LEUKOREDUCTION OF THE BLOOD SUPPLY

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In the summer of 1999, Canadian Blood Services (CBS) will implement pre-storage leukoreduction of red cells, in addition to the pre-storage leukoreduction of platelets, which has been available since February 1998. The paper below outlines some of the clinical benefits of leukoreduction, and the significance that they may have on your patients. A bibliography is attached for reference.

Introduction

Donor leukocytes, the residual white blood cells in platelet or red cell transfusions, are associated with potential adverse reactions. Leukocytes, with their specific allogeneic structure (exposing the HLA class I and class II antigens on their surface) are main targets of the recipient's immune system. Some transfusion recipients develop a fever after transfusion in response to the donor leukocytes. Repeated exposure to donor leukocytes may create an immune response that makes the recipient refractory to the donor platelets, thereby deriving no benefit from the transfusion event. In addition, some viruses, such as cytomegalovirus (CMV) are transmitted in donor leukocytes, in which they reside. Another less well-established effect of transfused leukocytes is a modulating influence they may have on the recipient's immune system, potentially compromising the recipient's ability to fight infection or prevent cancer recurrence.

In an effort to overcome these adverse effects, methods of removal of the donor leukocytes – leukoreduction or leukodepletion – have been developed. Today's technology permits removal of > 99.99% of donor leukocytes, usually by means of filtration of the red cells and or platelets. This filtration may occur shortly after donation and processing of the blood unit, and is called pre-storage leukoreduction. The filtration may occur in the hospital blood bank, just prior to the transfusion event, or may occur at the patient's bedside, the latter two being referred to as post-storage leukoreduction. Most data suggest that pre-storage leukoreduction is superior to any means of post-

storage leukoreduction, since the leukocytes have not yet begun to fragment or synthesize the variety of biological response modifiers (cytokines) they have been reported to produce. The membrane fragments and pre-formed biological response modifiers will generally pass through post-storage filters, and still are able to exert the adverse effects associated with intact white cells. Furthermore, pre-storage leukoreduction is associated with much better quality control of the process than post-storage or bedside filtration. Pre-storage leukoreduction necessitates implementation by the blood supplier, in this case CBS, while post-storage leukoreduction falls to hospitals to implement. .

In Canada, pre-storage leukoreduction of random donor platelets (derived from whole blood donations, but not apheresis platelets) was implemented in February 1998, making Canada one of the first countries in the world to process random donor platelets in this manner. Anecdotal evidence in Canada suggests that the adverse reactions that typically characterized frequent platelet transfusions are very substantially reduced (e.g. in Toronto hospitals from 27% to less than 15%), although it is too early to comment on the effect on platelet refractoriness.

Indications for use of leukoreduced blood components

Prevention of febrile transfusion reactions

A febrile transfusion reaction results from the production of cytokines by donor leukocytes in the transfused product (preformed at time of infusion), or from an interaction between leukocyte antigens and anti-leukocyte antibodies in the recipient. These cytokines produce a symptom complex of fever, chills, rigors, and are seen more often following platelet transfusions, but may occur following red cell transfusions, and are more common in multiply transfused individuals. While antipyretic medications (e.g. aspirin, non-steroidal anti-inflammatories, acetaminophen) may readily ameliorate these febrile transfusion reactions, prevention of the reaction by leukoreduction is clearly the preferable therapeutic intervention. While leukoreduction is very effective in reducing

the frequency of febrile transfusion reactions, this intervention is not absolutely effective. Antibodies in donor blood may interact with recipient white cells, or the residual white cells in the donor bag after leukoreduction may be sufficient to produce some cytokines, which, in sensitized individuals, may trigger febrile transfusion reactions. Nonetheless, numerous studies, including several from Canadian researchers have documented the effectiveness of leukoreduction in ameliorating febrile transfusion reactions.

Significance of leukoreduction for febrile transfusion reactions

While febrile transfusion reactions are rarely life-threatening, they may be quite debilitating, especially to very ill, chronically transfused patients (e.g. bone marrow transplant recipients). These reactions may also trigger expensive clinical investigations, since the symptom complex mimics that of sepsis, to which these patients may also be prone. The evidence in support of leukoreduction for reducing febrile transfusion reactions is overwhelming, with little, if any, data to the contrary.

