CHAPTER 24
Leukocyte-reduced blood components: laboratory and clinical aspects

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From a semantic viewpoint, it is obvious that “red cell concentrates” should contain red cells only, and the same is true for platelet concentrates—they should contain platelets and no other cells (e.g., contaminating leukocytes). Viable donor leukocytes contaminate blood components and are linked to a wide variety of acute and long-term transfusion complications, including febrile transfusion reactions, alloimmunization, transfusion-associated graft-versus-host disease (TA-GVHD), immunomodulation, and transmission of infectious diseases.¹ Consequently, removal of leukocytes from these concentrates should be an obvious consideration to diminish these complications.

By definition, a unit of leukocyte-reduced (LR) blood or blood component must contain no more than $1 \times 10^6$ according to European requirements and less than $5 \times 10^6$ according to US requirements.² In practice, there is no easy method to fulfill these requirements. For cellular blood components, an additional introduction step as filtration is needed to obtain a low level of contaminating leukocytes. For apheresis methods, some techniques provide significant leukocyte depletion of the red cell and platelet units collected, although all subsets of leukocytes are not reduced to the same degree.

The indications for prescribing LR blood components are not universally accepted. The guidelines for patients needing LR cellular blood components include patients who need chronic transfusions, patients with severe febrile transfusion reactions, patients with acute leukemia, patients needing cytomegalovirus (CMV)-negative blood components, and patients undergoing cardiac surgery. Some centers also include patients undergoing surgery in general in the guidelines, and some hospitals provide LR components to all pediatric transfusions. As the list of indications for LR components increases, many centers have implemented universal leukocyte reduction (ULR) to avoid difficulties with having two different inventories. Some countries in Europe opted for prestorage leukoreduction as a precautionary measure against variant Creutzfeldt–Jakob disease (vCJD), thus implementing ULR. In other countries, it was considered that more research was needed to justify the cost related to leukocyte reduction of all cellular components. The cost issue is essential, and some scientists argue that increased cost is the only argument against ULR.³ In the developing countries, the increased cost makes implementation of filtration procedures impossible.

In summary, even among countries with high healthcare costs, various guidelines are followed concerning indications for LR cellular blood components. Thus, there is a need to highlight different aspects of leukocyte contamination of cellular blood components.

How do leukocytes affect red cell and platelet storage?
It is well known that both red cells and platelets undergo changes that reduce their functional capabilities during storage. As these changes may occur due to both internal conditions as well as cell-to-cell interactions, leukocyte reduction of cellular components should provide an ideal environment for studying red cell–leukocyte and platelet–leukocyte interactions. After a regular whole blood donation, the unit normally contains $2–5 \times 10^9$ leukocytes. The number of leukocytes in a standard red cell concentrate is $5 \times 10^8$ and after buffy coat removal around $0.8 \times 10^8$. With modern filtration techniques, leukocyte contamination far below $1 \times 10^6$ may be achieved.

During the red cell storage, severe disturbances in cellular metabolism, rheological properties, oxidation and carboxylation stress, and cellular aging processes occur.⁴ Although the clinical importance of these changes is controversial, there are strong indications that the cell detriments as hemolysis, potassium release, and microvesicle formation cause reduced survival and increased adverse reactions in the recipients.⁵,⁶ Antonelou and her group have published an extensive paper on the effects of prestorage leukocyte reduction on stored red blood cells (RBCs), with the limitation that platelets were present in the nonfiltered group. They found significantly increased hemolysis, irreversible echinocytosis, microvesiculation, removal signaling, reactive oxygen species (ROS) and calcium accumulation, band 3-related senescence modifications, membrane proteome stress biomarkers, as well as the emergence of a senescence phenotype in the lower density RBCs in the non-LR concentrates.⁷ These results are in line with several earlier publications focusing on separate areas of red cell lesion. Reduced hemolysis in LR red cell concentrates has been reported in a large international study. Leukodepletion of AS-3 red cell concentrates prior to storage resulted in reduced glycolytic activity—and posttransfusion recovery of red cells stored for 42 days was significantly better in the prestorage leukocyte reduction group (84% vs. 82% in the group
receiving nonfiltered components). A Dutch group has published that red cell rheology is well preserved in LR red cell concentrates. However, it should not be forgotten that the impact of storage and leukocyte burden on adhesion molecules, glycoporphin A, and annexin V is also influenced by red cell age. Older RBCs showed significantly reduced expression of glycoporphin A during storage, and increasing annexin V concentrations were found in the supernatant of old red cells stored in the presence of leukocytes. In an in vitro study on procoagulant activity of fresh frozen plasma, leukocyte reduction resulted in lower hemostatic potential defined by thromboelastography (TEG) values and coagulation factor concentrations.

