

Role of Leukoreduction of Packed Red Blood Cell Units in Trauma Patients: A Review

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ABSTRACT

Hemorrhagic shock is a leading cause of mortality within the trauma population, and blood transfusion is the standard of care. Leukoreduction filters remove donor leukocytes prior to transfusion of blood products. While the benefits of leukocyte depletion are well documented in scientific literature, these benefits do not translate directly to the clinical setting. This review summarizes current research regarding leukoreduction in the clinical arena, as well as studies performed exclusively in the trauma population.

Key words: Leukocyte reduction; Blood transfusion; Erythrocyte transfusion; Trauma; Review

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INTRODUCTION

Trauma injury is leading cause of life years lost in the United States^[1,2]. At 30% of life years lost, this amounts to more than cancer, heart disease, and HIV combined^[2]. Among trauma patients, hemorrhage is responsible for 30-40% of all trauma-related mortality^[3]. Hemorrhagic shock is more than mere intravascular hypovolemia; it is accompanied by profound acidosis, coagulopathy, metabolic derangements, electrolyte abnormalities, and multisystem organ failure^[4,5]. The ideal treatment for hemorrhagic shock is transfusion of blood products^[6].

In the acutely hemorrhaging trauma patient, massive transfusion may be required to maintain normal vital and hematologic parameters until hemostasis is achieved. Massive transfusion is defined as transfusion of 10 units of packed red blood cells (RBCs) within 24 hours or the loss of 1-to-1.5 times the patient's entire blood volume^[7]. Hemorrhagic shock is frequently exacerbated by coagulopathy due to the consumption of coagulation factors and transfusion-associated hemodilution, in addition to the traumatic injury itself.

Currently, various transfusion strategies and institution-specific protocols are practiced in the hemorrhaging patient. While core principals remain similar across different practices, there is no definitive algorithm for optimal resuscitation. In light of this disparity, several studies have aimed to elucidate the optimal ratio of transfusion of various blood components during the acute phase. One such study, the Prospective Observational Multicenter Major Trauma Transfusion (PROMTTT) study, compared different blood component ratios and subsequent mortality rates at the 6-hour and 24-hour time points. This study included a total of 2150 adult trauma patients spanning ten level I centers across the US. At the 6-hour time point, the primary endpoint of in-hospital mortality was up to fourfold higher for those who received a lower ratio of plasma and platelets to RBCs compared to their counterpart (less than 1: 2 plasma or platelets-to-RBCs vs 1: 1 ratio). At the 24-hour mark, there