Prevention of HLA alloimmunization and platelet refractoriness

Donor antigen presenting cells in the transfused unit are able to present HLA class I and II antigens to a recipient's T-cells, which begins an immunological response in the host resulting in anti-HLA antibody production. The presence of these anti-HLA antibodies may inactivate platelet transfusions, rendering the recipient refractory to standard platelet transfusions. The extent to which leukoreduction reduces refractoriness to platelet transfusion varies between published studies, and may reflect the many compounding factors that influence platelet refractoriness (e.g. sepsis, antibiotic use, spleen size etc). Nonetheless, the common odds ratio in five studies published between 1983 and 1991 comparing unfiltered to filtered blood products was 0.27 (95% CI, 0.13-0.55) for alloimmunization and 0.28 (95% CI, 0.13-0.54) for refractoriness. The recently published Trial to Reduce Alloimmunization to Platelets (TRAP) study, perhaps the most important study addressing this question, confirmed that pre-storage leukoreduction was

highly effective at lowering HLA alloimmunization and platelet refractoriness in multiply transfused patients with acute leukemia.

Significance of leukoreduction for HLA alloimmunization & platelet refractoriness

There are several therapeutic interventions that may be tried to overcome platelet refractoriness, such as the use of HLA-matched single donor platelets or platelet cross matching. All are very cumbersome and costly, and in some patients may not result in provision of a clinically useful platelet product. Since these types of patients are at high risk of thrombocytopenic bleeding, platelet refractoriness may substantially increase morbidity and even be potentially life threatening. With the publication of the TRAP trial, evidence is overwhelming in favour of leukoreduction for its ability to lower both HLA alloimmunization and platelet refractoriness.

Prevention of leukocyte –transmitted infections

While transmission of viral disease has traditionally relied on serological screening assays, evidence now exists that leukoreduction can reduce the transmission of leukocyte-borne viruses, such as CMV, HTLV I or Epstein-Barr virus (EBV). Most of the studies have focussed on CMV transmission, since there is a high demand for CMV negative blood products for immunosuppressed patients, in whom CMV disease can be associated with high morbidity and mortality. While the literature is somewhat controversial in this area, most investigators now agree that if leukoreduction is practiced under cGMP conditions using third generation filters, then leukoreduction is equivalent in preventing transmission of CMV when compared to the use of blood products from CMV seronegative donors. Since testing for HTLV I and II is part of the screening of all blood donors, and since EBV transmission is of low clinical impact, the benefit afforded by leukoreduction to the transmission of these two viruses is small.

The transmission of viruses that are not exclusively carried intracellularly within leukocytes is not reduced by leukoreduction. HIV is such a virus. However, recent data suggests that HIV-infected individuals who receive leukoreduced blood products have slower progression of their disease and a slower virus doubling time, compared to HIV-infected individuals transfused with non-leukoreduced blood products. This phenomenon likely reflects the immunosuppressive effects of carrier leukocytes.

Significance of leukoreduction for viral transmission

CMV disease in certain immunocompromised transfusion recipients (e.g. neonates, bone marrow transplant patients) may cause significant morbidity and even mortality. In many centres up to 60% of the donor population is CMV seropositive, and it may be difficult to maintain sufficient inventory of CMV negative red cells and platelets. In such situations, evidence suggests that the use of leukoreduced blood products, prepared with 3rd generation filters, is at least equivalent to the provision of CMV negative units in preventing CMV disease transmission, and may be less expensive than maintaining a CMV negative inventory.

Prevention of bacterial / protozoal infections

The ability of leukocyte filters to remove contaminating bacteria or protozoa, such as *Trypanosoma cruzi*, the agent causing Chagas disease, from donor blood is questionable. Evidence suggests that certain strains of bacteria may be efficiently removed by leukoreduction, although conclusive experiments in donors and recipients are lacking, and much of the data derive from laboratory settings with blood units being spiked with exogenous bacteria. Leukoreduction is thought to reduce bacterial transmission in a number of ways. The leukocytes may ingest the contaminating bacteria, and then be cleared by filtering after a 6 – 24 hour period permitting phagocytosis. Alternatively, the filter medium itself may trap certain bacterial species. While leukoreduction cannot be claimed to be a primary means of preventing bacterial transmission, it may provide some

additional safety, since there are presently no licensed, effective ways of screening for or inactivating bacteria.

Significance of leukoreduction for bacterial transmission

The prevalence of bacterial transmission by blood products is not precisely known, but may be as high as 1 in 1000 units of platelets transfused. Platelets are more often implicated than red cells, because of they are stored at room temperature. Bacterial sepsis may cause significant morbidity and even mortality in critically ill transfusion recipients, and while reported cases of transfusion-related bacterial death are infrequent, they are well documented. There have been one or two cases per year reported in Canada. There may also be substantial underreporting of this phenomenon, because bacterial infection may arise from other sources, and clinicians may not link it to the transfused products. There are presently several techniques under investigation aimed at reducing the risk of bacterial transmission (e.g. use of psoralens and UV irradiation), but none is licensed. Leukoreduction may afford some protection in this regard, but its magnitude is difficult to quantify.

