Published in final edited form as:

Blood Cells Mol Dis. 2013 January ; 50(1): 61–68. doi:10.1016/j.bcmd.2012.08.009.

Transfusion immunomodulation - the case for leukoreduced and (perhaps) washed transfusions

Katie Lannan^a, Julie Sahler^a, Sherry L. Spinelli^b, Richard P. Phipps^{a,b,c}, and Neil Blumberg^b

^aDepartment of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY

^bDepartment of Pathology and Laboratory Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY

^cDepartment of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY

Abstract

During the last three decades, a growing body of clinical, basic science and animal model data has demonstrated that blood transfusions have important effects on the immune system. These effects include: dysregulation of inflammation and innate immunity leading to susceptibility to microbial infection, down-regulation of cellular (T and NK cell) host defenses against tumors, and enhanced B cell function that leads to alloimmunization to blood group, histocompatibility and other transfused antigens. Furthermore, transfusions alter the balance between hemostasis and thrombosis through inflammation, nitric oxide scavenging, altered rheologic properties of the blood, immune complex formation and, no doubt, several mechanisms not yet elucidated. The net effects are rarely beneficial to patients, unless they are in imminent danger of death due to exsanguination or life threatening anemia. These findings have led to appeals for more conservative transfusion practice, buttressed by randomized trials showing that patients do not benefit from aggressive transfusion practices. At the risk of hyperbole, one might suggest that if the 18th and 19th centuries were characterized by physicians unwittingly harming patients through venesection and bleeding, the 20th century was characterized by physicians unwittingly harming patients through current transfusion practices.

In addition to the movement to more parsimonious use of blood transfusions, an effort has been made to reduce the toxic effects of blood transfusions through modifications such as leukoreduction and saline washing. More recently, there is early evidence that reducing the storage period of red cells transfused might be a strategy for minimizing adverse outcomes such as infection, thrombosis, organ failure and mortality in critically ill patients particularly at risk for these hypothesized effects. The present review will focus on two approaches, leukoreduction and saline washing, as means to reduce adverse transfusion outcomes.

© 2012 Elsevier Inc. All rights reserved.

Correspondence to Neil Blumberg at: Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, 601 Elmwood Avenue, Box 608, Rochester, NY 14642, Telephone 585/275-3189, Fax 585/273-3002, neil_blumberg@urmc.rochester.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Contributions

KL and NB wrote the manuscript.

JS, SLS and RPP edited the manuscript and provided helpful comments.

Keywords

leukoreduction; red blood cell; washing; platelet; transfusion

Leukoreduction

The current main research emphasis on improving clinical results and reducing the toxicity of transfusion focuses on the duration of storage of red cell concentrates. The data available on red cell storage duration and its association with increased morbidity and mortality has been recently reviewed [1]. Randomized trials to determine whether susceptible patients can benefit from shorter storage periods for red cells are underway [2; 3]. Prior to this emphasis on storage duration research, the main thrust for improving clinical outcomes in transfused patients focused on leukoreduction--removal of the contaminating donor white cells and platelets in red cell concentrates.

In our view, leukoreduction has conclusively demonstrated itself to be the third great scientific advance in transfusion medicine in the last century, following the clinical and scientific triumphs of serologic testing for antibody responses to red cell antigens, and infectious disease testing to prevent transmission of blood-borne pathogens. Benefits of leukoreduction that are universally accepted include reductions in febrile nonhemolytic transfusion reactions (FNHTRs), CMV transmission and HLA antibody formation leading to platelet refractoriness. Most importantly, leukoreduced transfusions in randomized trials in cardiac surgery and observational studies in critically ill patients lead to striking decreases in mortality [4; 5]. Although leukoreduction removes 99.9% of contaminating white blood cells (WBCs) and platelets, thus preventing many transfusion-related complications, red cell transfusion remains an independent predictor of post-operative morbidity and mortality [6]. Changes during blood storage have been known for some time, but the exact changes and the associated clinical implications are not well understood. Here, we will make the case for the implementation of universal leukoreduction, as a means to improve patient care and decrease hospital costs, but with the understanding that universal leukoreduction is not by any means the end of the road for improving transfusion efficacy and safety.

It is well known that WBCs release many potentially harmful proinflammatory and prothrombotic mediators during storage, contributing to what is known as the “storage lesion” (Table I) [7; 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18; 19; 20; 21; 22; 23]. Removal of leukocytes before storage partially or completely prevents the accumulation of some of these proinflammatory mediators [24; 25]. Upon transfusion, cytokines made by leukocytes and platelets, such as interleukin-6, tumor necrosis factor alpha, interleukin-1 beta and CD40 ligand likely contribute to adverse transfusion reactions. Moreover, two recent studies by Phelan *et al* suggest that prestorage leukoreduction of red cells can prevent many of the detrimental effects of aging on stored blood in trauma patients, indicating that the WBCs themselves likely contribute to a substantial portion of the storage lesion [26; 27].

Leukoreduction – Observational and Retrospective Studies

Numerous teams have investigated the beneficial effects of leukoreduction of red cell transfusions in retrospective and observational studies, most of which demonstrate that leukoreduction is beneficial, and fewer or no transfusions are associated with decreased post-operative morbidity and mortality. The largest of these investigations was the Canadian “before and after” implementation trial, which examined the effects of converting to universal leukoreduction of the blood supply on post-operative infection rates and mortality [28]. Patients receiving leukoreduced transfusions had an absolute mortality rate of approximately 1% less than those patients receiving non-leukoreduced transfusions. Thus,

the benefits of reduced mortality with use of leukoreduced transfusions accrue to approximately 1 in 100 critically ill patients. Furthermore, a significant 2% absolute decrease in post-transfusion fevers was noted, as well as a concomitant 2% decrease in antibiotic usage. Consistent with numerous other findings of decreased FNHTRs in patients transfused with leukoreduced products [29; 30; 31], the authors speculate that decreased fevers led to decreased use of antibiotics in these patients and would thus lead to lower costs of care. With FNHTRs occurring in approximately 1 in 20 platelet transfusions and 1 in 330 red blood cell (RBC) transfusions, significant reductions would have a large impact on the transfused patient population [32; 33].