Table 1 Leukoreduction trials in the clinical setting

Trial	Year	Population	Endpoints	Results
Jensen <i>et al</i>	1996	Colorectal surgery (<i>n</i> = 260)	Wound infection [§]	0% [†] vs 12% [‡] (<i>P</i> < 0.0001)
			Intraabdominal abscesses [§]	0% [†] vs 5% [‡] (<i>P</i> = 0.017)
			Postoperative pneumonia [§]	3% [†] vs 23% [‡] (<i>P</i> < 0.001)
Van de Watering <i>et al</i>	1998	Cardiac surgery (<i>n</i> = 914)	Postoperative infection	16.9%/17.9% [†] vs 23% [‡] (<i>P</i> = 0.13)
			Postoperative mortality [§]	3.6%/3.3% [†] vs 7.8% [‡] (<i>P</i> = 0.015)
Uhlmann <i>et al</i>	2001	Hospital (<i>n</i> = 67 846)	Total reactions	0.20% [†] vs 0.25% [‡] (<i>P</i> = 0.41)
			FNHTR	0.08% [†] vs 0.12% [‡] (<i>P</i> = 0.33)
			ATR	0.06% [†] vs 0.04% [‡] (<i>P</i> = 0.43)
Dzik <i>et al</i>	2002	Hospital (<i>n</i> = 2 780)	In-hospital mortality	9.0% [†] vs 8.5% [‡] (<i>P</i> = 0.64)
			LOS (days)	6.3 [†] vs 6.4 [‡] (<i>P</i> = 0.21)
			Total hospital costs (\$)	19200 [†] vs 19500 [‡] (<i>P</i> = 0.24)
Bilgin <i>et al</i>	2004	Cardiac surgery (<i>n</i> = 496)	90-day mortality	8.4% [†] vs 12.7% [‡] , OR 1.52 (0.84-2.73) (<i>P</i> > 0.05)
			In-hospital mortality [§]	5.5% [†] vs 10.1% [‡] , OR 1.99 (0.99-4.00) (<i>P</i> < 0.05)
			Infection [§]	21.6% [†] vs 31.6% [‡] , OR 1.64 (1.08-2.49) (<i>P</i> < 0.05)
King <i>et al</i>	2004	Hospital (<i>n</i> = 36 162)	Total reactions [§]	0.40% [†] vs 0.56% [‡] (<i>P</i> = 0.024)
			FNHTR [§]	0.19% [†] vs 0.37% [‡] (<i>P</i> = 0.0008)
			ATR	0.17% [†] vs 0.15% [‡] (<i>P</i> = 0.59)
Llewelyn <i>et al</i>	2004	Cardiac and orthopedic surgery (<i>n</i> = 2 095)	Postoperative infection	20% [†] vs 21% [‡] , OR 0.83 (0.67-1.04) (<i>P</i> = 0.099)
			Postoperative LOS (days)	9.6 [†] vs 10.0 [‡] , OR 1.01 (0.92-1.10) (<i>P</i> = 0.905)
Paglino <i>et al</i>	2004	Hospital (<i>n</i> = 145 369)	FNHTR [§]	0.18% [†] vs 0.34% [‡] OR 1.70 (1.40-2.50) (<i>P</i> < 0.001)
			ATR	0.09% [†] vs 0.09% [‡] , OR 1.18 (0.66-2.10) (<i>P</i> > 0.05)
			FNHTR [§]	0.19% [†] vs 0.33% [‡] (<i>P</i> < 0.001)
Yazer <i>et al</i>	2004	Hospital (<i>n</i> = 143 345)	ATR	Not significant, data not reported
			TRALI [§]	Decreased, data not reported
			Hemolytic reaction	Not significant, data not reported
			Delayed reaction	Not significant, data not reported
Blumberg <i>et al</i>	2005	Hospital (<i>n</i> = 574 835)	Line-related infection [§]	0.033% [†] vs 0.053% [‡] (<i>P</i> = 0.0002)
Frietsch <i>et al</i>	2008	Orthopedic surgery (<i>n</i> = 1 089)	Postoperative infection	OR 1.20 (0.77-1.87) (<i>P</i> = 0.82)
			Postoperative LOS (days)	14.0 [†] vs 14.0 [‡] (<i>P</i> = 0.17)
Blumberg <i>et al</i>	2010	Hospital (<i>n</i> = 778 559)	TRALI cases [§]	OR 0.17 (0.04-0.80) (<i>P</i> = 0.01)
			TACO cases [§]	OR 0.52 (0.28-0.96) (<i>P</i> = 0.03)
			FNHTR [§]	OR 0.65 (0.56-0.76) (<i>P</i> < 0.0001)
			ATR	OR 0.99 (0.84-1.17) (<i>P</i> = 0.96)

Key: [†]leukoreduced arm, [‡]nonleukoreduced arm, [§]significant result.

was no significant difference in mortality, presumably because non-hemorrhagic causes of death outweighed hemorrhagic causes^[8].

