Original Article

Comparative Study of Predeposit and Bedside Leucodepletion Filters

Lt Col A Sen[*], Brig A Khetarpal, SM[+], Lt Col S Jetley (Retd)[#]

Abstract

Background: Removal of leucocytes from cellular blood components is associated with reduction of several transfusion associated adverse reactions.

Methods: A total of 400 units of packed red blood cells (RBCs) were subjected to leucodepletion at room temperature and 4°C using different commercially available prestorage and bedside filters (Terumo Penpol Immugard III and Pall Medical BPF-4). Pre-filtration and post-filtration parameters were compared to assess the efficacy of prestorage leucodepletion vis-à-vis bedside leucodepletion and the requirement of universal leucodepletion.

Result: Mean post-filtration red cell recovery ranged from 88.49-93.49% with all bags showing more than 85% red cell recovery. Mean post-filtration residual leucocyte count ranged from 0.205 x 10^6-0.338 x 10^6/ bag with all bags showing more than log 3 leucoreduction. Prestorage leucoreduction achieved by the polyurethane filter was better than that achieved by the polyester filter. Red cell recovery with the bedside filters at room temperature was significantly less than that with prestorage filters at either temperature.

Conclusion: This study suggests that prestorage leucoreduction is preferable over bedside leucoreduction and that polyurethane filters are better than polyester filters since leucodepletion achieved with the former is higher. We recommend selective log 3 leucodepletion using polyurethane prestorage filters for patients with specific indications.

MJAFI 2010; 66 : 142-146

Key Words : Leucodepletion; Pre-storage; Polyurethane

Introduction

Donor leucocytes with their specific allogenic structure, exposing the human leucocyte antigen (HLA) class I and class II antigens on their surface, are main targets of the recipient's immune system. The removal of these leucocytes from cellular blood components is associated with reduction of several transfusion associated adverse reactions. These include non-hemolytic febrile transfusion reaction (NHFTR), HLA alloimmunization with subsequent refractoriness to platelet transfusions, transfusion associated graft v/s host disease (GVHD), immuno-suppression and transmission of certain leucotropic agents such as cytomegalovirus (CMV) and human T-lymphotropic virus (HTLV) [1-4].

In an effort to overcome these adverse effects, methods of removal of the donor leucocytes– leucoreduction or leucodepletion – have been developed. Leucocyte reduction of blood components is a process whereby leucocytes are removed from a blood component. This process has been used for some time for select groups of patients. The removal of white blood cells (WBCs) from red blood cells (RBCs) units was first accomplished by differential centrifugation, then with the use of microaggregate filters and finally by more efficient and specific WBC reduction filters. This filtration may occur shortly after donation and processing of the blood unit and is then called pre-storage filtration, or it may occur at the patient's bedside when it is referred to as bedside filtration. WBC reduction by bedside filtration has been shown to be less efficient in preventing certain complications such as NHFTR as these reactions are caused by cytokines which are released from the leucocytes into the blood component during storage and cannot be removed by the bedside filters. Prestorage filters on the other hand remove the leucocytes before they can release the cytokines and are hence able to prevent NHFTR [5].

Current United States food and drug administration (FDA) guidelines [6,7] and American association of blood banks (AABB) standards define a leucocyte-reduced component as one with $< 5 \times 10^6$ residual donor leucocytes per final product (this includes RBCs and platelets). These standards currently define a WBC

[*]Classified Specialist (Pathology & Micro), [+]Consultant (Pathology), Army Hospital (R&R), Delhi Cantt-10. [#]Ex Classified Specialist (Pathology), Armed Forces Transfusion Centre, Delhi Cantt-10.

Received : 29.05.09; Accepted : 10.01.10 E-mail : aloksen19@yahoo.com

reduced RBC component as containing less than 5 x 10^8 WBCs when WBC reduction is intended to prevent NHFTR (log 1 or 90% WBC reduction) and less than 5 x 10^6 WBCs when it is intended to prevent HLA alloimmunization or CMV infection (log 3 or 99.9% WBC reduction) [8]. Quality control must indicate that 100% of units meet these criteria. Such components should also contain at least 80% of the original RBC content. By comparison, European guidelines define leucocyte-reduced components as those with $<1 \times 10^6$ residual leucocytes per unit. Most commercially available WBC reduction filters accomplish a log 3 to log 4 reduction in the WBC content of the RBC unit [9,10]. However, several studies have suggested that the efficacy of these filters is highly variable and may be affected by prolonged storage of the units at refrigerated temperature and by filtration of cold rather than room temperature units [11]. In addition, bedside filtration has been criticized as resulting in an unreliable and invalidated degree of WBC reduction.