In addition to the well-established benefit of decreasing the rate of FNHTRs, leukoreduction has been associated in observational and in some cases randomized trial data with reductions in many other adverse side effects of transfusion, including post-operative infections [6], acute kidney injury (AKI) [34], acute respiratory distress syndrome (ARDS) [35], acute lung injury (ALI) [36], transfusion related acute lung injury (TRALI) [14], transfusion-associated circulatory overload (TACO) [30], time on mechanical ventilation [37], length of hospital stay [38; 39] and mortality. Use of leukoreduced transfusions in critically ill trauma patients was associated with a 55% reduction in the incidence of late ARDS compared to patients receiving non-leukoreduced blood [35]. Similarly, our group recently published the results of an institutional implementation trial of leukoreduction, which supports the hypothesis that donor WBCs contribute to pulmonary complications [30]. The observed post-implementation incidence of TRALI decreased by 83% ($p=0.01$) and the incidence of TACO decreased by 49% ($p=0.03$). Consistent with many previous reports, transfusion-associated fever and/or rigor decreased by 35% ($p<0.0001$). In our institutional observational data in cardiac surgery, the mortality rate decreased from 5.3% to 3.2% following implementation of leukoreduced transfusions (not statistically significant) [40], comparable in magnitude to the decreases in mortality seen in three randomized trials [4; 5; 41]. Netzer and colleagues found that while RBC transfusion was associated with an increased risk of mortality in patients with ALI (OR=1.14), this association was reduced by more than half when only leukoreduced transfusions were given (OR=1.06) [36]. A retrospective before-and-after cohort study in trauma patients observed a significant association between implementation of leukoreduction of transfusions and incidence of infectious complications with a 45% reduction in pneumonia, but no concomitant reduction in mortality or length of hospital stay [42; 43]. The one randomized trial of leukoreduction in trauma patients found a modest advantage to leukoreduction that did not achieve statistical significance [44]. Collectively, these studies support the hypothesis that donor WBCs contribute to transfusion-related complications and increased length of hospital stay.

Some observations suggest a relationship between use of leukoreduced blood products and increased survival [4; 5; 28], although this area has been subject of different interpretations. It has been suggested that leukoreduction is most beneficial for patients who receive larger volumes of blood (*e.g.*, >6 units of red cells). Neonates and small infants receiving pre-storage leukoreduced products required shorter duration of mechanical ventilation and intensive care unit stays, and had decreased C-reactive protein levels compared to patients receiving blood that was leukoreduced post-storage [37]. Leukoreduction of transfusions to neonates and infants may provide a greater benefit, as blood transfusions in these small patients represent a much higher dose of leukocytes on a per kilogram basis compared to transfusions in adults.

One criticism of many implementation trials is the before-and-after nature of the studies. A major concern is the possibility that overall care improved over time, resulting in better outcomes for patients receiving leukoreduced transfusions that is coincidental rather than causal. This concern is addressed by the randomized clinical trials to be discussed, as well as

the implementation trials at the University of Pittsburgh Medical Center-Shadyside Hospital [38; 39]. Fung *et al* studied patients prospectively with the implementation of universal leukoreduction and subsequently reverted to transfusing non-leukoreduced blood in cardiac surgery. First, all patients undergoing cardiac surgery over a one-year period received leukoreduced transfusions and the clinical outcomes were compared to a historical cohort of patients who received non-leukoreduced transfusions. The implementation of universal leukoreduction was associated with a significant reduction in post-operative length of hospital stay, while there was no change in length of stay for patients who did not receive transfusions, suggesting a detrimental role for transfused allogeneic leukocytes in cardiac surgery. Five months later, presumably for cost reasons, this hospital reverted from leukoreduced to non-leukoreduced transfusions in cardiac surgery. A second clinical cohort study was performed to determine clinical outcomes in patients undergoing cardiac surgery. Reversion to non-leukoreduced blood was associated with a significant increase in post-operative length of hospital stay compared to the previous cohort of patients receiving leukoreduced transfusions. The authors also addressed the non-significant increase in mortality that was seen with the implementation of leukoreduction. A further increase in operative mortality and length of ICU stay was observed in patients receiving transfusions, as well as in non-transfused patients when the hospital reverted to non-leukoreduced transfusions, indicating that these increases in mortality and ICU stay were not likely due to the type of blood given. Instead, this result suggests that unknown factors (perhaps case mix) led to increases in mortality over time and this change could have masked any beneficial effect of leukoreduction on mortality.

Leukoreduction – Randomized Controlled Clinical Trials

Randomized trials and meta-analyses examining whether leukoreduction decreases post-operative infection, mortality and/or length of stay have come to varied conclusions [6; 45]. Five of the initial studies were performed with patients undergoing colorectal surgery and three out of five demonstrated clinical benefit of leukoreduction through decreased incidence of post-operative infections [46; 47; 48; 49; 50]. More recently, several studies have examined the effects of leukoreduction on post-operative morbidity and mortality in cardiac surgery and trauma patients. Several meta-analyses, all performed by the same group, have concluded that leukoreduction provides no significant benefit to patients, in terms of infectious complications and mortality [51; 52]. The limitation of these meta-analyses is the use of the intention-to-treat principle as opposed to an as-treated analysis. Specifically, the use of the intention-to-treat analysis in this instance included patients that did not receive transfusions, accounting for more than 10% of the analyzed patients in most cases, therefore diluting any potential beneficial effect of leukoreduction. For rigorous statistical analyses, inclusion of any patient that was randomized is usually critical. However exclusion of these patients provides a more scientifically valid examination of the outcomes between patients who have received non-leukoreduced or leukoreduced transfusions, excluding patients who received no transfusions at all. The material question is whether leukoreduced products produce superior clinical results to non-leukoreduced products. This was answered in a meta-analysis that included only patients who received transfusions (Figure 1) [6]. The analysis demonstrates a highly significant ($p=0.005$) benefit of reduced post-operative infections after leukoreduced transfusions in the settings of colorectal and cardiac surgery, with an absolute risk reduction of approximately 10% (i.e., 1 in 10 transfused patients benefit or number needed to treat for benefit = 10) and a relative risk reduction of approximately 36%.

Two important and frequently cited randomized controlled trials evaluating the effects of leukoreduced blood transfusions compared to unmodified transfusions have shown no clinical benefit of leukoreduction, although both found nonsignificant trends favoring

leukoreduction [31; 44]. Each study has significant differences from the previous studies that limit the interpretation of the results. The study by Nathans *et al*, though well designed, is underpowered to detect modest changes in clinical outcomes. In fact, the nonsignificant 16% relative risk reduction of infectious complications observed in this study of trauma patients would require nearly ten times as many subjects to demonstrate statistical significance [44]. Moreover, patients were only randomized to receive leukoreduced blood products for 28 days then were subsequently converted to non-leukoreduced RBCs, thus making it difficult to determine any longer-term benefits of leukoreduction. Studies showing significant reductions in mortality and infections have monitored patients for up to 90 days [4; 5]. Furthermore, the maximum storage duration of the red cell transfusions was 25 days, allowing for examination of the beneficial effects of leukoreduction only with shorter storage RBC units. Numerous studies have demonstrated that non-leukoreduced units accumulate detrimental mediators over time in storage and transfusion of older units is associated with worse clinical outcomes [53]. Speculatively, leukoreduction may play a more minor role in preventing adverse reactions from fresher RBC units and a greater effect may be seen with older units, consistent with other studies.