### Techniques for leukocyte removal from cellular blood components

#### Centrifugation

Standard centrifugation techniques for cellular component production do not provide results comparable with those of true leukocyte reduction. However, just by removing theuffy coat from whole blood after centrifugation, the residual leukocyte content in the corresponding red cell concentrate may be reduced by 70–80%. In combination with more sophisticated methods for leukocyte reduction for special patient groups, this may be a safe and low-cost alternative.

#### Filtration

Filtration has become the dominating method for leukocyte reduction of cellular blood components. Filters are made by cotton wool, cellulose acetate, polycarbonate, polyester, or polyurethane. The filters may be positively or negatively charged—or neutral. As the platelets and red cells adhere differently to foreign materials, filters for red cell concentrates and platelet concentrates cannot be used randomly. A whole blood platelet-sparing filter that also reduces leukocyte content in line with the requirements is available. Because of the introduction of leukocyte removal filters, the efficiency has improved from 1–2 log removal to 4–5 log removal of leukocytes. The filters may also, to some extent, remove bioactive substances and bacteria.

#### Mechanisms of filtration

The plastic housing that contains the filtration medium is designed so that blood encounters a large surface area of medium, the volume of blood retained by the filter (holdup volume) is minimal, and the medium fits tightly enough within the housing so that blood entering the device cannot bypass the filtration medium. Depletion of leukocytes results primarily from barrier retention in which the pore size of the filter medium is large enough to allow passage of red cells and platelets but small enough to impede passage of leukocytes. To achieve the small pore sizes required, manufacturers have used three different fabrication strategies. In one method, polyester is melted and extruded through fine nozzles into a turbulent gas stream at high velocity. In the process, akin to the formation of cotton candy, the polyester is stretched and cooled to form fine threads of microfibers. These fibers are matted together (like teased hair) and compressed to a controlled fiber density. An alternative approach produces coral-reef-like structures of porous polyurethane with an open-cell geometric configuration containing interconnecting channels that necessitate a circuitious path of flow.

![Figure 24.1 Electron micrograph shows fibers from the fine filter (downstream layer) of a leukocyte reduction filter designed for RBCs. The caliber of the fibers relative to that of individual cells is evident. (Source: Nishimura et al., 1989 [Brozovic B, Ed. The role of leucocyte depletion in blood transfusion practice. Oxford, UK: Blackwell, 1989:35—40.])](image323x578_to_563x738)

Polyester fiber filters consist of a series of layers of fiber material. At the upstream end of the filter, the fiber diameter and effective pore size are large. As blood passes through the layers of medium, the fiber diameter becomes smaller, and the pore size decreases to approximately 4 microns (Figure 24.1). Red cells, which are more deformable than leukocyte nuclei, can traverse these small pores. Because synthetic materials are naturally hydrophobic, they do not become “wet” easily in aqueous solutions. As a result, surface tension would prevent blood from flowing through very fine pore spaces under gravity. To overcome this obstacle, manufacturers have modified the surface of the filter medium fibers to increase their “wettability.”

#### Factors affecting filter performance—and clinical implications

Because of the complex physical and biologic forces involved in leukocyte retention by the filter medium, it is not surprising that several factors have been shown to affect the degree of leukocyte reduction obtained (Table 24.1).

The different filters have different optimal operating conditions and different capacities. It is therefore essential to perform filtration in a controlled laboratory or blood center environment according to the manufacturer’s instructions. In the first years of blood filtration, most filtration procedures had to be performed at refrigerated temperatures, but at present many procedures may be conducted at ambient temperature. The cellular composition of the blood also affects retention of leukocytes. Blood from donors with sickle cell trait often does not become adequately leukocyte reduced when filtered. Results of studies suggest that approximately 50% of

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**Table 24.1 Factors affecting filter performance**

- Capacity of the filter
- Input number of leukocytes
- Temperature (viscoelasticity)
- Flow rate, pressure, priming, and rinsing
- Presence of hemoglobin S
- Number and function of platelets
- Holding time between blood collection and filtration
- Plasma content of cell suspension media
units from donors with hemoglobin AS clog the filter and do not flow.\textsuperscript{15} Of the remaining 50%, approximately half of these flow normally but do not undergo adequate leukocyte reduction. The speed of filtration also has been shown to affect leukocyte reduction. If temperature is held constant, faster flow rates may result in decreased performance. This is presumed to result from high shear rates and decreased contact time with the medium. The filtration procedure may also have clinical consequences.