Transfusion-related immunomodulation

Several studies have demonstrated the immunosuppressive effects of allogeneic blood transfusion. This first became evident in renal transplant recipients, in whom heavily transfused patients had less allograft rejection than non-transfused patients. Allogeneic white cell immunotherapy has also been reported to have an effect in lowering rates of spontaneous recurrent abortions. The detrimental effects of leukocyte-mediated immunosuppression, however, remain uncertain. Transfusion-related immunosuppression has been implicated in the development or progression of viral disease, bacterial disease, malignant disease, increased recurrence of malignancy or metastases and possibly an increased overall mortality.

While there are several convincing animal experimental studies that demonstrate higher rates of tumor growth and recurrence with allogeneic transfusions and lower rates with leukoreduction, the randomized controlled trials in humans are still inconclusive. This may be due to lack of homogeneity among the studies, including type of product transfused, patient population and study design. A similar finding exists in studies examining the effect of allogeneic transfusions on the development of postoperative bacterial infection and the ability of leukoreduction to ameliorate this. A recent meta-analysis of unconfounded randomized controlled trials concluded that any possible immunomodulatory effect of transfusions would likely be smaller than 25% (relative risk reduction). Interestingly, a very recently published randomized controlled trial with 944 patients in cardiac surgery found a significant reduction in post-operative non-cardiac death for patients receiving filtered red cell transfusions compared to those receiving unfiltered blood products (with no difference between pre- or post-storage filtration).

Significance of leukoreduction for immunomodulation

There is still no convincing proof in human trials for an immunomodulatory effect of blood transfusion, and its amelioration by leukoreduction, and the precise mechanism by which this effect occurs is uncertain. On balance of evidence, however, it is probable that transfusion-related immunomodulation is a real entity, even if its magnitude is uncertain. While the relative risk reduction afforded by leukoreduction is likely to be small (< 25%), the huge numbers of surgery and cancer patients undergoing transfusion each year may translate into a very important benefit of universal leukoreduction. Thus, while immunomodulation cannot be used as a primary reason for instituting universal leukoreduction, there is absolutely no evidence that leukoreduction increases tumor growth or promotes bacterial or viral infection, and it may yet provide some undetermined degree of safety enhancement for the majority of transfusion recipients.

Prevention of Creutzfeldt-Jakob disease transmission

While there is no evidence that classical CJD can be transmitted by transfusion of blood or blood products, recent concern exists over the possible transmission of new variant CJD through B-lymphocytes. However, this data is based thus far on *in vitro* and animal experiments, and there are no human data concerning transmission of nvCJD. There is even less evidence, therefore, that leukoreduction may reduce what is presently a theoretical risk of transfusion transmission. While the decision to implement universal pre-storage leukoreduction has been made in other jurisdictions on the basis of risk reduction for nvCJD (e.g. United Kingdom), there is no scientific basis for this benefit, particularly since the agent responsible for nvCJD may possibly be found in plasma, on platelets and on WBC fragments.

Adverse effect of leukoreduction

While the benefits of leukoreduction very substantially outweigh any risks that may be associated with this intervention, leukoreduction should not be considered entirely risk free. Recently a number of cases of "red eye syndrome" were noted to occur in transfusion recipients who had received filtered red cells through one particular brand of filter. While the precise cause of this adverse reaction is not known, it is speculated that cellulose acetate, employed in that particular filter, may have had a causal role. The filter manufacturer has since changed its formulation, and no further cases of red eye syndrome have been reported since. Aside from this, there are no other documented adverse effects of leukoreduction.

Summary

Leukoreduction, the process of filtering passenger white cells from red cell or platelet transfusions, is associated with several well documented, some less well proven and some

potential, as yet unproven, benefits that may enhance the safety of the blood supply. While the benefits of leukoreduction are possibly more obvious in certain patient groups (e.g. regularly transfused, immunocompromised etc.), it is uncertain to what extent leukoreduction enhances the safety of the single or occasional transfusion event. There is no evidence that leukoreduction reduces the safety of the blood supply. Of the possible ways of using leukoreduction, pre-storage filtration appears to offer most benefit and afford most quality control of the process.

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