Dzik *et al* published a second prospective randomized trial that demonstrated little benefit of leukoreduction but also has several important limitations [31]. Although a large study, and thus powered to detect small benefits, Dzik and colleagues found no significant reductions in mortality with leukoreduction (non-leukoreduced=9%, leukoreduced=8.5%, $p=0.64$), nor reductions in length of hospital stay or costs. This study detected a reduction in the rate of FNHTRs (0.77% to 0.22%, $p=0.06$), which is a well documented, proven benefit of leukoreduction. One important confounding factor was that leukoreduced RBC units were on average 7 days older than control products, which in and of itself may have the potential to significantly affect clinical outcomes. For example, trauma patients who received blood that was on average 6 days older than control products were more likely to develop multiple organ failure and septic patients who received blood that was 8 days older were more likely to die [54; 55]. In the trial by Dzik and colleagues, the patients receiving leukoreduced products had reduced length of hospital stay, antibiotic usage and total hospital costs that were not statistically significantly less than those in patients receiving non-leukoreduced red cells. However the protocol violation rate for patients randomized to the leukoreduced arm (who incorrectly received non-leukoreduced red cells) was close to 13% and this was significantly higher than the protocol violation rate in non-leukoreduced arm. This receipt of non-leukoreduced red cell by patients in the leukoreduced arm and receipt of leukoreduced red cells by patients in the non-leukoreduced arm may well have reduced the power of this study to detect a difference in clinical outcomes.

Critical to any discussion of universal leukoreduction, several randomized controlled trials in cardiac surgery have found strikingly and statistically significant decreased post-operative mortality, infections and hospital length of stay with leukoreduced transfusions. Mortality rates after cardiac surgery were reduced from 7.8% to 3.5% and 10.1% to 5.5% in two randomized trials of leukoreduced transfusions in the Netherlands [4; 5]. An absolute decline in mortality rates of slightly less than 5% corresponds to approximately 1 in 20 patients who would have died who survived because they received leukoreduced transfusions. Leukoreduction of transfusions in cardiac surgery reduces the odds of post-operative bacterial infection, the single most frequent cause of serious morbidity and mortality in these patients, by approximately 24% to 32%. These two landmark studies support previous observational studies demonstrating a clear benefit of leukoreduction in preventing infections and mortality in patients undergoing cardiac surgery.

Benefits of leukoreduction, however, are not confined to the cardiac surgery setting. In another study, patients undergoing surgery for aortic aneurysm or gastrointestinal surgery

experienced significantly decreased incidences of multi-organ failure and length of hospital stay when receiving leukoreduced blood [41]. However, this study did not demonstrate a significant reduction in vascular surgery mortality, which was 10.3% in the non-leukoreduced group and 8.4% in the leukoreduced group ($p=0.20$), but was significantly reduced in the subset of patients undergoing gastrointestinal surgery (8% to 4%). Accounting for the number of aortic aneurysm and gastrointestinal surgeries each year and the cost of leukoreduction, the Dutch healthcare system could expect to save £2.9m per year as a result of a reduction in length of hospital stay associated with the use of leukoreduced products.

Leukoreduction – Cost considerations

The main concern with the implementation of universal leukoreduction is cost considerations. Many opponents of universal leukoreduction implementation argue that the cost is not worth the benefit. However, most of these arguments are formed empirically without global consideration for the numerous cost-saving benefits of leukoreduction, which include partial abrogation of transfusion immunomodulation effects, as well as the savings associated with decreased FNHTRs, CMV transmission, alloimmunization, platelet refractoriness and post-operative infections. Patients experiencing complications are likely to have longer hospital stays, which can account for a significant increase in cost, while a decrease in post-operative complications associated with leukoreduction could be expected to decrease hospital length of stay and therefore total costs. For example, post-operative infections can increase patient length of stay by several days or even weeks, amounting to thousands to tens of thousands of dollars in associated costs.

During our implementation trial of leukoreduced transfusions in cardiac surgery, although the mean cost per admission for non-transfused patients increased over time by \$4,000, the implementation of leukoreduced transfusions for cardiac surgery led to a decrease in mean costs per admission of \$1,700 [40]. If we extrapolate these numbers to a national level, an estimated 750,000 cardiac surgery cases nationwide multiplied by \$1,700 yields savings of \$1.28 billion dollars per year. Thus, the savings from leukoreduction in cardiac surgery are likely sufficient to pay for universal leukoreduction 2–3 times over. Post-operative infection has a mortality of 8–15% and is the leading cause of multi-organ failure. Each nosocomial infection has estimated costs on the order of \$30,000 per admission, dwarfing the modest costs per unit of leukoreduced blood at approximately \$20–30 per unit (Figure 2) [6; 56]. This is the primary reason that universal leukoreduction is actually cost saving rather than a net expenditure, unlike most health care innovations.

Most importantly, the estimated mortality potentially averted in surgical patients by leukoreduced transfusions is striking [57]. With an estimated 2 million surgeries including transfusion therapy, and 10% fewer post-operative infections with leukoreduction, one would predict 200,000 fewer surgical infections. Assuming 8–15% of infections lead to death, universal leukoreduction could theoretically lead to 16,000 to 30,000 fewer deaths per year. In cardiac surgery alone, an estimated 750,000 cases per year with 2–4% fewer deaths after universal leukoreduction, would correspond to 15,000 to 30,000 fewer deaths per year in the USA alone. Even if these estimates were off by a factor of two or three, the benefits of universal leukoreduction would affect tens of thousands of surgical patients each year in the USA. To put the utility of leukoreduction in perspective, we can compare the number of patients that need to be treated with leukoreduced transfusions to prevent one death in cardiac surgery patients (20 patients) with the number of units we need to test to prevent one HIV or HCV infection employing nucleic acid testing (500,000 to 1,000,000 units) [58]. Assuming fatality for those HIV and HCV infections, the cost of preventing one death is about \$2.5–5 million for HIV/HCV nucleic acid testing (NAT), versus about \$400–600 for

leukoreduction. In other words, leukoreduction is perhaps four orders of magnitude more cost effective than HIV/HCV nucleic acid testing in preventing a patient's death.