The most obvious effect is the loss of active substances: hemoglobin reduction of LR red cell concentrates and platelet loss from LR platelet concentrates. In a large French quality control database, the average hemoglobin content was 57.6 g in the nonfiltered versus 50.9 g in the LR red cell concentrates.\textsuperscript{16} The clinical significance of this difference may, however, be overshadowed by other factors that influence hemoglobin content and patient hemoglobin increment.\textsuperscript{17} In a clinical study, Sweeney et al. found a significant lower platelet dose in the LR platelet concentrates ($3.3 \times 10^{11}$) versus the nonfiltered concentrates ($4.0 \times 10^{11}$), but there was no significant effect on the corrected count increment (CCI).\textsuperscript{18} This observation is in line with the later outcome of a large platelet dosing study.\textsuperscript{19}

Significant clinical side effects are observed as a result of leukocyte reduction by filtration. Severe hypotensive reactions are the most prominent. The reactions are induced by generation of vasodilators as bradykinin, probably by donor plasma activation during filter transfer.\textsuperscript{20} For several years, this side effect was related to bedside leukocyte reduction procedures, but hypotensive reactions have also been reported after prestorage leukocyte reduction.\textsuperscript{21} In the beginning of the filtration era, the red eye syndrome, which also included eye pain, photophobia, and sometimes more generalized symptoms, occurred.\textsuperscript{22} This syndrome was linked to a cellulose acetate membrane that was used in some filters. When these filters were withdrawn, the syndrome disappeared.

**Apheresis devices to reduce leukocyte content**

Manufacturers of apheresis devices have modified the machines to collect platelets with low levels of residual donor leukocytes that require no further filtration to meet standards as LR platelet concentrates. These devices achieve a high degree of separation between the donor platelets and the donor leukocytes as a result of several design principles. Flow path geometry, counterflow centrifugation, elutriation, and fluid particle bed separation all are used to separate platelets from leukocytes on the basis of differences in cell mass. Despite advanced design, apheresis systems can fail to collect platelet concentrates that meet standards for being considered LR.

**Prestorage, poststorage, or bedside leukocyte reduction**

Leukocyte filtration may be performed in the blood bank prestorage or after storage of cellular blood components. Prestorage leukocyte reduction during or soon after component preparation has become the preferred method of leukocyte reduction and is ideally suited to process control. The leukocyte filtration is incorporated in the production process, whether this is performed on the donation day or after overnight holding of whole blood. For platelet concentrates, numerous investigators have documented that inflammatory cytokines, including interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-6, can accumulate during storage in some units. The extent of cytokine accumulation correlates with the leukocyte content of the unit and the duration of room temperature storage. For some transfusion recipients, the passively transferred cytokines result in febrile nonhemolytic transfusion reactions. Prestorage leukocyte reduction of platelet concentrates prevents accumulation of these cytokines. There is speculation that prestorage leukocyte reduction would have additional advantages by preventing transfusion of leukocyte fragments that would otherwise develop during storage. Results of laboratory studies and preclinical animal studies have suggested that leukocyte breakdown may contribute to HLA alloimmunization,\textsuperscript{23} release of intracellular viruses,\textsuperscript{24} and immunosuppression.\textsuperscript{25} However, none of these effects has been documented in clinical trials. Results of a large, randomized controlled study conducted in Europe with patients undergoing cardiac surgery showed no advantage of prestorage LR RBCs over poststorage LR RBCs for the prevention of postoperative infection, multiple organ failure, or death.\textsuperscript{26}

Poststorage leukocyte reduction is filtration of components shortly before issue from the blood bank. Poststorage filtration is easy to standardize, can be incorporated into the laboratory procedure in a manner similar to that for other manipulations of blood components, and is easily adapted into a program of process control. For whole blood–derived platelet concentrates, poststorage leukocyte reduction has the key disadvantage that cytokines that accumulate during storage are not removed by filtration. For RBCs, there is no known disadvantage to poststorage leukocyte reduction.

In the beginning of the filtration era, bedside filtration procedures were common. However, quality control of the procedure was in practice impossible, and this came in conflict with the Good Manufacturing Practice requirements. Even more important was the publication of a large randomized study that showed that bedside filtration did not prevent HLA immunization.\textsuperscript{27} Moreover, for some recipients taking angiotensin-converting enzyme (ACE) inhibitors, bedside filtration has been associated with hypotensive transfusion reactions. For these reasons, bedside leukocyte reduction is no longer recommended.

