Leucodepletion and Blood Products

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Abstract

Background : Leucoreduction of blood products is increasingly being employed to produce blood products with residual WBCs < 5×10^6 per unit (99.9 percent or a log 3 leucoreduction). Clinical data suggests that non-haemolytic febrile transfusion reactions can be prevented by leucodepletion. The procedure also prevents alloimmunisation to HLA antigens in patients who will repeatedly require transfusion of blood/blood products.

Method : Of the methods available to reduce the number of WBC in blood products washing of red cells, freezing and deglycerolisation are effective and yield a product with only a 24 hour shelf life. Other methods such as leucodepletion filters are relatively inexpensive, simple and the final product has a normal shelf life. Modern generation of leucoreduction filters and apheresis machines can provide greater than 4 log reduction of WBC.

Results : After the introduction of leucodepletion of blood for Thalassemics at our center in 2003, the incidence of non haemolytic febrile transfusion reactions (NHFT) fell from 4% in 2002 to 1% in 2003.

Conclusion : In patients undergoing long-term blood transfusion therapy e.g. Thalassemics, alloimmunisation against the HLA antigens on donor white cells is prevented by leucodepletion and prevents NHFTs.

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Key Words : Leucoreduction; leucodepletion; alloimmunisation.

Introduction

Leucodepletion is a process by which leucocytes are removed from donated blood. It has been estimated that the average content of leucocytes in donated human whole blood is 10^9 /unit. By the current standards, the total content leucocytes in a blood unit should be less than 5×10^6 /unit after preparation and a minimum of 85% of whole blood or red cells is retained [1]. This process has been in use for some time for a select group of patients e.g. the multi-transfused patients, the immunocompromised and malignancy patients. It has been well established that febrile reactions occurring in most cases while transferring blood is due to immune reaction of the recipient against donor leucocytes [2]. The original leucocyte depletion filter contained cotton wool as the filtering agent and was designed by Diepenhorst who published his work in 1972 [3]. Subsequently, cellulose acetate filters were discovered and found to be more suitable. Other methods include red cell washing, centrifugation and buffy coat removal, freezing and deglycerolisation of red cells and apheresis [4,5]. The initial standards for leucodepletion required removal of at least 70% white blood cells and retention of 70% of the original red cells [4]. These values have been under review constantly, and presently, blood is considered leucodepleted if the total leucocyte content

per unit of blood is $<5 \times 10^6$ and the red cell product should have retained at least 85% of the original red cells [4].

It has been observed that antibodies against histocompatibility antigens tend to develop in the recipient, if the leucocyte content in a unit of transfused blood exceeds the figure stated above. The same observation has been made with respect to WBCs contaminating units of platelet concentrates. The current consensus is that, antigens on the platelets, by themselves are not capable of initiating a primary immune response. However, even with their weaker antigenic capability they can propagate a secondary immune response [6]. Therefore to avoid primary alloimmunisation against the histocompatibility antigens, the total content of leucocytes in a unit of red cells should be less than 5×10^6 per unit. To qualify as leukocyte reduced platelets, pheresis units should also have less than 5×10^6 leukocyte per bag. For platelet concentrates derived from whole blood, the standard requires that each unit contains no more than 0.83×10^5 WBC per unit, so that a pool of six units would contain no more than 5×10^6 WBC per unit [7].

Leucocyte antibodies

The role of leucocyte antibodies in causation of

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transfusion reactions was suggested while observing better tolerance of leucocyte poor blood by patients who had a history of severe febrile reactions following blood transfusions [2,8]. The patients characteristically had flushing and a feeling of warmth shortly following transfusion. Subsequently, the patients felt better for a period varying between 40-60 minutes before developing a severe febrile reaction. Antibodies produced consequent to alloimmunisation, may react both with the HLA antigens and granulocyte specific antigens. In a study, sera from 101 patients presenting with a history of non haemolytic transfusion reactions, reacted strongly against leucocytes [9]. In another study, it was observed that HLA antibodies were found frequently than granulocyte specific antibodies. Alloantibody of one kind or the other was found in every single case [10].