Importantly, most centers have not experienced significant increases in cost of care when leukoreduction was utilized, but instead show cost savings or no net difference. Cost-effectiveness of leukoreduction was analyzed in a randomized clinical trial of patients undergoing valve surgery using clinical outcomes and per patient cost-data [59]. Although this trial was not powered to detect significant changes in cost, leukoreduced blood saved on average \$214 per patient, while patients receiving more than four units of blood had an even more favorable economic profile. In conjunction with the cost-savings observed in our implementation trial, these data suggest that leukoreduction in patients undergoing cardiac surgery almost certainly costs the health care system less overall than using non-leukoreduced products.

In a prospective randomized controlled trial evaluating all admitted patients requiring a blood transfusion, no statistically significant decrease in the total hospital costs was observed for patients who were randomized to receive leukoreduced products compared to the control group (control = \$19,500 vs. leukoreduced = \$19,200, $p=0.24$) [31]. Although no significant cost-savings was seen, one could conclude that in the general patient population, leukoreduction did not increase total hospital expenditures. Furthermore, as mentioned previously, it has been estimated that leukoreduction for gastrointestinal and aneurysm surgeries could expect to save the Dutch healthcare system £2,900,000 per year due to a decreased length of hospital stay alone [41]. These data suggest that leukoreduction, at worst, will not change the cost of care and at best, will result in substantial cost savings while reducing morbidity and mortality, the proverbial "free lunch."

Leukoreduction Summary

It is a serious error and oversight that universal leukoreduction of transfusions has not been introduced in the United States in the 12–13 years since it was introduced in Canada, the United Kingdom and France, amongst other national blood systems. Leukoreduction not only reduces morbidity and mortality in transfusion recipients, but actually is that rare therapeutic advance that saves the health care system money rather than requiring net new expenditures.

Transfusion Immunomodulation Despite Leukoreduction

Allogeneic transfusion (but probably not autologous transfusion) has profound immunomodulatory effects on some patients that are broad and serious. Leukoreduction no doubt mitigates rather than abrogates these effects. What is known of the mechanisms of the residual immunomodulatory effects of leukoreduced red cell transfusions at this point in time? Multiple studies in animals and patients demonstrate that allogeneic (but not autologous) transfusion down regulates cellular immunity (*e.g.*, cytotoxic T cell and macrophage functions) and dysregulates inflammatory innate immunity (*e.g.*, natural killer cells, macrophages) [60]. These underlying immunomodulatory effects may explain the dose-dependent deleterious increases in post-operative infection, tumor recurrence, multi-organ failure, thrombosis and beneficial increases in kidney allograft survival seen in epidemiologic studies.

However, no single comprehensive theory is likely to explain the diverse effects of transfusion on the recipient's immune system. We and others have provided preliminary data in support of the hypothesis that immune deviation towards type 2 immune responses (of helper and cytotoxic T cells and monocytes/macrophages) and corresponding inhibition of type 1 responses may explain transfusion's adverse effects on post-operative infection

and cancer recurrence, as well as the favorable effects of transfusion on inflammatory bowel disease, kidney allograft survival and repetitive spontaneous abortion [61; 62]. This hypothesis suggests that following allogeneic transfusion, allergic and antibody driven immunity are increased (*i.e.*, type 2 responses) while cytotoxic T cell function and host defenses against bacteria and viruses are decreased (type 1 responses). In this fashion, transfusion may be immunologically similar to pregnancy, which slightly increases the risk of cancer and infections, while allowing tolerance of the fetus as an allograft. Additionally, increases in T regulatory cells has been demonstrated *in vitro* after allogeneic exposure to stored RBCs [63]. Promotion of T regulatory cells by allogeneic transfusion is another potential mechanism to account for the associations of transfusions with decreased allograft rejection, mitigation of autoimmune disease and increased risks of infection and tumor progression.

Harmful Mediators in Stored Blood

While leukoreduction ameliorates some of the immunomodulatory effects of transfusion in both experimental animal models and in clinical settings, there are clearly additional residual toxic effects of transfusion. In fact, associations have been made between transfusion and increased morbidity and mortality despite leukoreduction in certain patient populations [64]. Three recent studies have shown transfusion to be independently associated with increased complications following surgery in patients with colon [65], oral [66] or lung cancer [67]. Moreover, the risk of complications increased with the dose of blood transfused. These studies suggest that leukoreduced blood transfusions still contain harmful mediators that can adversely affect patient outcomes. Is it possible that the stored supernatant of red cells and platelets, for example, contains mediators, microparticles, *etc.* that alter recipient immune function in undesirable ways? [68] This is certainly true after irradiation of RBCs, which can result in increased potassium concentrations in the supernatants [69]. Transfusion of toxic amounts of potassium through blood transfusion predisposes some patients to arrhythmias and even asystole. In this setting, washing of irradiated RBCs had proven efficacious in preventing hyperkalemia after large volume transfusions during cardiopulmonary bypass.

There is also mounting evidence suggesting that unirradiated RBC and platelet units accumulate harmful supernatant mediators, such as lipids, free hemoglobin (RBCs), cytokines and microparticles over time [70]. Accumulation of these mediators has the theoretical potential to adversely affect the recipient after transfusion by altering hemostasis and inflammation. Despite leukoreduction at the time of collection to prevent WBC- and platelet-secreted cytokines from accumulating in stored blood, RBCs and particularly platelets can also contribute to the accumulation of many mediators during storage. For example, platelets in platelet concentrates release large amounts of sCD40L during storage, which can bind to the CD40 receptor on immune cells, such as PMNs and B-lymphocytes [11; 71]. Neutrophil priming is proposed to be a crucial event in the pathogenesis of TRALI, which can occur through ligation of the CD40 receptor on the neutrophil membrane. Moreover, *in vitro* and animal model studies demonstrate that ALI can be mediated by neutrophils primed by lysophosphatidylcholines, which accumulate in stored blood products [14]. These data suggest that washing of stored blood in order to remove the soluble mediators may be an effective way to minimize TRALI and other immune effects in transfused patients [72; 73].

Lipids, as well as cell-free hemoglobin, are known to alter endothelial cell and vascular function and also play an important role in platelet activation. Infusion of high concentrations of these mediators has the potential to adversely affect the recipient, thus washing of stored blood products to remove these mediators may be beneficial for selected high-risk patients. Moreover, damage to RBC and platelet integrity during storage leads to

the accumulation of microparticles over time [74]. Microparticles have been shown to alter hemostasis, inflammation and thrombosis, possibly by modifying platelet-WBC and platelet-endothelial cell interactions [75; 76]. Finally, microparticles possibly may participate in transcellular communication whereby mediators from stored cells may be packaged into microparticles and transferred to cells of the transfused recipient. With the knowledge that potentially harmful mediators accumulate in stored blood components, there is the possibility that there is a direct transfer of mediators to cells of the transfused patient.