**Process control of leukocyte-reduced components**

The concentration of white blood cells (WBCs) in a 300-mL LR blood component with $10^9$ WBCs per unit is only 3.3 WBCs/$\mu$L. Because traditional automated cell counters are not accurate at leukocyte concentrations less than 100 WBCs/$\mu$L, special techniques are required to accurately count residual WBCs in LR components. Several methods have been evaluated for their abilities to count such low numbers of leukocytes accurately. In a large comparison study, the Nageotte hemocytometer and four platforms based on fluorescent staining of nuclei were tested.\textsuperscript{28} Although there were significant variances in performance between the automated methods, the flow cytometry–based testing platforms performed best, and the Nageotte method performed poorly in terms of accuracy and precision. However, the use of larger volumes, the concentration of leukocytes, and accurate methodology may improve the performance of the Nageotte method.\textsuperscript{29} Recently, a new microscopic method for counting of residual leukocytes in filtered blood components has been evaluated.\textsuperscript{30}

As the testing for leukocyte contamination is laborious and costly—and it is important to ensure the stability of the process—strict validation and process control are necessary. A practical guideline for these purposes has been developed by the Biomedical Excellence for Safer Transfusion (BEST) society.\textsuperscript{31}
Clinical indications for leukocyte reduction

In the first years, leukocyte reduction was selectively used for special patient groups: to avoid HLA immunization in patients receiving multiple platelet transfusions during cytotoxic therapy, to avoid transmission of CMV, and to prevent febrile transfusion reactions. Later, several other clinical indications were introduced, leading to increased use of leukocyte reduction filters. When it was speculated that leukocyte reduction could reduce risk for vCJD, ULR was implemented in many countries. Thus, it may be argued that ULR sometimes was introduced because it was logistically difficult to administer two parallel inventories—and that the scientific rationale was weak.3

HLA immunization

The threshold level of leukocytes needed to provoke primary HLA alloimmunization is not well defined. During the 1990s, the standard for LR blood components was set to $5 \times 10^6$ WBCs per transfusion, and this threshold value is still used in the United States. However, in Europe, maximum leukocyte contamination was set at $1 \times 10^6$ WBCs per transfusion.

Mechanism

Decades of research provide unequivocal evidence that passenger leukocytes, which express donor HLA antigens, are a prime cause of recipient sensitization to HLA alloantigens. Experiments by Claas et al.32 with a rodent transfusion model showed that residual donor leukocytes provoked major histocompatibility complex (MHC) alloimmunization and that leukocyte reduction could decrease the incidence of primary sensitization to MHC antigens. These investigators further documented that even very low numbers of donor leukocytes in previously sensitized animals induced secondary immunization, suggesting that leukocyte reduction would be less effective in the previously sensitized patient. The importance of donor leukocytes was confirmed in experiments by Kao33 and Blajchman et al.23 who also provided evidence for the possible role of antigens on microparticles in plasma.34

Alloimmunization can occur by means of both direct and indirect immune recognition.35 Direct allorecognition is the process by which recipient immune cells respond directly to donor HLA antigens without the processing of donor antigens by recipient antigen-presenting cells (APCs). The mixed lymphocyte reaction is an in vitro example of direct T-cell recognition. Direct allosensitization requires at least three fundamental elements—binding of the antigen to the antigen receptor, binding of costimulatory molecules mediating cell–cell contact, and local elaboration of cytokines and appropriate cytokine receptors. Recipients may recognize intact Class I structures on the surface of donor leukocytes (Figure 24.2). Alternatively, Class II–positive donor APCs may carry, within the peptide-binding groove, oligopeptides that represent the cell’s own HLA Class I antigen (Figure 24.3). In either case, depletion of Class II–positive donor APCs would reduce the chance of direct HLA immunization to Class I antigens. The failure of the recipient to recognize donor Class I antigens on platelets may reflect the fact that platelets lack critical costimulatory molecules required for direct allostimulation.

