Leukocytes in blood transfusion: adverse effects and their prevention

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Leukocyte contamination during blood transfusion can cause many adverse effects, such as the transmission of cell-associated infectious agents, febrile non-haemolytic reactions, graft-versus-host disease, and immunosuppression. While using leukodepleted blood components can minimise some of these adverse effects, the leukodepletion of all cellular blood components is costly. A more cost-effective alternative would be to supply leukodepleted blood components to at-risk patients only.

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Introduction

White blood cells or leukocytes are present in all cellular blood components that are prepared by standard techniques. Studies have increasingly shown that leukocyte contamination of erythrocyte or platelet preparations can cause a wide variety of side effects after their transfusion. Examples of adverse effects of leukocyte contamination are the transmission of cell-associated infectious agents, febrile non-haemolytic transfusion reactions, refractoriness to platelet transfusion, graft-versus-host disease, generalised immunosuppression, and an increased graft rejection rate of marrow or kidney transplantations.

There are few benefits of retaining leukocytes in transfused blood products, except from extending kidney transplant survival. As a consequence, the use of leukodepleted blood components is widely practised and recommended.[1] The current trend in industrialised countries is that all cellular blood components to be transfused are leukodepleted.

Adverse effects of blood transfusion and their prevention by leukodepletion

Transmission of cell-associated infectious agents

Cytomegalovirus transmission

Cytomegalovirus (CMV) can be transmitted during the transfusion of cellular blood products and can cause significant morbidity and mortality in immunocompromised CMV-seronegative patients, such as CMV-seronegative pregnant women, premature infants (birthweight <1.2 kg) born to CMV-seronegative women, CMV-seronegative recipients of allogeneic bone marrow transplants from CMV-seronegative donors, and CMV-seronegative patients with acquired immunodeficiency syndrome.[1] Transfused erythrocytes, platelet concentrates, and granulocyte concentrates have all been implicated as the cause of infection by transfusion-transmitted CMV (TT-CMV), while fresh frozen plasma and cryoprecipitate have not been reported to cause CMV transmission.[2] The cells that serve as reservoirs for CMV have not been identified, but monocytes have been considered to be the dominant cell-type that is infected in peripheral blood.[3-6]

The use of CMV-seronegative blood products has been the 'gold standard' method of preventing TT-CMV infection. Even so, they are still associated with a risk of TT-CMV of approximately 4%.[7] It has recently been demonstrated that latent CMV in recipients' leukocytes can be reactivated from leukocytes of healthy seronegative blood donors.[8] In some of the CMV-seronegative individuals who harboured infectious CMV, a significant anti-CMV antibody response either may have diminished with time or may have never been present.[8] These possibilities could explain, at least in part, the residual risk of TT-CMV following the transfusion of seronegative blood products. On the other hand, some CMV-seropositive blood may not be infectious. Since the prevalence of antibody to CMV ranges from 60% to more than 90%, eliminating all CMV-seropositive donations to prevent TT-CMV would deplete the supply of blood available

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for transfusion. In addition, as the CMV-seropositivity rate among the Hong Kong population is more than 90%, obtaining CMV-seronegative blood or blood components may be difficult.

Studies have found that depleting blood components of leukocytes is successful in preventing TT-CMV infection in neonates, patients with acute leukaemia, and bone marrow transplant recipients.[9-11] Using leukodepleted blood components is thus an effective alternative method to using CMV-seronegative blood components to prevent TT-CMV infection to at-risk patients.

Transmission of other herpesviruses

Besides CMV, other herpesviruses such as Epstein-Barr virus (EBV), human herpesvirus (HHV)-6, HHV-7, and HHV-8 (or Kaposi's sarcoma–related herpesvirus [KSHV]) are associated with leukocyte contamination during transfusion. Primary EBV infections from blood transfusions may cause clinical problems in EBV-seronegative transfusion recipients.[12] Thus, in high-risk patients, leukocyte depletion to prevent primary EBV infection might be indicated. The clinical relevance of the transmission of HHV-6, HHV-7, and HHV-8/KSHV through blood transfusion is unclear and requires further investigation.

Human T-cell leukaemia/lymphoma virus types I and II

Human T-cell leukaemia/lymphoma virus (HTLV) types I and II target T lymphocytes and are solely transmitted by cellular blood components.[13] Transfusion-transmitted HTLV-I/II, however, is a very rare event and is even more rarely associated with progression to clinical disease. The current strategy of limiting transfusion-transmitted HTLV-I/II is to serologically screen all donor blood. As data are limited and no comparison exists, the Canadian Coordinating Office for Health Technology Assessment recently concluded that "leukodepletion cannot currently be used as a substitute for HTLV-I testing for this rare disease...."[14]

Bacterial contamination

The rate of the bacterial contamination of platelet components is at least 10- to 100-times greater than the rate of contamination by many viruses (human immunodeficiency virus [HIV] 1 or 2, hepatitis B and C viruses, HTLV-I/II). Studies have shown that approximately one in every 1500 to one in every 2000 units of platelets are contaminated with bacteria,[15,16] although contamination does not always result in signs and symptoms of sepsis. Leukocyte depletion filters can directly and indirectly remove bacteria from platelet and erythrocyte preparations. Bacteria adhere to the filter matrix, while phagocytic leukocytes, which adhere to or ingest bacteria, are retained by the filters. However, the clinical efficacy of leukodepletion for bacterial removal is not known.