Washing – Evidence for Benefit

Are stored, aged red cells and platelets toxic in and of themselves or do supernatant mediators cause harm to the recipient? There is evidence for both of these possibilities. In our hospital, over a 14-year period, the incidence of TACO (transfusion associated cardiac overload—e.g., congestive failure) and TRALI (transfusion related acute lung injury) following transfusion of leukoreduced platelets and red cells was 11 of 319,161 components transfused, which was significantly lower than that reported with non-leukoreduced transfusions. During that same period, the incidence of TACO and TRALI due to washed, leukoreduced platelets and red cells was zero of 97,445 components transfused ($p = 0.049$ compared with the incidence seen with red cell and platelets that were only leukoreduced) [30]. This reduced reporting of both TRALI and TACO with washed transfusions suggests that perhaps TACO is sometimes misdiagnosed TRALI, and/or that TACO may be caused or exacerbated by soluble inflammatory mediators, as has been hypothesized for congestive heart failure in general. Furthermore, the incidence of febrile reactions to platelet transfusions, which have both been leukoreduced and washed, is close to zero, compared with 1–2% in leukoreduced, non-washed transfusions. These observations are consistent with the previously mentioned *in vitro* studies demonstrating the ability of lipids from stored leukoreduced RBCs to cause ALI in experimental animals and pyrogenic mediators to cause fever and rigors.

A small randomized trial demonstrated that adult patients with acute leukemia receiving washed, leukoreduced ABO identical transfusions had improved survival compared with those receiving transfusions that were only leukoreduced and ABO identical [77; 78]. Cholette *et al* at our center completed a randomized trial demonstrating that, during pediatric cardiac surgery, the use of washed, leukoreduced red cell transfusions was associated with lower levels of inflammatory markers post-operatively than transfusions that were only leukoreduced [7]. There was a non-significant trend toward reduced mortality and blood component needs in the washed arm of the study. *In vitro* studies demonstrate increased surface expression of the activation marker, CD62P, after washing of platelet concentrates, indicating that the act of washing may activate platelets [79; 80]. It will be important to further investigate platelet activation post-washing and whether this is associated with any important adverse clinical outcomes. Our small study suggests that the benefit gained from removing potentially harmful mediators in the supernatants outweighs any possible harm due to platelet activation during washing. Additional investigations in larger populations are needed in both critically ill and hematology-oncology patients to determine if there is truly a benefit from routine use of plasma-depleted or washed transfusions. Removing the plasma from red cells and platelets prior to transfusion would almost certainly strikingly reduce or eliminate the risk of TACO and TRALI due to these components, but more evidence will be needed before the additional expense and difficulty of an entirely supernatant-depleted blood supply could be justified.

Conclusions

In summary, allogeneic transfusions can cause post-operative bacterial infection, cancer recurrence, lung injury, inflammation, multi-organ failure and possibly thrombosis. Leukoreduction substantially reduces the risks of post-operative infection, multi-organ failure and death in cardiac surgery, and may reduce the risk of lung injury and inflammation in certain patient groups. Leukoreduction also reduces CMV transmission, febrile reactions and HLA alloimmunization, none of which are desirable outcomes. Universal leukoreduction is long overdue in the United States for these reasons, and should be mandated by regulatory (FDA) and accrediting organizations (AAAB, Joint Commission) to improve the quality of patient care, improve outcomes and reduce health care costs. Additionally, saline washing may reduce the risk of death in younger patients with acute leukemia and reduces inflammatory markers in pediatric cardiac surgery, but these results need to be confirmed by others and studied in larger cohorts. Transfusions dramatically alter the immune system of recipients and the mechanisms by which this occurs are the main research challenges of the coming decades in transfusion medicine. Overall, leukoreduction is a simple and obvious answer towards mitigating immunomodulation and improving current transfusion therapies. In the future, strategies such as washing, rejuvenation, renitrosylation and shorter storage periods may be strategies for reducing the unfavorable immunologic, rheologic and vascular effects of blood transfusions.

Acknowledgments

Grant Support

Supported in part by NIH grants ES01247, HL078603, HL095467, HL100051.

Disclosure of Conflicts of Interest

NB has received lecture honoraria, consulting fees and research support from Pall Biomedical and Fenwal, manufacturers of leukoreduction filters, as well as from Caridian (now Terumo), manufacturers of cell washing equipment and supplies.

Abbreviations

AABB	the voluntary accrediting organization formerly known as the American Association of Blood Banks
AKI	acute kidney injury
ALI	acute lung injury
ARDS	acute respiratory distress syndrome
CMV	cytomegalovirus
FDA	Food and Drug Administration
FNHTR	febrile nonhemolytic transfusion reactions
HCV	hepatitis C
HIV	human immunodeficiency virus
HLA	Human Leukocyte Antigen
OR	odds ratio
TACO	transfusion associated circulatory overload
TRALI	transfusion related acute lung injury