Indirect allorecognition is the process by which recipient APCs first engulf donor cells and then process donor antigen for redisplay to the recipient immune system. Donor cells, cell fragments, or soluble donor antigens are engulfed and degraded within lysosomes of the recipient APCs. Small peptide fragments corresponding to the alloantigenic region of donor HLA are then deposited in the peptide-binding groove of Class II structures on recipient APCs (Figure 24.4).35 Recipient T and B cells then interact with recipient APCs in an MHC-restricted manner and respond to the donor peptide antigens displayed by the APCs. Recipient helper T-cell receptor-mediated recognition of this complex results in elaboration of cytokines by the donor APC (IL1 and IL12), which in turn results in APC surface expression of CD80 and CD86. These molecules bind to CD28, the central costimulatory molecule on helper T cells. T cells are further activated and elaborate cytokines such as CD40 ligand, IL4, IL5, and IL10, which provide the critical help to B lymphocytes for their proliferation and antibody production.35 Ultraviolet-B (UVB) irradiation of APCs disrupts costimulatory signals35 and thus may account for the effectiveness of this method for reducing the rate of HLA alloimmunization observed in
Will leukocyte reduction prevent secondary alloimmunization?

Because a secondary or anamnestic immune response requires a far smaller antigenic challenge than does a primary response, the secondary antibody recall to MHC antigens is difficult to prevent with LR blood components. The issue of secondary alloimmunization was directly addressed in the study by Sintnicolaas et al.11 Female patients (n = 75) with hematologic malignant disease and a history of previous pregnancy were randomly assigned to receive platelet concentrate support with either unmodified or LR apheresis platelet concentrates. Among the evaluable patients, HLA alloimmunization developed in 43% (9 of 21) of the standard platelet concentrate group and in 44% (11 of 25) of the filtration group. Refractoriness occurred in 41% (14 of 34) of women in the standard platelet concentrate group and in 29% (8 of 28) of the filtration group (p = 0.52). The time to development of refractoriness was similar in the two groups. The authors concluded that leukocyte reduction to less than 5 × 10^6 WBCs per transfusion did not prevent alloimmunization and refractoriness among previously sensitized recipients.

In the TRAP study, however, analysis of patients who had been pregnant showed some benefit, albeit decreased, from the use of LR blood. In that study, 62% of patients with a history of pregnancy had HLA alloantibodies after transfusion of routine components; 33% did so after transfusion of LR components. Seftel et al.42 analyzed 106 women with hematologic malignancy and a history of pregnancy treated before and after the introduction of ULR in Canada. They found that leukocyte reduction did not significantly change the rate of alloimmunization (15% vs. 10%) or platelet refractoriness (11% vs. 9%), although the surprisingly low rate of alloimmunization in control patients may have accounted for the lack of observed benefit of leukocyte reduction. However, when previous transfusion was studied as an immunizing event, leukocyte reduction was highly effective in reducing the rate of alloimmunization (29% vs. 2%). Thus, it appears that patients with hematologic malignant disease and a history of exposure to foreign HLA antigens from a previous pregnancy derive diminished benefit from the use of LR components.

Whether leukocyte reduction reduces alloimmunization to platelet-specific antigens or red cell antigens is unclear. In the Canadian study that concluded that ULR reduces alloimmunization and refractoriness, platelet-specific antibodies were not included.32 Concerning red cell alloimmunization, a retrospective cohort study by Blumberg et al. reported a significant decrease in alloimmunization in acute myeloid leukemia patients.43 In a Dutch study, these results could not be reproduced,44 but this study was related to universal leukocyte reduction, and the “nonfiltered” units were buffy coat-depleted.

Nonhemolytic febrile transfusion reactions

The nonhemolytic febrile transfusion reaction is the most common transfusion reaction as reported from many hemovigilance systems. The febrile transfusion reaction involves patient antibodies reacting with donor lymphocytes, causing release of cytokines from the donor cells, inflammatory cytokines released from recipient cells in response to antigen–antibody complex formation, and passive transfer of cytokines accumulated in the blood component. Among the cytokines involved are IL1β, IL6, IL8, TNFα, and CD40 L.11

Given these mechanisms, it seems obvious that the febrile transfusion reactions should be reduced by leukocyte reduction, especially if the filtration is performed prestorage.
Several clinical trials have confirmed that prestorage leukocyte reduction is effective in reducing the rate of FNHTRs to red cells by approximately 50%, with residual rates well below 1%.