Another multicenter prospective trial, the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) study, investigated the ideal ratio of blood components for the hemorrhagic patient. Among 680 patients across 12 level I trauma centers, transfusion of 1: 1: 1 ratio vs 1: 1: 2 ratio of plasma to platelets to RBCs was compared, with primary endpoints of 24-hour and 30-day all-cause mortality. While there were no significant differences in mortality at either time point, significantly higher rates of hemostasis and fewer deaths due to exsanguination were observed in the 1: 1: 1 group^[9]. Both the PROMTTT and PROPPR studies suggest a critical role of transfusion of plasma and platelets along with packed RBCs in the acutely hemorrhaging trauma patient.

Unfortunately, transfusion of large volumes of blood may be associated with significant complications. Common transfusion-associated reactions include hemolytic reactions, febrile non-hemolytic transfusion reactions (FNHTR), allergic transfusion reactions (ATR), transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), nosocomial infections likely secondary to immunosuppression, and reperfusion injury^[10-12]. These complications are attributed to the transfused blood itself. Several factors have been implicated as the injurious element, including the RBC storage lesion, RBC-derived microparticles, stored cytokines, donor antigens, and donor leukocytes^[13]. RBC-derived microparticles, for example, activate lung endothelial cells and provoke lung injury in animal models^[14,15].

Leukoreduction

Leukoreduction (LR) aims to attenuate transfusion-associated reactions by filtering donor leukocytes from packed RBC units. In order to achieve leukoreduction, freshly collected RBC units are passed through a filter (Figure 1) that operates by two mechanisms: barrier filtration and cell adhesion. Barrier filtration partitions cell types based on size. Modern LR filters have a pore size of 4 μm, which is sufficient to allow platelet and deformable RBC passage but retains leukocytes. Cellular adhesion between the filter medium and the leukocytes themselves also contributes to leukocyte retention^[16].

For each unit of RBCs filtered, approximately 100 μL of leukocytes are extracted^[16]. Prior to undergoing LR, RBC units carry 2-5 billion leukocytes per unit. After LR, this count is brought to less than one million per unit, which is compliant with current Food and Drug Administration (FDA) regulations^[17,18].

LR may be performed either at the time of blood product manufacturing, referred to as prestorage LR, or immediately prior to transfusion, termed poststorage LR. Each approach has its advantages and disadvantages. Prestorage LR has the distinct advantage of removing leukocytes before they can contribute to the RBC storage lesion. The process is more streamlined, and thus, better quality control measures may be implemented. Poststorage LR, on the other hand, is cheaper than its counterpart, and bears the theoretical advantage of filtering nucleocytosements accumulated during the storage period^[19].

Advantages and disadvantages of leukoreduction

Filtering pRBC units does more than removing donor leukocytes. LR also filters pro-inflammatory mediators, prevents transmission of

Table 2 Leukoreduction trials in the trauma population

Trial	Year	Population	Endpoints	Results
Nathens <i>et al</i>	2006	Trauma (<i>n</i> = 268)	28-day infection rate	RR 1.20 (0.55-1.3)
			Febrile episodes	RR 1.01 (0.89-1.2)
			Organ dysfunction (mean Marshall score)	5.9 [†] vs 6.6 [‡] (<i>P</i> = 0.26)
			Overall mortality	RR 1.20 (0.74-1.9)
Utter <i>et al</i>	2006	Trauma (<i>n</i> = 68)	TAMC	37% [†] vs 28% [‡] (<i>P</i> = 0.43)
Phelan <i>et al</i>	2007	Trauma (<i>n</i> = 679)	Overall mortality	7.9% [†] vs 7.1% [‡] (<i>P</i> = 0.68)
			LOS (days)	12.0 [†] vs 12.0 [‡] (<i>P</i> = 0.46)
Watkins <i>et al</i>	2008	Trauma (<i>n</i> = 268)	ALL, early (< 72 hr)	RR 1.06 (0.69-1.64)
			ARDS, early (< 72 hr)	RR 0.96 (0.48-1.91)
			ALL, late (≥ 72 hr)	RR 0.88 (0.54-1.44)
			ARDS, late (≥ 72 hr)	RR 0.95 (0.58-1.57)

Key: [†]leukoreduced arm, [‡]nonleukoreduced arm, [§]significant result.