Transmission of *Toxoplasma gondii*

Primary toxoplasmosis has been transmitted by whole-blood and granulocyte transfusions and from the transplantation of organs from seropositive donors to immunocompromised recipients.[17] *Toxoplasma gondii* can survive in refrigerated blood components.[18] As with other leukocyte-associated agents, *T gondii* transmission can be minimised by transfusing leukocyte-depleted blood components. Since the seroprevalence to the organism is high, however, most transfusion recipients are not at risk.

Transmission of prions

Prions cause neurodegenerative disorders in both human and animals, such as kuru, Creutzfeldt-Jakob disease (CJD), bovine spongiform encephalopathy, and the new-variant CJD (nvCJD).[19] The abnormal prion-related protein can be found in the tonsils and the spleen of patients with nvCJD, but not in those with classical CJD. Hence, circulating B lymphocytes might harbour the agent responsible for the development of nvCJD.[20-24] However, no data are available on the likely transmissibility of CJD or nvCJD by blood transfusion.

Febrile non-haemolytic transfusion reactions

Febrile non-haemolytic transfusion reactions (FNHTRs) have been reported to occur with an incidence of 6.8% after erythrocyte transfusion and 37.5% after platelet transfusion.[25] The major cause of severe FNHTRs to erythrocytes is human leukocyte antigen (HLA) alloimmunisation. A reduction in the number of leukocytes to 5×10^8 leukocytes per unit of blood component is sufficient to prevent FNHTRs in most cases. It is generally agreed that patients requiring long-term red blood cell support (eg those with beta thalassaemia major) should receive erythrocyte concentrates in which leukocyte levels are below 5×10^8 per unit to prevent FNHTRs.[26] This level of leukodepletion can be achieved by the use of buffy coat–depleted red blood cell concentrates or by transfusing red blood cells through bedside leukocyte filters. If FNHTRs continue despite these measures, leukodepleted erythrocyte concentrates that have a much lower level of leukocytes should be used.

There is increasing evidence that the major cause of FNHTRs after platelet transfusion is due to the

presence of pyrogenic cytokines, especially IL-1α, that are released from leukocytes during the storage of platelets at 22°C.[27] Hence, FNHTRs following platelet transfusion are not reliably prevented by the bedside leukocyte filtration of platelet concentrates. In contrast, performing leukodepletion before storage not only removes the leukocytes, but can also markedly reduce the level of cytokines in platelet concentrates.[28]

The third mechanism of the onset of FNHTRs is relatively more common after the transfusion of platelets than after that of erythrocytes. The adverse reaction is due to the formation of immune complexes of the recipient's antibodies with cells or proteins in the blood product, which triggers the recipient's immune system to release cytokines. Leukocyte depletion would therefore not prevent FNHTRs that occur due to this mechanism.

Refractoriness to platelet transfusion

Platelet refractoriness is the repeated failure to obtain satisfactory responses to platelet transfusion and is a common problem for patients receiving multiple transfusions. Platelet refractoriness can arise due to immune or non-immune causes. Non-immune causes can include septicaemia, fever, disseminated intravascular coagulation, and splenomegaly. The main immune cause is HLA alloimmunisation.

The development of an immune response to transfused platelets depends mainly on the interaction of the donor's transfused antigen-presenting cells (APCs) with the recipient's T cells, which then signal the recipient's B cells to produce alloantibodies. It has been postulated that removing the donor's leukocytes (including APCs: mainly dendritic cells, monocytes, and B lymphocytes) prior to transfusion may reduce the rates of platelet antigen alloimmunisation. The accumulated data suggest that leukodepletion is relatively effective in preventing platelet antigen alloimmunisation among patients with acute myeloid leukaemia who are receiving induction chemotherapy.[29]

The effectiveness of leukodepleted blood components in the prevention of HLA alloimmunisation among patients who may have been sensitised by previous transfusion or pregnancies is still controversial.[30] There is also currently no convincing evidence that the routine leukodepletion of blood components produces clinical benefits for patients who are receiving multiple platelet transfusions, although HLA alloimminisation and platelet refractoriness may be reduced.[1]

Transfusion-associated graft-versus-host disease

A rare but usually fatal complication of transfusion is transfusion-associated graft-versus-host disease (TA-GVHD). The risk associated with an individual transfusion depends on the number and viability of contaminating lymphocytes, the susceptibility of the patient's immune system to their engraftment, and the degree of the immunological (HLA) disparity between the donor and recipient. The transfused viable T lymphocytes, under certain circumstances, engraft and proliferate in the recipient. The interaction between donor T lymphocytes and recipient cells bearing either class I or class II HLA antigens results in cellular damage, which may be mediated by natural killer cells. Major target tissues include skin, thymus, gastro-intestinal tract, liver, spleen, and bone marrow. The risks of TA-GVHD are highest in recipients who have an immunodeficiency or who are immunosuppressed, although TA-GVHD has not been described in patients who are infected with HIV.