A major disadvantage is the small number of randomized clinical trials evaluating effects of leukocyte reduction. Another difficulty is that the components investigated in the studies differ (e.g., it is probable that the leukocyte reduction obtained by buffy coat removal by itself reduces febrile reactions compared with standard cellular concentrates). In their recent review article, Bilgin et al.11 accordingly refer to large “before and after universal leukocyte reduction” observational studies from the United States (35,000 RBC transfusions) and Canada (140,000 RBC transfusions and 57,000 platelet transfusions) to conclude that the number of febrile transfusion reactions is reduced by over 50%—provided prestorage leukocyte reduction is performed. Studies based on bedside filtration do not show the same effects, and these studies also contributed to the discontinuation of this procedure.35,46

**Immunomodulation**

For many years, it has been known that exposure of large amounts of allogeneic cells may lead to hyporesponsiveness as easy as antibody formation.47 Studies on effects of allogeneic transfusions have led to the term *transfusion-related immunomodulation* (TRIM). Originally, the effects were described as beneficial—such as increased renal transplant survival. Later, deleterious effects have been attributed to TRIM—like increased recurrence rate of malignancies, increased rate of postoperative bacterial infections, and increased short-term mortality.48 The mechanisms behind TRIM are complex, and the effects may be mediated by allogeneic mononuclear cells, leukocyte-derived soluble mediators, and soluble HLA peptides.48

**Clinical studies on immunomodulation**

**Posttransfusion infection**

Several randomized controlled trials (RCTs) have been performed to examine postoperative infection rates among recipients of LR or non-LR blood. These studies have provided conflicting results; some show benefits of leukocyte reduction, and others do not. An “intention to treat” meta-analysis of eight studies (excluding the report of Van Hilten et al.50) indicated that there was no significant TRIM effect.49 However, when the three cardiac surgery studies were analyzed separately, a statistically significant TRIM effect was observed with a summary odds ratio (OR) of 1.39 (95% CI, 1.08–1.80), indicating that cardiac surgery patients transfused with non-LR components had a 39% higher risk of postoperative infection. A subsequent meta-analysis of these eight studies and the additional RCT by van Hilten51 included only those patients who were actually transfused and found a statistically significant (*p = 0.005*) OR of 0.52 across all studies, indicating a nearly 50% reduction in the risk of postoperative infection associated with the use of LR components.51 Subset analysis indicated that the three studies in patients undergoing cardiac surgery were the primary contributors to the overall statistical significance. One limitation of these meta-analyses is that the studies differed in the type of component assigned to the non-LR control with some using standard red cells and others using buffy-coat-depleted red cells. Overall, however, these data increasingly suggest a beneficial role of LR blood in reducing the risk of postoperative infections, particularly in patients undergoing cardiac surgery.

**Pretransplantation blood transfusion**

Several lines of evidence have suggested that recipient exposure to donor WBCs can result in improved survival of subsequent renal transplants. For example, the original studies of Opelz and Ter-aski52 showed that the use of frozen-deglycerolized (and thus LR) blood did not protect against rejection of renal transplants as well as did other RBC preparations. In 1993, a prospective, double-blind, randomized trial showed that patients given postoperative transfusions of non-LR fresh RBCs had better renal allograft survival than did patients given frozen deglycerolized RBCs.53 Because cyclosporine and tacrolimus have become widely adopted as immunosuppressive agents for organ transplantation, the contribution of transfusion to graft survival has declined. Because the risk of HLA alloimmunization associated with using leukocyte-rich blood components outweighs any benefit from potential immune tolerance, LR components are indicated for organ transplant candidates.

**Morbidity and mortality after surgery**

LR blood components have been reported to be associated with improved patient outcomes, specifically reduced mortality and/or hospital length of stay unrelated to postoperative infection. This effect has been primarily studied in cardiac surgery patients. A prospective study of three serial periods of transfusion strategies in cardiac surgery patients using non-LR components, LR components, and then non-LR components showed that hospital length of stay declined from 10.1 to 9.5 days with the use of LR components and then returned to 10.8 days with the reintroduction of non-LR components. There was no change in length of stay in control patients who did not require transfusions.54 A meta-analysis of three RCTs studying LR components in cardiac surgery showed that leukocyte reduction was associated with reduced mortality as well as a reduced risk of postoperative infection.55 Later, numerous publications indicated a significant benefit of LR components for patients undergoing cardiac surgery, with a short-term mortality reduction of up to 50%.56 Although the explanations for the beneficial effects may be complex, these data provide convincing evidence in support of the use of LR components in patients undergoing cardiac surgery.