Platelet products invariably get contaminated with leucocytes. It is unfortunate, that 30 to 50 percent of patients who receive multiple platelet transfusions become alloimmunised to HLA antigens, and subsequently do not respond to pooled random donor platelets and become platelet refractory [11]. Therefore, prevention of transfusion induced HLA alloimmunization could be of significant benefit in transfusion medicine. Formation of antibodies against histocompatibility antigens occurs if platelets are contaminated with leucocytes. Platelets per se, alone are only capable of inducing a secondary immune response. The induction of a primary immune response is dependant on the presentation of foreign class I antigens by transfused white blood cells [12]. It therefore implies that, alloimmunization against class I antigens can be prevented by leucodepletion of platelet concentrates. There is ample evidence that primary HLA immunization does not occur when less than 5×10^6 leucocytes per unit are transfused [6]. This value also applies to simple whole blood, the value is $<8.3 \times 10^5$ in all bags. Since the final pooled component also must have 5×10^6 leucocytes. This implies that the final pool size should not exceed 6 units [1].

Preparation methods

Various methods have been employed for leucodepletion of blood. A clear distinction can be made into two types.

1. An open system of leucocyte removal where the closed system is entered as a consequence of which the blood unit has a shelf life of only 24 hours. e.g., manual and automated cell washing, freezing and de-glycerolisation of red cells.
2. A closed system, wherein, the normal shelf life of the blood / blood product is maintained. Here the processing for leucodepletion is done without

entering the original blood collection pack or with entry of the system only during the course of transfusion e.g. centrifugation and buffy coat removal.

i) Cell washing- Cell washing done by automated machines or manual methods, removes not only the leucocytes, but also the plasma. The automated systems are also very expensive and washed red cells have a shelf life of only 24 hours.

ii) Centrifugation and buffy coat removal- After packing, red cells are subjected to visually controlled expression of the buffy coat using a plasma expresser. In this technique, leucodepletion is proportional to red cell loss, with a cut off point subjective to each operator. This method has therefore lost its popularity. The only virtue of this method is that it is a closed system [13].

iii) Freezing and Deglycerolisation- Glycerol serves as a cryo-protectant, and is added to packed red cells. These cells are then frozen to -60°C or below for storage. Both glycerolization and freezing destroys the leucocytes. After the red cells are thawed, the glycerol is removed by washing [14].

iv) Leucocyte filters – The leucocyte removal filters contain multiple layers of synthetic polyester non-woven fibres that selectively retain white cells while allowing red cells and / or platelets to flow through. Lymphocytes and monocytes are passively held in the filter, while granulocytes in addition are also trapped by adhesion [15]. Filters are less expensive as compared to other methods as automated cell washing, freezing and de-glycerolisation. It has therefore emerged as an effective and reliable way for leucodepletion [13]. Advances in filtration techniques and type of filters have resulted in reducing WBCs in red cell and platelet concentrates to 99.9 percent (less than 5×10^6 WBC per unit) and more [16-18].

Leucocyte filters are available commercially in a number of configurations. They can be used to perform leucodepletion before storage (on-line leucodepletion), or in the laboratory (pre-storage) or it may even be carried out at the bedside during blood transfusion. The timing of leucodepletion is important. It is well known that leucocyte removal is better if the time span between blood collection and leucodepletion is shortened. This is so because, during storage, leucocytes degranulate, fragment or die thereby releasing their contents that could result in febrile and allergic transfusion reactions. The cytokines especially IL-8 that accumulate during storage have been implicated for some failures of bedside filtration to prevent febrile reaction [18].

Counting Methods

Special methods of cell counting are required to detect such a low number of leukocytes in leucocyte depleted blood. Automated haematology cell counting devices may not accurately determine the residual leukocytes in leucocyte-reduced products. The methods used are as follows-

Manual methods - Methods using Nageotte counting chamber and Turk's or Plaxan's staining solutions alone or in conjunction with Zap-oglobin® or centrifugation are comparable in sensitivity and reliability for counting less than 5 leukocytes/uL [17-20].