RBC	red blood cell
WBC	white blood cell

References

1. Wang D, Sun J, Solomon SB, Klein HG, Natanson C. Transfusion of older stored blood and risk of death: a meta-analysis. *Transfusion*. 2012; 52:1184–1195. [PubMed: 22188419]
2. Steiner ME, Assmann SF, Levy JH, et al. Addressing the question of the effect of RBC storage on clinical outcomes: the Red Cell Storage Duration Study (RECESS) (Section 7). *Transfus Apher Sci*. 2010; 43:107–116. [PubMed: 20655807]
3. Lacroix J, Hébert P, Fergusson D, et al. The Age of Blood Evaluation (ABLE) randomized controlled trial: study design. *Transfus Med Rev*. 2011; 25:197–205. [PubMed: 21550205]
4. van de Watering LM, Hermans J, Houbiers JG, et al. Beneficial effects of leukocyte depletion of transfused blood on postoperative complications in patients undergoing cardiac surgery: a randomized clinical trial. *Circulation*. 1998; 97:562–568. [PubMed: 9494026]
5. Bilgin YM, van de Watering LM, Eijssman L, et al. Double-blind, randomized controlled trial on the effect of leukocyte-depleted erythrocyte transfusions in cardiac valve surgery. *Circulation*. 2004; 109:2755–2760. [PubMed: 15148271]
6. 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; 47:573–581. [PubMed: 17381614]
7. Cholette JM, Henrichs KF, Alfieri GM, et al. Washing red blood cells and platelets transfused in cardiac surgery reduces postoperative inflammation and number of transfusions: Results of a prospective, randomized, controlled clinical trial*. *Pediatr Crit Care Med*. 2012; 13:290–299. [PubMed: 21926663]
8. Sparrow RL, Patton KA. Supernatant from stored red blood cell primes inflammatory cells: influence of prestorage white cell reduction. *Transfusion*. 2004; 44:722–730. [PubMed: 15104654]
9. Benson DD, Beck AW, Burdine MS, et al. Accumulation of pro-cancer cytokines in the plasma fraction of stored packed red cells. *J Gastrointest Surg*. 2012; 16:460–468. [PubMed: 22246854]
10. Davenport RD, Kunkel SL. Cytokine roles in hemolytic and nonhemolytic transfusion reactions. *Transfus Med Rev*. 1994; 8:157–168. [PubMed: 8081078]
11. Kaufman J, Spinelli SL, Schultz E, Blumberg N, Phipps RP. Release of biologically active CD154 during collection and storage of platelet concentrates prepared for transfusion. *J Thromb Haemost*. 2007; 5:788–796. [PubMed: 17403203]
12. Phipps RP, Kaufman J, Blumberg N. Platelet derived CD154 (CD40 ligand) and febrile responses to transfusion. *Lancet*. 2001; 357:2023–2024. [PubMed: 11438137]
13. Yin K, Halushka PV, Yan YT, Wong PY. Antiaggregatory activity of 8-epiprostaglandin F2 alpha and other F-series prostanoids and their binding to thromboxane A2/prostaglandin H2 receptors in human platelets. *J Pharmacol Exp Ther*. 1994; 270:1192–1196. [PubMed: 7932171]
14. Silliman CC, Moore EE, Kelher MR, et al. Identification of lipids that accumulate during the routine storage of prestorage leukoreduced red blood cells and cause acute lung injury. *Transfusion*. 2011; 51:2549–2554. [PubMed: 21615744]
15. Villagra J, Shiva S, Hunter LA, et al. Platelet activation in patients with sickle disease, hemolysis-associated pulmonary hypertension, and nitric oxide scavenging by cell-free hemoglobin. *Blood*. 2007; 110:2166–2172. [PubMed: 17536019]
16. Winterbourn CC. Free-radical production and oxidative reactions of hemoglobin. *Environ Health Perspect*. 1985; 64:321–330. [PubMed: 3007097]
17. Xiong Z, Cavaretta J, Qu L, et al. Red blood cell microparticles show altered inflammatory chemokine binding and release ligand upon interaction with platelets. *Transfusion*. 2010
18. Burnier L, Fontana P, Kwak BR, Angelillo-Scherrer A, et al. Cell-derived microparticles in haemostasis and vascular medicine. *Thromb Haemost*. 2009; 101:439–451. [PubMed: 19277403]

19. Thomas S, Beard M, Garwood M, Callaert M, Cardigan R. Platelet concentrates produced from whole blood using the Atreus processing system. *Vox Sang.* 2009; 97:93–101. [PubMed: 19392785]
20. Lawrie AS, Albany A, Cardigan RA, Mackie IJ, Harrison P. Microparticle sizing by dynamic light scattering in fresh-frozen plasma. *Vox Sang.* 2009; 96:206–212. [PubMed: 19175566]
21. Moroff G, Holme S, Keegan T, Heaton A. Storage of ADSOL-preserved red cells at 2.5 and 5.5 degrees C: comparable retention of in vitro properties. *Vox Sang.* 1990; 59:136–139. [PubMed: 2124752]
22. Bonaventura J. Clinical implications of the loss of vasoactive nitric oxide during red blood cell storage. *Proc Natl Acad Sci U S A.* 2007; 104:19165–19166. [PubMed: 18048331]
23. Bosman GJ, Werre JM, Willekens FL, Novotný VM. Erythrocyte ageing in vivo and in vitro: structural aspects and implications for transfusion. *Transfus Med.* 2008; 18:335–347. [PubMed: 19140816]
24. Wadhwa M, Seghatchian MJ, Dilger P, et al. Cytokines in WBC-reduced apheresis PCs during storage: a comparison of two WBC-reduction methods. *Transfusion.* 2000; 40:1118–1126. [PubMed: 10988316]
25. Wadhwa M, Krailadsiri P, Dilger P, et al. Cytokine levels as performance indicators for white blood cell reduction of platelet concentrates. *Vox Sang.* 2002; 83:125–136. [PubMed: 12201842]
26. Phelan HA, Gonzalez RP, Patel HD, et al. Prestorage leukoreduction ameliorates the effects of aging on banked blood. *J Trauma.* 2010; 69:330–337. [PubMed: 20699741]
27. Phelan HA, Eastman AL, Aldy K, et al. Prestorage leukoreduction abrogates the detrimental effect of aging on packed red cells transfused after trauma: a prospective cohort study. *Am J Surg.* 2012; 203:198–204. [PubMed: 21924400]
28. Hébert PC, Fergusson D, Blajchman MA, et al. Clinical outcomes following institution of the Canadian universal leukoreduction program for red blood cell transfusions. *JAMA.* 2003; 289:1941–1949. [PubMed: 12697796]
29. King KE, Shirey RS, Thoman SK, et al. Universal leukoreduction decreases the incidence of febrile nonhemolytic transfusion reactions to RBCs. *Transfusion.* 2004; 44:25–29. [PubMed: 14692963]
30. Blumberg N, Heal JM, Gettings KF, et al. An association between decreased cardiopulmonary complications (transfusion-related acute lung injury and transfusion-associated circulatory overload) and implementation of universal leukoreduction of blood transfusions. *Transfusion.* 2010; 50:2738–2744. [PubMed: 20561296]
31. Dzik WH, Anderson JK, O'Neill EM, et al. A prospective, randomized clinical trial of universal WBC reduction. *Transfusion.* 2002; 42:1114–1122. [PubMed: 12430666]
32. Geiger TL, Howard SC. Acetaminophen and diphenhydramine premedication for allergic and febrile nonhemolytic transfusion reactions: good prophylaxis or bad practice? *Transfus Med Rev.* 2007; 21:1–12. [PubMed: 17174216]
33. Hendrickson JE, Hillyer CD. Noninfectious serious hazards of transfusion. *Anesth Analg.* 2009; 108:759–769. [PubMed: 19224780]
34. Romano G, Mastroianni C, Bancone C, et al. Leukoreduction program for red blood cell transfusions in coronary surgery: association with reduced acute kidney injury and in-hospital mortality. *J Thorac Cardiovasc Surg.* 2010; 140:188–195. [PubMed: 20416894]
35. Plurad D, Belzberg H, Schulman I, et al. Leukoreduction is associated with a decreased incidence of late onset acute respiratory distress syndrome after injury. *Am Surg.* 2008; 74:117–123. [PubMed: 18306860]
36. Netzer G, Shah CV, Iwashyna TJ, et al. Association of RBC transfusion with mortality in patients with acute lung injury. *Chest.* 2007; 132:1116–1123. [PubMed: 17505028]
37. Miyaji K, Miyamoto T, Kohira S, et al. The effectiveness of prestorage leukocyte-reduced red blood cell transfusion on perioperative inflammatory response with a miniaturized biocompatible bypass system. *J Thorac Cardiovasc Surg.* 2010; 139:1561–1567. [PubMed: 20038472]
38. Fung MK, Rao N, Rice J, et al. Leukoreduction in the setting of open heart surgery: a prospective cohort-controlled study. *Transfusion.* 2004; 44:30–35. [PubMed: 14692964]