**Transfusion-associated graft-versus-host disease**

TA-GVHD results from clonal expansion of allogeneic donor leukocytes (see Chapter 54). Patients who experience TA-GVHD either do not eliminate donor leukocytes because of severe immunosuppression or do not recognize donor cells as foreign because of HLA similarity between the donor and recipient. The threshold number of donor leukocytes required to provoke human TA-GVHD cannot be determined and likely varies among different donor–recipient pairs. Because TA-GVHD depends on recipient exposure to viable allogeneic donor leukocytes, it might be anticipated that sufficient leukocyte reduction would reduce the risk of TA-GVHD. However, there are no conclusive clinical data to support this notion,56 and TA-GVHD has been reported among isolated patients who received transfusion of LR blood components.57 Therefore, γ-irradiation is a necessary means to prevent TA-GVHD. The pathogen reduction technologies available for platelet concentrates disable leukocyte DNA replication, and these technologies therefore also protect efficiently against TA-GVHD.58

**Transmission of CMV infection**

When it became known that deadly CMV infection in premature children and immunosuppressed patients could be related to transmission from blood donors, this became a major driving force for
leukocyte reduction of cellular blood components. After CMV infection, the virus is lifelong present in mononuclear lymphocytes.

Studies were performed to document the effectiveness of leukocyte depletion to prevent CMV transmission to at-risk neonates. Gilbert et al. conducted a prospective, randomized, blinded trial in which 515 newborns were randomly assigned to receive transfusion support with either unmodified RBCs or RBCs filtered through an IG500 (Terumo Corporation) filter capable of 1–1.5 log leukocyte removal. In the control arm, 29 infants weighed less than 1500 g, and CMV infection developed in nine (33%). In the filtration arm, among 24 neonates weighing less than 1500 g who were CMV seronegative and received CMV-serositive filtered blood, none acquired CMV infection. In another study, Eisenfield et al. used less advanced filtration technology and documented prevention of CMV transmission to at-risk neonates. In contrast, Ohto et al. performed a prospective randomized study of LR blood components in 52 newborn infants, of whom 43 weighed <1500 g. There was no effect of leukocyte reduction on the rate of CMV infection; however, the geographic region in Japan where this study was conducted had a high rate of CMV among the mothers (89%) and the blood donors (89%), and, importantly, CMV infection likely was due to breastfeeding by mothers secreting the virus into their milk—an observation made by others.

As patients undergoing cytotherapy for hematological malignancies and bone marrow transplantation are severely immunosuppressed, it is important to clarify if leukocyte reduction of cellular blood components transfused to these patients is sufficient to protect against CMV infection. Bowden et al. conducted a large, randomized, prospective trial with CMV-negative patients who received transplants of CMV-negative marrow. The investigators compared support with CMV-seronegative blood with support with filtered blood from untested donors. They randomly assigned 502 CMV-seronegative patients undergoing autologous or allogeneic marrow transplantation to two treatment groups. Among 252 patients receiving CMV-seronegative blood components, two had CMV infection; none had CMV disease. Among 250 patients receiving filtered CMV-untested components, three CMV infections (three with CMV disease) were observed. Two additional CMV infections in the seronegative group and three in the filtration group occurred within 21 days of entrance to the study and were considered the result of transfusions before enrollment. The survival rate was equal in the two study arms.

Because current filters are considerably more effective at leukocyte reduction than were those used in the published studies, most centers use leukocyte reduction as the only preventive measure against CMV infection, and this policy is supported by several recent studies. As there is a relatively high “seroconversion rate” in the donor population, the collection of blood from donors with negative test results for antibodies to CMV is not completely safe. Sensitive polymerase chain reaction (PCR) techniques are needed to demonstrate the CMV genome in the blood of seropositive healthy donors. Studies on blood from infected donors showed that the CMV PCR signal is lost or greatly reduced when blood was leukocyte reduced by means of filtration or apheresis processing. However, as the use of CMV-seronegative donors or leukocyte reduction does not completely eliminate the risk of CMV infection, the term reduction of risk should be used—as in leukocyte reduction and pathogen reduction technologies.

Another concern is that transfusion of donor leukocytes could reactivate endogenous viral infection. The clinical relevance of these observations was specifically addressed in a large, multicenter RCT—the Viral Activation by Transfusion Study (VATS). The study was conducted to evaluate the ability of LR blood to prevent either HIV or CMV activation among a cohort of patients with HIV infection undergoing therapy for the infection. The study showed no beneficial effect of leukocyte reduction for prevention of early death, virus activation, or time to first infection-related complication.

Vamvakas has published a systematic review of studies related to bone marrow recipients, and the conclusion was that both CMV screening of the donors and leukocyte reduction of the components reduced the transfusion-associated infection rate by 92–93%. Based on the clinical data available, leukocyte reduction alone seems to be the most practical and economical method for prevention of CMV infection. CMV-antibody testing is of uncertain value, sensitive PCR techniques are expensive, and the testing may delay the transfusions.