The present study was aimed to compare the efficacy of leucoreduction during prestorage filtration vis-à-vis bedside filtration, to evaluate the leucoreduction carried out by different, commercially available leucocyte filters and at different temperatures and finally to establish whether universal leucocyte reduction (ULR) be implemented keeping in view the cost factor.

Material and Methods

Four hundred units of blood (packed RBCs) were subjected to leucocyte reduction using two types of filters i.e. prestorage filters and bedside filters. Filters were used from different commercially available brands like Immugard III – RC (Terumo Penpol) and BPF 4 (Pall Medical). Various parameters like pre-filtration haematocrit, total WBC count with mononuclear cell and granulocyte percentage were compared with post-filtration counts. The filtration was carried out both at room temperature and at 4°C on random samples.

Donors were selected based on following criteria: weight > 50 kg, age 18 to 60 years, at least three months from last donation/ three days from last platelet pheresis, haemoglobin ≥12.5 gm/dl, platelet count ≥ 150 x 10^3/cmm, absence of any illness, no consumption of non-steroidal anti-inflammatory drugs (NSAIDs) for last seven days and negative test for human immunodeficiency disease (HIV), hepatitis B, hepatitis C, syphilis and malaria. The units of blood were collected over a period of six months. Whole blood (350 ml) was drawn into 49 ml of citrate phosphate dextrose anticoagulant (CPDA) and sent to the component lab for processing. The bags were centrifuged at 3800 rpm for nine minutes and the plasma removed. A Day 0 sample for cell counts was obtained and the units were weighed. The blood bags were randomly distributed into eight groups of 50 each as per the Table 1.

A total of 200 units of packed cells were taken up for prestorage filtration as per the details given in the Table 1 (Group 1-4). The units were randomly divided into two groups; 100 units were filtered at room temperature and 100 units at 4°C. The filters were connected to the blood bag/ product bag using a sterile connecting device and filtered by gravity into the product bag. The final product was weighed and a sample for cell counts was obtained.

A total of 200 units of packed cells were stored for 14 days at 4°C. After 14 days' storage, 100 units were taken out and filtration through bedside filters was carried out in the laboratory with the blood at 4°C. The filters were connected to the blood bag/product bag using a sterile connecting device and filtered by gravity into the product bag. The remaining

Table 1

Data related to type of filter and filtration conditions

Group	Type of filtration	Number of units	Storage interval (collection to filtration)	Temperature of filtration
1	Prestorage immugard III – RC (Terumo Penpol)	50	Within 2 hrs of collection	22°C (room temperature)
2	Prestorage BPF-4 (Pall Medical)	50	Within 2 hrs of collection	22°C (room temperature)
3	Prestorage immugard III – RC (Terumo Penpol)	50	6-8 hrs after collection	4°C (placed in blood storage cabinet for 4-6 hrs before filtration)
4	Prestorage BPF-4 (Pall Medical)	50	6-8 hrs after collection	4°C (placed in blood storage cabinet for 4-6 hrs before filtration
5	Bedside (Terumo Penpol)	50	14 days	22°C (brought to room temperature before filtration)
6	Bedside (Pall Medical)	50	14 days	22°C (brought to room temperature before filtration)
7	Bedside (Terumo Penpol)	50	14 days	4°C (taken out from blood storage cabinet and filtered)
8	Bedside (Pall Medical)	50	14 days	4°C (taken out from blood storage cabinet and filtered)

100 units destined for simulated bedside filtration were brought to 22°C and then filtered. The final product was weighed and a sample for cell counts was obtained.

RBC counts, WBC counts, platelet counts and hematocrit of the pre-filtration samples were estimated using the Sysmex KX 21 haematology autoanalyser. The volume of the unit was calculated by dividing the net weight of the content of the bags by the specific gravity of the unit (1.06) and the volume of RBCs in the unit calculated by multiplying the hematocrit and the total volume of the unit. The RBC counts, platelet counts and hematocrit of the post-filtration samples were also estimated using the Sysmex KX 21 haematology autoanalyser. The residual WBC counts in the post-filtration samples were counted using a Nageotte chamber since the autoanalyser cannot give accurate counts if the WBC count is below 100/µl.

