Original article

Indian Journal of Pathology: Research and Practice

Prestorage Leuco Reduction of Blood Components

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Accepted on 15.08.2012

Abstract

Introduction: Non haemolytic febrile transfusion reactions can be prevented by leucodepletion. In spite of effective leucodepletion, presence of platelet derived cytokines will limit the lifespan of platelets.

Objective: To analyse the effect of pre storage leuco reduction by using clinical and laboratory parameters on packed cells and platelets.

Materials and methods: 250 units of packed cells and 250 units of platelet concentrates which were prepared by TACE (quadruple bags were included for the study. Pre leucocyte count was done by automated cell counter and neubauer chamber. After separation of packed cells and platelets, post leukocyte count was done. As the number of cells was too low in platelet concentrate, Nagotte chamber was used for counting cells. After transfusion, occurrence of NHTFR was watched for and increment of haemoglobin was noted whenever possible.

Results: The leucoreduction was in the range of 45% to 80% in packed cell preparation and 98-99.7% in platelet preparation. The reduction in platelet was achieved irrespective of the level of reduction in the packed cell concentrate. However no incidence of NHTFR was noted in n 500 transfusions up followed up in both packed cells and platelet concentrate.

Conclusions: Reduction of residual leucocytes is important in the preparation of blood components. Utilisation of new generation filters or leuco depletion processes with better performance characteristics may help to reduce specific leukocyte subsets as well as activation of inflammatory system such as cytokines, which will improve the quality of the component prepared.

Keywords: Packed cells; Platelet concentrate; Leucoreduction; Blood components.

Introduction

The improvement of transfusion medicine technology is an ongoing process primarily directed at increasing the safety of allogeneic blood component transfusions for recipients[1]. Febrile non-haemolytic transfusion reactions due to leukoagglutinins are frequently seen in patients who have been given multiple blood transfusions[2]. Multiple blood transfusions may lead to the production of leukocyte antibody which in many cases is responsible for non-haemolytic febrile transfusion reaction. The severity of these reactions depends on the number of leukocytes present in the transfused blood[3]. Leukocytes have the ability to distinguish between self-cells (body's own cells) and foreign (allogeneic) cells on the basis of human leukocyte antigen(HLA) proteins that are present on cell membrane and are effectively unique to a person. During allogeneic

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blood transfusion person receives a large number of allogeneic donor leukocytes and these are recognized as foreign cells by recipient immune system which leads to several adverse reactions. Leukocyte depleted blood transfusion is recommended to avoid such leukocyte mediated adverse reactions[4].

Leukocytes can be separated on the basis of their size, dielectric properties, affinity separation, freeze thawing and centrifugation, but all these methods are time consuming and costly. Filtration is another method for leukocyte depletion, which is comparatively less expensive and more efficient as it gives more than 90% of leukodepletion of blood along with minimal loss of cells. However, present filtration procedures also have some limitations as they work efficiently with blood components but not with whole blood and show nonspecific adhesion of large number of platelets and red blood cells along with leukocytes. With this background, we evaluated the effect of pre-storage leukoreduction by using clinical and laboratory parameters on packed red cells and platelets, which are the most common components used.

Material and methods

The study was prospective in nature and was done during the period from December 1 2009 to November 30, 2010 at St.John's Medical College Hospital, Bangalore.

Leukodepletion

At the time of component preparation leuko reduction was done using T-ACE automatic component separator. The samples were collected before and after leuko reduction to assess the level of leuko reduction.

After collection of samples the following parameters were evaluated.

1. Haemoglobin by automated cell counter, SLS-Haemoglobin method.
2. Total WBC count by using Neubauer counting chamber for leuko reduced packed RBCs.
3. Total WBC count using Negotte counting chamber for leuko reduced platelets.

After the issue of packed red cells and platelets which were assessed for leuko reduction, the patients to whom the units were issued were followed up.

The clinical parameters included

1. The demographic details of the patient transfused
2. Haemoglobin increment.
3. Presence or absence of any febrile non-haemolytic reactions and time of onset. Follow up was done for 48 hours after transfusion.

The clinical and laboratory parameters thus obtained were correlated to study the level and effect of leuko reduction.

Results

A total of 500 units (250 units of Packed cells and 250 units of platelets) randomly selected during the study period were included. All units were screened for HIV, Hepatitis B and C, Malaria and for syphilis by VDRL as per the standard protocols followed in the blood bank.

Packed red cell concentrates

Leukocyte depletion of the 250 packed cells was in the range of 45% to 80%. After calculating the level of depletion from the samples, the units were categorized into 4 types to see the efficacy of the process.

1. Bags with a leukoreduction of < 1000 cells/ micro litre
2. Bags with a leukoreduction > 1000 but < 2000 cells/ micro litre
3. Bags with a leukoreduction of >2000 but <4000 cells/micro litre
4. Bags with a leukoreduction of > 4000 Cells/ micro litre

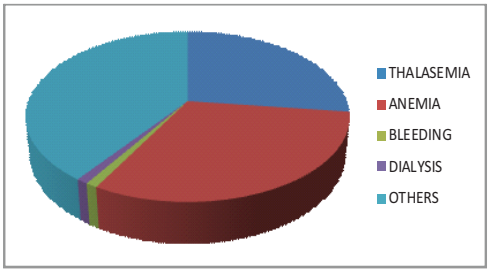
There were 29 bags in the first category, 77 bags in the second category, 95 bags in the third

category and 51 bags in the fourth category. This indicated only in 20% of units, the maximum level was achieved. This was probably due to the some retained buffy coat sticking to the primary bag and in the tube after the first spin which will get mixed with packed cell concentrate.