Lymphocyte viability is retained in stored erythrocytes for at least 3 weeks, and cases of TA-GVHD following the transfusion of whole blood, red blood cells, platelets, and granulocytes have been reported. Transfusing granulocytes poses a particular risk owing to the freshness and number of contaminating lymphocytes, as well as the likelihood that the recipient is immunoincompetent. No cases of TA-GVHD have been described following the transfusion of frozen deglycerolized cells, cryoprecipitate, fresh frozen plasma, or fractionated plasma products such as clotting factor concentrates, albumin, and intravenous immunoglobulin.

The 'threshold' dose of lymphocytes required for the development of TA-GVHD in humans is unknown and may depend on the recipient's ability to reject transfused lymphocytes. In one case, the disease developed after the transfusion of only 8×10^4 lymphocytes per kilogram. The successful prevention of TA-GVHD thus depends on either the physical removal of donor lymphocytes or the destruction of their proliferative potential. Current filtration technology cannot consistently produce the levels of lymphocyte removal required. The current mainstay of preventing lymphocyte proliferation continues to be gamma irradiation. The recommended minimum dose to prevent TA-GVHD is 25 Gy.[31] It has also been shown that TA-GVHD can be transmitted to immunocompetent recipients when the blood donor has a homozygous HLA haplotype that is identical to one of the recipient's HLA type.

Generalised immunosuppression after transfusion

Allogeneic blood transfusions produce a variety of effects on the recipient's immunological functions, such as the decreased function of natural killer cells, macrophage migration to sites of injury, lymphocyte proliferation, and cutaneous delayed hypersensitivity. The presence of donor leukocytes in allogeneic blood may play a role in suppressing cellular immune function. Reports have suggested that allogeneic blood transfusions increase the incidence of postoperative infection and the tumour recurrence rate. These reports attribute the postoperative morbidities to the immunomodulatory effects of blood transfusion.[32,33] This association remains unproven, however, and there is currently insufficient evidence to recommend the routine use of leukodepleted blood components for surgical patients to prevent either postoperative infection or tumour recurrence.

Graft rejection rates after organ transplantation

The sensitisation of an individual to transplantation antigens by preceding transfusions can lead to graft rejection after organ transplantation. Allogeneic blood containing leukocytes has been shown to have an adverse effect in patients with aplastic anaemia who undergo bone marrow transplantation[34] and in renal transplant patients. The sensitisation to transplantation antigens can potentially be prevented by leukodepleting blood components that are to be used in pretransplantation transfusions. Liver transplantation, however, does not seem to require HLA matching or lymphocyte crossmatching before transplantation; hence, leukodepleted blood components are not indicated in transfusions before this procedure.[35] For heart transplantations, there is currently no available information about the possible benefit of preventing HLA alloimmunisation by leukodepleting blood components that are to be used for pretransplantation transfusions.

Leukodepleting blood components

As leukocytes in the blood can cause so many undesirable effects, leukodepletion is the best method of preventing or delaying such effects. The United States and many European countries have now decided to leukodeplete all cellular blood components. However, it is still controversial as to whether it is cost-effective to practise universal leukodepletion.

A leukodepleted blood component is most commonly defined as containing fewer than 5×10^6 white blood cells per unit of component. Blood filtration by using leukocyte filters is a commonly used method of leukodepleting blood components. The filtration can be performed either at the bedside during transfusion or in the component-processing laboratory. The latter mode of filtration (prestorage filtration) is superior to bedside filtration because leukocytes are removed before storage, thus preventing further biological changes associated with the storage of these cells, and because quality assurance can be guaranteed. Performing leukodepletion within a relatively short time—usually within 48 hours after blood collection—also eliminates leukocytes before they release cytokines, fragments of cell membrane, and possibly intracellular viruses. These factors may not be removable by bedside filtration. The cytokines and cell membrane fragments may lead to FNHTRs and primary HLA alloimmunisation,[36] respectively, even if the blood is transfused through a bedside leukocyte filter.

The temperature of filtration is also an important factor in determining the efficiency of leukocyte depletion. Studies have shown that colder temperatures result in the better leukodepletion of red blood cell concentrates.[37] Because the transfusion of erythrocytes usually occurs over a few hours, however, the blood will warm to room temperature, which will reduce the efficiency of leukodepletion. Thus, filters used at the bedside may not perform as well as those used in the laboratory. For the production of leukocyte-depleted platelet concentrates, apheresis technology can be used and circumvents any need for filtration or further processing.

Conclusion

Leukocytes in erythrocyte and platelet concentrates can be considered as a contaminant that may lead to serious morbidity and even mortality in at-risk recipients. Some western countries have adopted a universal leukodepletion policy for cellular blood components. However, universal leukodepletion is costly and, consequently, it may be more cost-effective to reserve leukodepleted blood components for at-risk recipients.

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