39. Fung MK, Moore K, Ridenour M, Mook W, Triulzi DJ. Clinical effects of reverting from leukoreduced to nonleukoreduced blood in cardiac surgery. *Transfusion*. 2006; 46:386–391. [PubMed: 16533280]
40. Blumberg N, Heal JM, Cowles JW, et al. Leukocyte-reduced transfusions in cardiac surgery results of an implementation trial. *Am J Clin Pathol*. 2002; 118:376–381. [PubMed: 12219779]
41. van Hilten JA, van de Watering LM, van Bockel JH, et al. Effects of transfusion with red cells filtered to remove leucocytes: randomised controlled trial in patients undergoing major surgery. *BMJ*. 2004; 328:1281. [PubMed: 15142885]
42. Friese RS, Sperry JL, Phelan HA, Gentilello LM. The use of leukoreduced red blood cell products is associated with fewer infectious complications in trauma patients. *Am J Surg*. 2008; 196:56–61. [PubMed: 18513694]
43. Phelan HA, Sperry JL, Friese RS. Leukoreduction before red blood cell transfusion has no impact on mortality in trauma patients. *J Surg Res*. 2007; 138:32–36. [PubMed: 17161430]
44. Nathens AB, Nester TA, Rubenfeld GD, Nirula R, Gernsheimer TB. The effects of leukoreduced blood transfusion on infection risk following injury: a randomized controlled trial. *Shock*. 2006; 26:342–347. [PubMed: 16980879]
45. Vamvakas EC. Why have meta-analyses of randomized controlled trials of the association between non-white-blood-cell-reduced allogeneic blood transfusion and postoperative infection produced discordant results? *Vox Sang*. 2007; 93:196–207. [PubMed: 17845256]
46. Jensen LS, Andersen AJ, Christiansen PM, et al. Postoperative infection and natural killer cell function following blood transfusion in patients undergoing elective colorectal surgery. *Br J Surg*. 1992; 79:513–516. [PubMed: 1611441]
47. Jensen LS, Kissmeyer-Nielsen P, Wolff B, Qvist N. Randomised comparison of leucocyte-depleted versus buffy-coat-poor blood transfusion and complications after colorectal surgery. *Lancet*. 1996; 348:841–845. [PubMed: 8826808]
48. Houbiers JG, Brand A, van de Watering LM, et al. Randomised controlled trial comparing transfusion of leucocyte-depleted or buffy-coat-depleted blood in surgery for colorectal cancer. *Lancet*. 1994; 344:573–578. [PubMed: 7914960]
49. Tartter PI, Mohandas K, Azar P, et al. Randomized trial comparing packed red cell blood transfusion with and without leukocyte depletion for gastrointestinal surgery. *Am J Surg*. 1998; 176:462–466. [PubMed: 9874434]
50. Titlestad IL, Ebbesen LS, Ainsworth AP, et al. Leukocyte-depletion of blood components does not significantly reduce the risk of infectious complications. Results of a double-blinded, randomized study. *Int J Colorectal Dis*. 2001; 16:147–153. [PubMed: 11459288]
51. Vamvakas EC, Blajchman MA. Prestorage versus poststorage white cell reduction for the prevention of the deleterious immunomodulatory effects of allogeneic blood transfusion. *Transfus Med Rev*. 2000; 14:23–33. [PubMed: 10669938]
52. Vamvakas EC. Meta-analysis of randomized controlled trials investigating the risk of postoperative infection in association with white blood cell-containing allogeneic blood transfusion: the effects of the type of transfused red blood cell product and surgical setting. *Transfus Med Rev*. 2002; 16:304–314. [PubMed: 12415516]
53. Vandromme MJ, McGwin G, Weinberg JA. Blood transfusion in the critically ill: does storage age matter? *Scand J Trauma Resusc Emerg Med*. 2009; 17:35. [PubMed: 19678943]
54. Zallen G, Offner PJ, Moore EE, et al. Age of transfused blood is an independent risk factor for postinjury multiple organ failure. *Am J Surg*. 1999; 178:570–572. [PubMed: 10670874]
55. Offner PJ, Moore EE, Biffl WL, Johnson JL, Silliman CC. Increased rate of infection associated with transfusion of old blood after severe injury. *Arch Surg*. 2002; 137:711–716. discussion 716–7. [PubMed: 12049543]
56. Stone PW, Larson E, Kowar LN. A systematic audit of economic evidence linking nosocomial infections and infection control interventions: 1990–2000. *Am J Infect Control*. 2002; 30:145–152. [PubMed: 11988708]
57. Blumberg N, Heal JM. Blood transfusion immunomodulation: the silent epidemic. *Arch Pathol Lab Med*. 1998; 122:117–119. [PubMed: 9499352]