The maximum utilization of packed cells was from the OPD, where children with thalassemia received the units. The next ones which utilized maximum were Paediatric ICU (22 units) and Obstetric ICU (25units). A total of 100 units were issued to all other wards combined including surgery department and operation Theatre. The other wards include cardio thoracic ward, paediatric intensive care, urology, emergency medicine, orthopaedics, gynaecology, neonatal wards, oncology, emergency, and those which are released to outside the hospital. The indications given is represented as pie chart (Figure 1) As mentioned earlier, the maximum utilization was in the OPD for thalassemia children followed by obstetrics and female medical ward for anaemia.

The Haemoglobin increment was followed up whenever possible. An increment of 0.9 g/dl to 1.0 g/dl of haemoglobin increment was

Figure 1: Indications for utilisation



The indications are represented as a pie chart. Anaemia ranked 1st followed by others.

observed in this study with an average of 0.7g/dl increment. When the weight of the bag was correlated with haemoglobin increment, the units which have weight more than 270mg showed an increment of 1.0g/dl of haemoglobin probably because they had a higher haematocrit, whereas it decreased in units less than 255mg with an average increment of 0.7 to 0.8g/dl. Post transfusion haemolytic or non haemolytic febrile transfusion in patients who has been transfused with these units was also observed in this study. No such reactions were reported during this study.

In platelet concentrates the leuko reduction was divided into 2 categories- Units with a total leukocyte count of less than 25 cells /cu mm and units with total leukocyte count of greater than 25 cells /cu mm. In 25% of the units, the maximum reduction was achieved. As the numbers of cells were very less, Nagotte chamber was used. As the platelet concentrates were given as two to five units per patient, the increment could not be calculated per bag. The leuko reduced platelet concentrates were also followed for any reaction in the patient, but no adverse reactions were observed.

Discussion

The reasons of reduced efficiency of leuko reduction in packed red cell concentrates may be several. The study was done in quadruple blood units only, so that both packed red cells and platelets can be prepared from same unit. All the bags which used for blood collection did not have an in built filter. TACE–I exerts pressure on the main bag to separate the components after centrifugation. The instrument pushes the bag from top to bottom which will allows the flow of plasma first followed by buffy coat to the buffy coat bag. The amount of buffy coat collected is dependent on the weight of buffy coat bag. There are no optical sensors for the buffy coat bag. During separation time, some of the buffy coat sticks to the primary bag and some amount of buffy coat layer get retained in the tubing and in the primary bag. After plasma separation, when the residual plasma in the platelet bag is allowed to flow into the primary bag, this may gets mixed up with the retained buffy coat layers in the tubing from the primary bag.

In contrast a higher level of leuko depletion was obtained in platelet concentrates. The platelet concentrates were prepared by buffy coat method. In this method the buffy coat bag was allowed to hang for a at least one hour before centrifugation. The low spin centrifugation of the buffy coat bag resulted in settling of WBCs leaving behind plasma rich in platelets and thus a higher amount of leukodepletion is achieved.

Factors influencing the occurrence of reactions

Irrespective of the level of leukoreduction, no reactions were observed during the study. The practice of use of anti histamine drugs as pre medication to the recipient before transfusion may be one of the reasons for non occurrence of such reactions. The sample size of the study was only 250 units of packed red cell concentrates and 250 units of platelet concentrates. Since this sample size is only a small fraction of the total number of transfusions per year in the hospital, the probability of getting a transfusion reaction also is lower.

The largest prospective randomized study of leukoreduction which enrolled 2780 patients documented no differences in the outcome measures studied, including in-hospital mortality, hospital length of stay, intensive care unit length of stay, and postoperative length of stay, antibiotic usage, and readmission rate. Subgroup analyses based on age, sex, amount of blood transfused, and category of surgical procedure showed no effect of leukocyte reduction. The patients who received leukocyte- reduced blood exhibited a lower incidence of febrile reactions ($p =< 0.06$)[5].

Pathogen inactivation

The concept of pathogen inactivation in blood components is to reduce the residual risk of known pathogens and to effectively eliminate new, yet unknown pathogens. However, the different approaches advocated such as use of toxic or mutagenic chemicals increase the blood safety without compromising the product efficacy or causing adverse effects, The choice of a pathogen reduction approach depends on whether it is used to treat components for transfusion such as RBC, PLT and plasma, or for products manufactured from the plasma. Two distinct methods, methylene blue (MB) and solvent-detergent (SD) are currently employed for the treatment of plasma intended for transfusion[6]. MB is a phenothiazine colorant that inactivates most viruses and bacteria after exposure to visible light. While it has the advantage of being useful for single plasma units, its ineffectiveness against intracellular pathogens and probable interaction with coagulation factors considerably reduce its efficacy. The SD approach acts by disrupting the envelope proteins of targeted pathogens, thus compromising the integrity of the pathogen and rendering it non infectious. This approach is used on small pools of plasma. The limitation of this technique is that it is not active against non-enveloped pathogens, and that levels of coagulation factors such as protein S may be decreased significantly by some of the SD treatment methods.

All the units were screened for transfusion transmitted diseases as per protocol and none of the units had been positive for any of them. Finally, other than the reduction of incidence of adverse reactions, utilisation of new generation filters or leuko reduction processes with better performance characteristics may help to reduce specific leukocyte subsets as well as activation of inflammatory system which will improve the quality of the component prepared.

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