Results

The pre and post-filtration characteristics of the red cell units are tabulated in Table 2 and 3. The average pre-filtration volume of all the 400 units of packed red cells ranged from 203.5-212.5 ml with a mean hematocrit ranging from 66.6-69.1%. The mean pre-filtration red cell volume in the bags ranged from 127.9-137.6 ml with a mean pre-filtration leucocyte count of 8694-12057/µl and a mean platelet count ranging from 174.9 x 10^3-213.8 x 10^3/ µl.

The average post-filtration volume of all the 400 units of packed red cells ranged from 171.9-184.4 ml with a mean hematocrit ranging from 66.7-69.4%. The mean post-filtration red cell volume in the bags ranged from 118.3-127.9 ml with a mean post-filtration leucocyte count of 1.12-1.88/ µl and a mean platelet count ranging from 6.0 x 10^3 – 8.9 x 10^3/ µl. The number of leucocytes in the post-filtration product was too

Table 2

Pre-filtration characteristics of the red cell units

Type of filtration	Temp	No of bags	Pre Filtration Mean Counts					
			Wt (gm)	Vol (ml)	HCT (%)	Red cell Vol	TLC (/µl)	Platelet (x10^3/µl)
Prestorage Immugard III (Terumo Penpol)	22°C	50	210.7	198.7	68.9	136.9	11250	191.4
Prestorage BPF-4 (Pall Medical)	22°C	50	207.1	195.4	68.4	133.5	10600	174.9
Prestorage Immugard III (Terumo Penpol)	4°C	50	211.1	199.2	69.1	137.6	12057	184.2
Prestorage BPF-4 (Pall Medical)	4°C	50	208.9	197.1	67.7	133.4	8694	192.7
Bedside Immugard III (Terumo Penpol)	22°C	50	212.5	201.5	68.2	137.4	9355	196.2
Bedside (Pall Medical)	22°C	50	209.7	197.9	67.8	134.1	9384	206.8
Bedside Immugard III (Terumo Penpol)	4°C	50	205.8	194.2	68.2	132.5	10550	213.8
Bedside (Pall Medical)	4°C	50	203.5	191.9	66.6	127.9	9560	185.1

Table 3

Post-filtration characteristics of the red cell units

Type of filtration	Temp	No of bags	Post Filtration Mean Counts								
			Wt (gm)	Vol (ml)	HCT (%)	Red cell Vol	Red cell Recovery (%)	TLC (/µl)	Platelet (x10^3/µl)	Leucocyte Removal %/ Log	Residual WBCs (x10^6)
Prestorage Immugard III (Terumo Penpol)	22°C	50	195	183.9	69.2	127.3	92.97	1.12	6.1	99.990% > Log 4	0.205
Prestorage BPF-4 (Pall Medical)	22°C	50	190.6	179.8	69.3	124.8	93.49	1.88	8.9	99.982% Log 3 – 4	0.338
Prestorage Immugard III (Terumo Penpol)	4°C	50	195.4	184.4	69.4	127.9	92.95	1.14	6.4	99.990% > Log 4	0.210
Prestorage BPF-4 (Pall Medical)	4°C	50	194.4	183.4	66.7	122.5	91.80	1.27	8.3	99.985% Log 3 – 4	0.232
Bedside Immugard III (Terumo Penpol)	22°C	50	186.4	175.9	69.1	121.6	88.49	1.28	6.0	99.986% Log 3 – 4	0.225
Bedside (Pall Medical)	22°C	50	182.2	171.9	68.8	118.3	88.21	1.29	6.4	99.986% Log 3 – 4	0.221
Bedside Immugard III (Terumo Penpol)	4°C	50	188.3	177.7	68.5	121.7	91.78	1.33	6.6	99.987% Log 3 – 4	0.236
Bedside (Pall Medical)	4°C	50	187.2	176.6	67.6	119.4	93.39	1.59	6.2	99.983% Log 3 – 4	0.280

small (1-3.2/ µl) for the mononuclear cell and granulocyte percentage to be significantly assessed.