58. Blumberg N, Heal JM. Mortality risks, costs, and decision making in transfusion medicine. *Am J Clin Pathol.* 2000; 114:934–937. [PubMed: 11338483]
59. van Hulst M, Bilgin YM, van de Watering LM, et al. Cost-effectiveness of leucocyte-depleted erythrocyte transfusion in cardiac valve surgery. *Transfus Med.* 2005; 15:209–217. [PubMed: 15943705]
60. Blumberg N, Heal JM. Immunomodulation by blood transfusion: an evolving scientific and clinical challenge. *Am J Med.* 1996; 101:299–308. [PubMed: 8873492]
61. Babcock GF, Alexander JW. The effects of blood transfusion on cytokine production by TH1 and TH2 lymphocytes in the mouse. *Transplantation.* 1996; 61:465–468. [PubMed: 8610362]
62. Blumberg N, Heal JM. The transfusion immunomodulation theory: the Th1/Th2 paradigm and an analogy with pregnancy as a unifying mechanism. *Semin Hematol.* 1996; 33:329–340. [PubMed: 8916306]
63. Baumgartner JM, Silliman CC, Moore EE, Banerjee A, McCarter MD. Stored red blood cell transfusion induces regulatory T cells. *J Am Coll Surg.* 2009; 208:110–119. [PubMed: 19228512]
64. Weinberg JA, McGwin G, Griffin RL, et al. Age of transfused blood: an independent predictor of mortality despite universal leukoreduction. *J Trauma.* 2008; 65:279–282. discussion 282–4. [PubMed: 18695462]
65. Koch M, Antolovic D, Reissfelder C, et al. Leucocyte-depleted blood transfusion is an independent predictor of surgical morbidity in patients undergoing elective colon cancer surgery—a single-center analysis of 531 patients. *Ann Surg Oncol.* 2011; 18:1404–1411. [PubMed: 21153884]
66. Perisanidis C, Dettke M, Papadogeorgakis N, et al. Transfusion of allogenic leukocyte-depleted packed red blood cells is associated with postoperative morbidity in patients undergoing oral and oropharyngeal cancer surgery. *Oral Oncol.* 2012; 48:372–378. [PubMed: 22182932]
67. Ng T, Ryder BA, Chern H, et al. Leukocyte-depleted blood transfusion is associated with decreased survival in resected early-stage lung cancer. *J Thorac Cardiovasc Surg.* 2012; 143:815–819. [PubMed: 22325327]
68. Ness PM. Does transfusion of stored red blood cells cause clinically important adverse effects? A critical question in search of an answer and a plan. *Transfusion.* 2011; 51:666–667. [PubMed: 21496035]
69. Swindell CG, Barker TA, McGuirk SP, et al. Washing of irradiated red blood cells prevents hyperkalemia during cardiopulmonary bypass in neonates and infants undergoing surgery for complex congenital heart disease. *Eur J Cardiothorac Surg.* 2007; 31:659–664. [PubMed: 17291775]
70. Grimshaw K, Sahler J, Spinelli SL, Phipps RP, Blumberg N. New frontiers in transfusion biology: identification and significance of mediators of morbidity and mortality in stored red blood cells. *Transfusion.* 2011; 51:874–880. [PubMed: 21496049]
71. Khan SY, Kelher MR, Heal JM, et al. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. *Blood.* 2006; 108:2455–2462. [PubMed: 16772606]
72. Vlaar AP, Hofstra JJ, Levi M, et al. Supernatant of aged erythrocytes causes lung inflammation and coagulopathy in a "two-hit" in vivo syngeneic transfusion model. *Anesthesiology.* 2010; 113:92–103. [PubMed: 20508493]
73. Vlaar AP, Hofstra JJ, Kulik W, et al. Supernatant of stored platelets causes lung inflammation and coagulopathy in a novel in vivo transfusion model. *Blood.* 2010; 116:1360–1368. [PubMed: 20479286]
74. Rubin O, Crettaz D, Canellini G, Tissot JD, Lion N. Microparticles in stored red blood cells: an approach using flow cytometry and proteomic tools. *Vox Sang.* 2008; 95:288–297. [PubMed: 19138258]
75. Rubin O, Crettaz D, Tissot JD, Lion N. Microparticles in stored red blood cells: submicron clotting bombs? *Blood Transfus.* 2010; 8(Suppl 3):s31–s38. [PubMed: 20606747]
76. Tissot JD, Rubin O, Canellini G. Analysis and clinical relevance of microparticles from red blood cells. *Curr Opin Hematol.* 2010; 17:571–577. [PubMed: 20960973]

77. Blumberg N, Heal JM, Rowe JM. A randomized trial of washed red blood cell and platelet transfusions in adult acute leukemia [ISRCTN76536440]. *BMC Blood Disord.* 2004; 4:6. [PubMed: 15588315]
78. Blumberg N, Heal JM, Liesveld JL, Phillips GL, Rowe JM. Platelet transfusion and survival in adults with acute leukemia. *Leukemia.* 2008; 22:631–635. [PubMed: 17805333]
79. Tynngård N, Trinks M, Berlin G. Platelet quality after washing: the effect of storage time before washing. *Transfusion.* 2010; 50:2745–2752. [PubMed: 20576018]
80. Tynngård N, Trinks M, Berlin G. In vitro quality of platelets during prolonged storage after washing with three platelet additive solutions. *Vox Sang.* 2012; 102:32–39. [PubMed: 21534983]

Figure 1. Leukoreduction of blood transfusions reduces the risk of postoperative infection by approximately 36 percent

A forest plot of a meta-analysis of randomized trials is shown for 3093 patients who actually received transfusions ($p=0.005$). Reproduced from Blumberg, H. Zhao, H. Wang, et al. Transfusion 47 (2007) 573-81.

Figure 2. Estimated potential cost savings in the USA of universal leukoreduction for transfusions to surgical patients as opposed to use of exclusively non-leukoreduced transfusions

Table 1
Harmful biological mediators accumulate during the storage of blood

WBCs, RBCs and platelets produce soluble mediators during storage that may lead to adverse outcomes in the transfused recipient. Some of these adverse reactions can be mitigated, at least in part, through pre-storage leukoreduction and/or post-storage washing of cells prior to transfusion. Microparticles and storage induced aging may also contribute to the adverse immunologic, rheologic and vascular effects of transfusions.

Mediator	Cell Origin	Postulated Mechanism of Action	Potential Clinical Effects	Prevention Strategy	Reference
IL-6	WBCs	Mediates acute phase response Activates platelets	Inflammation Fever	Leukoreduction Washing	[7]
IL-8	WBCs	Recruits neutrophils	Inflammation	Leukoreduction Washing	[8]
MCP-1	WBCs	Recruits monocytes, T cells & dendritic cells	Inflammation Mediates hemolytic transfusion reaction symptoms	Leukoreduction Washing	[9; 10]
sCD40L	Platelets	Primes neutrophils Activates platelets, B cells & endothelial cells	Inflammation TRALI	Leukoreduction Washing	[11; 12]
Lipid Mediators	WBCs, RBCs & platelets	Prime neutrophils Activates platelets	Inflammation TRALI	Leukoreduction Washing	[13; 14]
Cell-free hemoglobin	RBCs	Generates free radicals Provides iron load Activates platelets	Alters redox state Fosters infection and vascular dysfunction	Washing Shorter storage	[15; 16]
Microparticles	WBCs, RBCs & platelets	Activate endothelial cells, platelets and host immune cells Transfers cellular contents to host cells	Thrombosis Inflammation	Leukoreduction Washing	[17; 18; 19; 20]
Storage Damaged/Altered RBCs	RBCs	Inadequate 2,3 BPG, nitric oxide Altered adhesive & rheologic properties	Dysregulated oxygen release Vascular dysfunction Thrombosis Sludging	Rejuvenation Renitrosylation Shorter storage	[21; 22; 23]