Transfusion-related acute lung injury (TRALI)

TRALI is an acute, immune-mediated transfusion reaction characterized by dyspnea, hypoxia, pulmonary edema, low pulmonary capillary wedge pressure, and alveolar infiltrates on chest radiographs (see Chapter 59). Two different mechanisms may contribute to the development of TRALI. First, results of serologic investigations suggest that TRALI occurs when donor plasma contains antibodies reactive against the recipient’s HLA type or against recipient non-HLA leukocyte antigens. Some have postulated that TRALI may result when recipient leukocyte antibodies react with residual donor leukocytes, although solid evidence is lacking. However, an FNHTR reaction is more common in this setting. A second mechanism proposes a two-hit model involving lipid agents in donor blood that prime recipient neutrophils in the presence of specific cytokine activation. Thus, leukocyte reduction is not expected to play an important role in the prevention of TRALI. However, in a large “before-and-after” study of acute transfusion reactions, Blumberg et al. found an 83% reduction in TRALI after ULR (2.8 cases per 100,000 components vs. 0.48 after ULR).

Bacterial overgrowth

It is unlikely that prestorage removal of leukocytes would have any measurable effect on the incidence of bacterial overgrowth in blood components. The mechanisms by which leukocyte reduction filters may deplete blood components of low levels of contaminating bacteria have been reviewed. In a European multicenter study, Seghatchian et al. found that leukocyte reduction was not effective to protect against bacterial contamination of cellular components, even if the filtration process were postponed for eight hours to allow for phagocytosis. Because bacterial overgrowth is infrequent, a clinical study would require an enormous number of observations. The hemovigilance network in France reported an analysis of reported cases of bacterial sepsis before and after introduction of ULR. The study found that bacterial sepsis decreased from 3.8% to 1.7% (p < 0.001) of all reported adverse events.

Cost-effectiveness of leukocyte reduction

Since the 1990s, there has been a substantial debate about whether leukocyte reduction should be implemented as a universal measure (ULR) or if leukocyte reduction of cellular blood components should be reserved for special clinical indications. In many Western countries, the ULR approach has been implemented. In economically poorer countries, the cost of the procedures has made ULR completely irrelevant.
The main disadvantage of the leukocyte reduction procedure is, accordingly, the cost. The filter and processing costs will differ, but reasonable ranges are US$20–40 and EUR 20–35 per unit. The literature on cost-effectiveness is very heterogeneous. In a review paper on estimating the cost of blood, Shander et al. have found that the cost per quality-adjusted life years (QALYs) ranges from US $2470 if the risk is high (1.85 relative risk) to US$3.4 million if there is no infection risk. In 1995, Blumberg et al. conducted a cost study on transfusion of LR red cell concentrates in a patient population with malignant diseases, and reported a “significant cost reduction” due to leukocyte filtration. A study on the cost-effectiveness of leukoreduction for prevention of febrile nonhemolytic transfusion reactions (REFs) concluded that the cost per prevented FNHT was EUR 6916. In a review of blood transfusion practices in Spain 1997–2007, Garcia-Erce et al. concluded against ULR, “which has led to an incremental cost for unknown, but probably slight, benefits for patients.”

Despite the uncertainties concerning the cost-effectiveness issue, the trend is in favor of ULR in economically wealthy countries. In Europe, the blood directive is focusing on maximal patient safety, and this leukocyte reduction is regarded by many as a step to achieve this goal. In the United States, the trend is that the larger hospitals support ULR. Canada has implemented ULR; a publication based on retrospective data reported significantly reduced in-hospital mortality and suggested that one life was saved for every 120 patients who received LR blood compared with standard blood components. In the less economic-rich countries, ULR is not implemented. A paper from India concludes that despite the advantages of leukocyte reduction, it is not practically feasible to implement this policy in developing countries and other underresourced nations.

Summary

The majority of cellular blood components transfused in the Western world are leukocyte reduced. High-performance blood filters and low-leukocyte apheresis devices have made LR components available to all transfusion facilities. The technology represents an important advance in the preparation of blood components. Several European nations and Canada have adopted ULR of the blood supply. However, leukocyte reduction represents a large increase of cost in blood component production. Although experimental data point to many possible advantages for the patients, the clinically proven beneficial indications for LR are relatively few. Because ULR already is so widespread, it is possible that the desired, large randomized control studies to evaluate the full clinical picture of leukocyte reduction of cellular blood components will never be conducted. For blood component production in developing countries and other underresourced nations, Buffy coat removal or similar leukocyte reduction steps could provide a good and cheap alternative to reduce donor-leukocyte-associated transfusion complications.

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Key references

A full reference list for this chapter is available at: http://www.wiley.com/go/simon/transfusion