The mean post-filtration red cell recovery ranged from 88.49-93.49% with all 400 bags showing more than 85% red cell recovery. The mean post-filtration residual leucocyte count in the blood bags ranged from 0.205 x 10^6-0.338 x 10^6/ bag with all the bags showing more than log 3 leucocyte reduction.

Discussion

In this study we evaluated the leucoreduction carried out by different, commercially available leucocyte filters at different temperatures and storage times. The red cell recovery and leucocyte reduction in all 400 bags was above the minimum international standards of 80% red cell recovery and < 5 x 10^6 leucocytes/ bag.

The performance of both the prestorage filters that were assessed in this study was almost at par with each other, both at 4°C and at room temperature. The Terumo Immugard prestorage filter, a polyurethane filter, showed a mean red cell recovery of 92.97% at room temperature and 92.95% at 4°C. In comparison, the Pall BPF-4 prestorage filter, a polyester filter, showed a marginally higher mean red cell recovery of 93.49% at room temperature. There was a drop in mean red cell recovery to 91.80% when the filtration was carried out at 4°C. On comparing the leucocyte reduction of the two prestorage filters at different temperatures, it was seen that the Terumo Immugard prestorage polyurethane filter showed a log 4 leucoreduction with 99.990% mean leucocyte reduction at both room temperature and at 4°C while the Pall BPF-4 polyester prestorage filter showed a slightly lower mean leucocyte reduction of 99.985% at 4°C which went further down to 99.982% at room temperature. However, all the filters achieved greater than log 3 leucocyte reduction in all bags and the residual leucocytes were much lower than the minimum international standards.

On assessing the performance of the two bedside filters, it was seen that both the filters showed a better performance at 4°C as compared to room temperature. The Terumo Immugard bedside filter, showed a mean red cell recovery of 91.78% at 4°C which dropped to 88.49% when the filtration was done at room temperature. In comparison, the Pall BPF-4 bedside filter, showed a marginally higher mean red cell recovery of 93.39% at 4°C which dropped to an even lower figure of 88.21% when the filtration was carried out at room temperature. On comparing the leucocyte reduction of the two bedside filters at different temperatures it was seen that the mean leucocyte reduction of both filters was almost identical varying from 99.983 – 99.986% irrespective of the temperature. Again, all the filters achieved greater than log 3 leucocyte reduction in all bags and the residual leucocytes were much lower than the minimum international standards.

In this study we found that the red cell recovery with the prestorage filters was significantly better than that achieved with the bedside filters. Further, it was seen that the red cell recovery with the bedside filters decreased even more when the bedside filters were used at room temperature. Considering that the bedside filters would normally be used on products at room temperature since they are used at the time of the transfusion, the decreased red cell recovery with the bedside filters at room temperature is very significant. Similar results were reported by Sawant et al [12] who also found that prestorage leucodepletion filters had better outcome measures as compared to bedside filters. Out of the four filters assessed, the best leucocyte reduction was achieved with the Terumo Immugard prestorage filter which was the only filter to show a log 4 leucocyte reduction.

There is a consensus in majority of previous studies that the quality of the cellular blood components improves when leucocytes are removed prior to storage and the same result was found in this study as well. Based on the benefits of leucodepletion, there are generally accepted specific indications for the same. However, there is still no consensus of opinion on whether leucocyte reduction should be universal and if it should, then to what level – log 1 or log 3 [13].

In certain countries such as Canada, Germany, New Zealand, United Kingdom, Ireland, Portugal and France universal prestorage leucocyte depletion with residual leucocytes < 1 x 10^6 per bag (> log 3) in at least 90% of the tested units is mandatory. In other countries leucocyte depletion is restricted to the well known indications mainly because of the cost factor. In most countries leucocyte depletion is performed prestorage, the main justification being that it allows for much better quality control of the blood component. The established indications for using leucocyte depleted red cells are for patients of thalassaemia major, aplastic anemia, sickle cell anemia, leukemia requiring multiple blood transfusions, patients for organ transplantation, patients on dialysis and fetal/ neonatal transfusions.