Automated methods - Flow cytometry, using propidium iodide alone or combined with thiazole orange, compares favourably in sensitivity to the Nageotte chamber method and provides the advantages of automation and the ability to determine phenotypes of residual leukocytes which may be of interest to clinicians [21].

Indications for leucodepleted transfusion

Leucodepletion is generally indicated in-

- Reduction of non-haemolytic transfusion reactions.
- Reduction of HLA alloimmunisation that may lead to patients becoming refractory to platelet transfusions e.g. patients requiring long term platelet therapy as in Aplastic anaemia and MDS.
- Reduction of CMV transmission e.g. in patients awaiting organ transplantation.
- Reduction in the risk of *Yersinia enterocolitica* contamination of RBC.
- Possible reduction of Prion Disease.
- Reduction in the incidence of transfusion associated GVHD (TA-GVHD).

Efficacy of leucocyte filters

Various trials for validating the efficacy of leucocyte filters have been carried out. The leucocyte filters currently available are capable of providing the desired level of leucodepletion. Present day filters provide a minimum of 3 log depletion of WBC and many filters are commercially available which can provide 4 log depletion and more [22-23]. It has been observed that loss of red cells while filtering blood ranges from 30 – 65 ml depending from filter to filter and about 12 to 14 percent of the platelets are also lost. The losses are more when packed red cells in CPDA are filtered vis-à-vis packed red cells in Adsol/SAGM. The product loss during filtration of Single Donor Platelets (SDP) can range to anything from 45 to 50 ml. Leucodepletion filters for platelets are different as they are supposed to retain platelets during filtration. Unfortunately, the loss of

platelets during this procedure has been reported to be anything from 9 to 35 percent. This is quite high and may result in an increased frequency of transfusions and donor exposures to compensate for the loss during processing [24]. The time taken for filtration varies from filter to filter and can take anything from 10 to 20 min. Current generation of apheresis machines and disposable sets used are capable of achieving a 3 log leucodepletion, obviating the need for filtration [25-26].

Our experience at Dept of Transfusion Medicine, AFMC.

Current therapy for thalassemia syndromes consists of regular red cell transfusions to maintain a baseline hemoglobin level > 9g/dl, coupled with intensive parenteral chelation therapy with desferoxamine. These patients receive long-term multiple transfusion and the common transfusion reactions noted are non-haemolytic febrile transfusion reaction (NHFT) and allergic reactions. Others include iso-immunisation, autoimmune haemolytic anaemia proven with circulating autoantibodies, post-transfusional hypertension, etc. [27]. In our center, since 2003, leucodepletion of blood is routinely being done using third generation pre-storage filters (PALL, USA) before transfusion to thalassemias. Of the 303 transfusions given to Thalassemics, in 2003, only 03 patients (1%) had NHFT after use of leucodepleted blood in comparison to 10 out of 252 (4%) in 2002 who received non-leucodepleted blood. All units of blood were tested for pre and post leucoreduction WBC count. The requisite standard of leucoreduction were achieved ($p < 0.001$).

Conclusion

Leucocyte removal from blood components before transfusion by leucocyte depletion filters is known to prevent or at least delay leucocyte mediated adverse reactions. By use of third generation leucodepleted filters, the WBC count in the red cell and platelet concentrates has reduced to greater than 99.9%. The increasing use of leucocyte-depleted products increases the need for quality assurance. The American standards specify that leucodepleted blood should have less than 5×10^6 WBC per unit of blood. Food and Drug administration guidelines (FDA) recommend testing of 1% of leucodepleted units of which 100 percent should contain less than 5×10^6 WBC per unit of blood. It is to be noted that quality control procedures cannot be carried out satisfactorily on bedside leucodepletion filters and therefore bedside leucodepletion should only be carried out when other options for leucodepletion cannot be utilized. Universal leucodepletion is the best method in transfusion practice, but in developing countries like ours, it is not cost effective. To conclude, the aim should be

to constantly prepare WBC reduced blood products with less than 5×10^6 residual WBC so that primary alloimmunisation to HLA antigens is prevented and transfusion is made safer.

Conflicts of Interest

None identified

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