Figure 1 Leukoreduction filter. Standard leukoreduction filter. Pink filter is used to filter red blood cells, and white filter is used to filter platelets. Image courtesy of Robert Giuletto at Hoxworth blood center.

blood borne infectious agents, and reduces human leukocyte antigen (HLA) antibody production in sensitized transfusion recipients^[20].

Stored RBC units carry inflammatory cytokines that accrue throughout the storage period, such as tumor necrosis factor (TNF- α), interleukin-1 (IL-1), and IL-8^[21]. In addition, stored RBC units can prime unstimulated neutrophils *in vitro*^[22]. LR reduces the amount of inflammatory cytokine accumulated during storage and abrogates this inflammatory response^[22,23]. Prestorage LR is more effective in reducing cytokines than poststorage LR, suggesting that these cytokines may be generated from donor leukocytes themselves^[24]. LR also removes human neutrophil peptides, the major antimicrobial peptides of neutrophils^[25].

LR filters may restrict the transmission of infectious agents stored in the blood. Viral agents commonly transmitted via leukocytes include Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human T-cell lymphocytic virus (HTLV)-I. LR is an effective alternative to CMV-seronegative blood products in preventing transfusion-associated CMV infection in bone marrow recipients^[26,27]. EBV titers are also significantly reduced by RBC filtration, rendering most filtered units EBV-negative^[28]. HTLV-I titers decline as well after LR, though not completely^[29]. Other blood borne infectious diseases curbed by RBC filtration include malaria, leishmaniasis, human granulocytic anaplasmosis, and *Yersinia enterocolitidis*^[30-33].

Despite these benefits, leukocyte depletion is not without its drawbacks. Up to 10% of RBCs may be inadvertently removed during the filtering process^[34]. Considering 12-14 million units of blood are donated each year, that amounts to a considerable fraction of discarded cells^[35]. RBCs have also been shown to hemolyze during processing, further reducing their oxygen-delivering capacity^[36]. The acceptable level of hemolysis is currently set at 1% in the United States^[36]. In response to this concern, Ghandi *et al* reported

in 2006 that leukoreduced RBCs did not demonstrate a significant difference in hemolyzed cells when compared to nonleukoreduced RBCs^[37]. Altogether, the advantages of LR far outweigh the technical difficulties, at least on a scientific level.

Should all pRBC units be leukoreduced?

While the advantages of LR are well documented, the cost-to-benefit ratio of universal leukoreduction (ULR) remains a topic of controversy^[38-40]. Approximately 3-4 million patients receive blood transfusions each year, and the cost of LR amounts to approximately \$30 per unit^[41]. This calculates to an annual expenditure of over \$500 million^[42]. Critics argue that this cost may not be worth the clinical benefits, and that LR should be considered on a case-by-case basis. Proponents nevertheless insist that the United States is lagging behind Europe and Canada, who have already instituted ULR into their blood-banking industries.

One such proponent, the Advisory Committee on Blood Safety and Availability from the Department of Health and Human Services (DHHS), issued a formal statement in 2001 recommending ULR to the FDA. Today, nearly fifteen years later, ULR has yet to be adopted. Nevertheless, approximately 80% of all RBC units transfused in the United States are leukoreduced. The Red Cross, who supplies nearly half of the blood transfused, has incorporated ULR into its practices. United Blood Services, which provides 10% of transfused blood in the United States, also follows ULR standards. Among the independent services, which account for the remainder of the blood banking industry, almost half abide by ULR standards.