In order to overcome the cost factor, some countries are advocating universal log 1 (90% leucocyte removal) leucocyte reduction along with selective log 3 (99.9% leucocyte removal) leucocyte reduction for patients who fall within the established indications for using leucocyte depleted red cells. Log 1 or 90% WBC reduction is intended to prevent NHFTR whereas log 3 or 99.9% WBC reduction is intended to prevent HLA alloimmunization or CMV infection. The log 1 reduction is achieved by removing the leucocytes at the time of

preparation of the components using an automatic component extractor which separates the buffy coat from the red cells. Since this step is done at the prestorage stage before the leucocytes can release their cytokines, it results in the elimination of NHFTR, one of the most commonly seen transfusion reactions. The log 3 leucocyte reduction is achieved by using leucocyte depletion filters.

Based on the cost factor and the results of this study where we evaluated two different types of prestorage and bedside filters under different temperatures and storage conditions, we recommend that selective log 3 or 99.9% leucocyte reduction using leucocyte depletion filters should be implemented for patients who fall within the established indications for using leucocyte depleted red cells. Based on the findings of this study, we further recommend that prestorage filtration is definitely preferable over bedside filtration since it results in a higher post-filtration red cell yield and allows for much better quality control of the blood component. Of the two prestorage filters assessed in this study, we recommend that the use of polyurethane prestorage filter is preferable over the polyester prestorage filter since the leucocyte reduction achieved with the polyurethane filter was higher than that achieved with the polyester filter.

Conflicts of Interest

This study has been funded by research grants from the O/o DGAFMS.

Intellectual Contribution of Authors

Study Concept : Lt Col A Sen, Brig A Khetarpal, SM
Drafting & Manuscript Revision : Lt Col A Sen,
Lt Col S Jetley (Retd)
Statistical Analysis : Lt Col A Sen
Study Supervision : Brig A Khetarpal, SM, Lt Col S Jetley (Retd)

References

1. Wenz B. Clinical and laboratory precautions that reduce the adverse reactions, alloimmunization, infectivity, and possibly immuno-modulation associated with homologous transfusion. Transfus Med Rev 1990; 4: 3-7.
2. van Marwij KM, Proojen HC, Moes M, Bosma-Stants I, Akkerman JW. Use of leucocyte- depleted platelet concentrates for the prevention of refractoriness and primary HLA alloimmunisation: a prospective randomized trial. Blood 1991; 77: 201-5.
3. Moroff G, Luban N. Prevention of transfusion-associated graft-versus host disease. Transfusion 1992; 32:102-3.
4. Bowden RA, Slicmer SJ, Sayers M. A comparison of filtered leukocyte-reduced and cytomegalovirus (CMV) seronegative blood products for the prevention of transfusion-associated CMV infection after marrow transplant. Blood 1996; 86:591-603.
5. Aye MT, Polmer DS, Giulivi A. Effect of filtration of platelet concentrates on the accumulation of cytokines and platelet release factors during storage. Transfusion 1995; 35: 117–24.
6. Food and Drug Administration. Memorandum: Recommendations and license requirements for leukocyte-reduced blood products. Rockville, MD: CBER Office of Communication, Training and Manufacturers Assistance, 1996.
7. Food and Drug Administration. Draft guidance: For the submission of chemistry, manufacturing and controls and establishment description information for human blood and blood components intended for transfusion or for further manufacture and for the completion of the FDA form 356h "Application to market a new drug, biologic or antibiotic drug for human use;" part I section III.B-leukocyte reduction. (July 10, 1998) Rockville, MD: CBER Office of Communication, Training, and Manufacturers Assistance, 1998.
8. Menitove JE, editors. Standards for blood banks and transfusion services. 21st ed. Bethesda: American Association of Blood Banks, 2002.
9. Heaton WA, Holme S, Smith K. Effects of 3-5 log prestorage depletion on red cell storage and metabolism. Br J Hematol 1994; 87: 363-8.
10. Bontadini A, Fruet P, Conte R. A new tool in white blood cell reduction for packed RBCs: 5 log depletion. Tranfus Med 1997; 7: 29-32.
11. Beaujean F, Segier J M, le Forestier C. Leucocyte depletion of red cell concentrate by filtration: Influence of blood product temperature. Vox sang 1992;62: 242-3.
12. Sawant B, Jathar S, Kadam P. A comparison of leucodepletion filters (laboratory and bedside use) for filtration of red blood cells. Vox sang 2006; 91: 220.
13. Dzik S, Aubuchon J, Jeffries L. Leucocyte reduction of blood components: public policy and new technology. Transfus Med Rev 2000;14:34-52.