestorage filtration may reduce pro-inflammatory activity in the RBC supernatant and prevent TRALI[119]. They demonstrated that the experimental filters developed for small-volume filtration removed more than 96% of IgG, 93% of antibodies to HLA class I antigens, and 99% of antibodies to HLA class II antigens. In addition, such removal of antibodies inhibited acute lung injury in a two-event, *in vivo,* animal model of TRALI thus supporting the efficacy of LR in preventing TRALI in an experimental model.

In addition, some recent studies confirmed that residual leucocytes and platelets are a stressful storage factor in stored RBC, especially if non-leucoreduced. LR may have a positive effect on some RBC senescence phenomena such as haemolysis, irreversible echinocytosis, microvesiculation, accumulation of reactive oxygen species/calcium, band 3-related senescence modifications, and membrane proteome stress biomarkers[119-122].

TA-GVHD may occur in immunocompromised patients such as transplant recipients, onco-haematological patients, foetuses or premature infants. It may also develop in immunocompetent transfusion recipients who are heterozygous for an HLA haplotype for which the donor is homozygous[123]. As TA-GVHD is correlated to exposure to allogeneic donor leucocytes, it might be useful to reduce leucocytes in blood components. However, it is not possible to advocate LR to prevent TA-GVHD because this disease also occurs in patients transfused with leucoreduced blood components[124].

In conclusion, the prevention of TRALI is based on the exclusion from clinical use of high-plasma volume blood components donated by multiparous and/or transfused donors, as well as donors involved in TRALI cases or with proven antibodies to HLA or human neutrophil antigens. As far as concerns the prevention of TA-GVHD, leucocyte inactivation and gamma-irradiation have proven efficacy[125]. LR does not have an evidence-based role in the prevention of TRALI and TA-GVHD.

Evaluation of costs

As mentioned above, LR prevents some transfusion reactions but analyses on its cost-effectiveness are scarce and mainly based on observational data, as only a limited number of RCT on this issue have been carried out. In Canada, the introduction of universal LR caused reductions in the mortality rate[126] and the rate of alloimmunisation against HLA antigens[127]. In Germany, universal LR gave a substantial contribution to reducing bacterial contamination of blood components[128]. Contrariwise, in Spain universal LR is not encouraged and costs from LR are considered excessive, when compared with the clinical benefits[129].

Cost-effectiveness was analysed in a RCT in patients undergoing cardiac valve surgery. In this trial, the use of leucoreduced blood components saved 214 US$ per patient, when more than four RBC units were transfused[130]. Clinical outcomes such as mortality, duration of hospitalisation, infections and development of multi-organ failure were assessed. Two additional RCT published in the Netherlands showed that LR was cost-effective: the benefit of LR of RBC ranged between 220 USD and 310 USD per life-year gained in patients undergoing coronary artery bypass grafting[131,132].

The costs of LR were also evaluated in another RCT performed in the USA[133]. In this trial, which was carried out in a general hospital excluding patients with established medical indications for leucoreduced blood components, the primary outcomes such as in-hospital death, duration of hospitalisation from first transfusion event, and total hospitalisation costs were not statistically significantly different (p=0.64, p=0.21, and p=0.24, respectively) between patients and controls. No additional costs were associated with LR but there were no clinical benefits either. Recently, Tsantes *et al.*[134] showed that LR did not have a favourable cost-effectiveness ratio

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in preventing FNHTR. They analysed more than 80,000 RBC transfusions, of which 63% were leucoreduced: the calculated incremental cost-effectiveness ratio (ICER) was € 6,916, which is the cost of preventing one FNHTR.

In conclusion, although the cost-effectiveness remains controversial, as cost-effectiveness analyses relate the costs of a programme/technique to its key outcomes or benefits, we deem that health technology assessment should include all the aforementioned potential advantages of LR (in unselected populations of patients) in the projected benefits. This approach would probably provide sufficient evidence to support a recommendation aiming at including this policy in the current standard of care.

Conclusions

From the analysis of the available information, it seems fair to conclude that LR can be recommended for the prevention of HLA and HPA immunisation and platelet refractoriness. In addition, WBC-depleted platelets should be used as equivalent products to apheresis platelets. The current available literature data provides robust evidence supporting an equivalent role of CMV-seronegative and leucodepleted blood components in preventing the risk of CMV transfusion transmission. A consistent wealth of observational data shows that LR is effective in reducing the rate of FNHTR. LR is also effective in reducing post-operative infections and mortality in cardiac surgery patients and can play a key role in the prevention of transfusion-transmitted EBV and HTLV infections. Finally, experimental data from animal studies exploiting affinity prion reduction filters provide very promising results for the prevention of transfusion-transmitted vCJD.

On these bases, several European countries[135], as well as Canada, introduced universal LR about 10 years ago. Current evidence on a poor cost/benefit of LR is widely disputable, as it does not include balanced evaluations encompassing the extensive list of its proven and probable benefits[136].

The Authors declare no conflict of interest.

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