Indications for leukoreduction

In response to the debate over ULR, the University Health System Consortium (UHC) convened an Expert Panel to establish consensus recommendations for the use of leukoreduced blood products. Their evidence-based indications for LR are as follows^[40]:

1. Patients that require long-term platelet support in order to decrease refractory platelet transfusion due to HLA alloimmunization;
2. CMV-seronegative patients who require reduced risk of CMV transmission.
3. Patients with documented FNHTR, in order to prevent future episodes.
4. Solid organ transplant candidates (nonhepatic), in order to decrease the incidence of HLA alloimmunization.

The UHC Expert Panel also established nonindications for LR based on clinical data available in 2001^[40]:

1. Prevention of viral reactivation in CMV or HIV-positive patients.
2. Prevention of general immunomodulatory effects, including cancer recurrence, postoperative infection, and postoperative mortality.

3. Reduction of hospital length of stay (LOS).
4. Prevention of transfusion-associated graft-versus-host disease (GVHD) or TRALI.
5. Prevention of bacterial sepsis.
6. Prevention of transfusion-related infections including HTLV-II, EBV, human herpesvirus-8 (HHV-8), and other blood borne infections.
7. Prevention of acquired prion diseases such as variable Creutzfeldt-Jakob disease (vCJD).

Their unanimous conclusion was that the cost-to-benefit ratio for ULR was not justified, and that the FDA should not mandate ULR based on their evidence-based review.

Leukoreduction in the clinical setting

Since the establishment of the UHC Expert Panel guidelines in 2001, many clinical trials and retrospective analyses have been performed comparing filtered versus unfiltered blood products. Various endpoints have been scrutinized in determining whether the scientific advantages of leukocyte filtration translate to the clinical setting. These endpoints include transfusion reactions, infection rates, and hospital-related parameters. Clinical studies are summarized in Table 1.

FNHTR is the most common acute transfusion reaction, and the most common endpoint analyzed across all studies. It is defined as a $\geq 1^\circ\text{C}$ temperature increase above baseline within three hours of transfusion, which is not attributed to separate cause^[43]. Because FNHTR is associated with the presence of donor leukocytes and platelets, many have postulated that LR prevents FNHTR through the removal of released cytokines^[43-45]. In concordance with these scientific findings, five large studies have found significantly decreased rates of FNHTR associated with leukoreduced blood ($n = 2780, 36\ 162, 145\ 369, 143\ 345, 778\ 559$)^[46-50]. A sixth study noted a similar but nonsignificant decrease in febrile reactions from 0.12% to 0.08% with LR ($n = 67846$)^[51].

ATRs encompass a host of reactions, ranging from mild urticaria to anaphylactic shock. These are mostly immunoglobulin E (IgE)-mediated type I hypersensitivity reactions leading to mast cell degranulation. Antibodies against plasma proteins are responsible for severe ATRs, often observed in IgA-deficient patients^[52]. Four separate studies noted no significant correlation between leukocyte depletion and rates of ATR ($n = 67\ 846, 36\ 162, 145\ 369, 778\ 559$)^[47-49,51].

TRALI is the leading cause of transfusion-related mortality. Defined as acute onset non-cardiogenic pulmonary edema following transfusion, it is often confused with TACO, which is acute onset cardiogenic pulmonary edema due to large volume transfusion. LR was associated with decreased TRALI cases in two studies ($n = 143\ 345, 778\ 559$) and decreased TACO cases in one study ($n = 778\ 559$)^[49,50].

Blood transfusion is associated with higher infection rates in intensive care unit (ICU) patients, colorectal surgery patients, and cardiac surgery patients^[11,53,54]. Additionally, the rate of nosocomial infections in the ICU is linked to increased mortality and LOS^[10]. Six separate studies were carried out in various patient populations, analyzing whether LR had any relationship to rate of infection. In the colorectal surgery population, LR was associated with decreased rates of wound infection, intraabdominal abscesses, and postoperative pneumonia ($n = 260$)^[55]. In the orthopedic population, LR had no significant relationship to postoperative infection rates ($n = 1\ 089, 2\ 095$)^[56,57]. Both hospital and ICU patients had decreased line-related infection rates when using filtered versus nonfiltered blood products ($n = 574\ 835$)^[58,59]. Among the cardiac surgery population, one study

observed decreased infection rates ($n = 496$), while two others noted no significant correlation ($n = 2\ 095, 914$)^[57,60,61].

Hospital parameters analyzed include in-hospital mortality, LOS, total hospital costs, and readmission rate. LR was not found to correlate with in-hospital mortality in the general hospital population ($n = 2\ 780$), but reduced in-hospital mortality among cardiac surgery patients ($n = 496, 914$)^[46,60,61]. LR did not affect hospital LOS ($n = 2\ 780, 1\ 089, 2\ 095$), total hospital costs ($n = 2\ 780$), or readmission rates ($n = 2\ 780$)^[46,56,57].

Leukoreduction for transfusion in trauma patients

Since 2001, four randomized controlled trials have been performed regarding LR in the trauma population, and can be found in Table 2. In 2006, Nathens *et al* conducted a single-center, double-blinded randomized control trial comparing transfusion of leukocyte depleted versus non-depleted blood products ($n = 268$). He noted no significance in 28-day rate of infection (primary endpoint), febrile episodes, organ dysfunction scores, or overall mortality rates^[62]. A similar study was carried out by Phelan *et al.*, observing no significant difference in hospital LOS or overall mortality rates ($n = 679$)^[63].

In 2006, Utter *et al* performed a study on the development of transfusion-associated microchimerism (TAMC) within the trauma population. TAMC is defined as the long-term survival of donor leukocytes in the recipient's blood, and is thought to play a role in the development of autoimmune disorders such as graft-versus-host disease (GVHD). Given that LR removes donor leukocytes prior to blood transfusion, Utter postulated that LR may attenuate the development of TAMC and GVHD. At least one month following hospital discharge, trauma patients were evaluated using blood tests and a survey for GVHD symptoms. Neither endpoint was found to be significantly different between the leukoreduced versus non-reduced arms ($n = 67$)^[64].

In 2008, a single-center, double-blinded randomized control trial was performed evaluating whether LR affected the incidence of acute respiratory distress syndrome (ARDS) or acute lung injury (ALI, now classified as mild ARDS). Watkins *et al* found that LR did not significantly affect the incidence of early (< 72 hours) or late ALI/ARDS (≥ 72 hours). In addition, secondary endpoints such as ventilator parameters and ventilator-free days were not significantly different among the two groups ($n = 268$)^[65].

CONCLUSION

As long as traumatic injury exists, hemorrhagic shock will remain a leading cause of death, and patients will continue to require blood transfusion. The benefits of filtering these blood products far outweigh its disadvantages from a scientific perspective. As evidenced by many clinical trials, these benefits do not always clearly translate to the clinical setting. Thus, the cost-effectiveness of LR remains a topic for debate.

Among the trauma population, none of the studies performed to date have noted any significant effect of LR on the various endpoints. These studies have mostly been limited to smaller patient populations, however, in comparison to other clinical studies. One reason for this is the implementation of leukoreduction across various institutions. Larger non-trauma clinical studies have been able to retrospectively analyze their endpoints between pre-ULR and post-ULR periods, while the smaller trauma-related trials are limited to prospective investigation.

Another reason for the lack of significant results in the trauma

studies may be the patient demographic. The average trauma patient is younger and healthier compared to the general hospital population, and may be better equipped to handle minor inflammatory insults. A third reason may be that the smaller effects of unfiltered erythrocytes are outweighed by the huge physiological stress inherent to traumatic injury. The hypermetabolic and endocrine responses to trauma are well known to caregivers of this patient population.

In conclusion, further and better-powered studies are necessary to evaluate the benefits, if any, of leukocyte depletion in the trauma population.

CONFLICT OF INTEREST

There are no conflicts of interest.

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