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A COMPARISON OF DIFFERENT FILTERS FOR WHITE CELL REDUCTION*

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Removal of white blood cells (WBCs) from blood components before transfusion by filters with at least 3 log_{10} depletion may prevent or delay leukocyte-associated transfusion reactions such as HLA alloimmunization, non-hemolytic febrile reactions, transmitted infections (e.g., CMV, HTLV-1), and immunomodulation. The aim of this study was to compare the leukocyte removal efficiency (LRE) of six commercial bedside filters that are said to achieve 3 log_{10} (Bio R-01, Leucostop 4LT-1, Pall RC 50) and 4 log_{10} (Bio R-01 Plus, Pall RC 400, Pall RC XL-1) WBC depletion. A total of 430 units of whole blood ranging from 32 to 92 for each filter type were analyzed by an automated counter before and after filtration. Postfiltration blood samples were also evaluated for WBCs by Nageotte chamber. All the filters demonstrated leukocyte removal about 1 log_{10} less than the manufacturer's claim. The fourth generation filters showed a better performance than the third generation filters. Of them, Pall RC XL-1 showed the best efficacy with 99.93 percent leukocyte removal and a median residual WBCs of 1.6×10^6 per unit. These results indicate that the fourth generation filters, which are designed for the filtration of packed red cells, in particular Pall RC XL-1, are also able to reduce WBCs from whole blood below the critical antigenic leukocyte load (5×10^6), and can be efficiently used for polytransfused patients to prevent alloimmunization. *Key words: leukocyte removal efficiency, bedside filters, thalassemia, allimmunization, blood transfusion.*

The use of WBC-reduced red blood cells (RBCs) has been shown to prevent or delay a wide range of adverse transfusion effects, including recurrent non-hemolytic febrile reactions, refractoriness to platelet transfusion, alloimmunization to leukocyte antigens, transmission of cell-associated viruses (e.g. cytomegalovirus, human T-lymphotropic virus-1), and possibly immunosuppression[1-6].

In the 1980's, different filtration techniques were used in order to reduce WBCs from the blood components, such as the spin-cool-filter technique and microaggregate filtration[7-9]. Recently, different polyester filters suitable for use both in the laboratory and at the bedside became available[1, 10]. These filters

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do not require prefiltration procedures and are claimed to be more efficient[1,2]. Third generation filters are said to deplete WBCs by 3 $\log_{10}$ with a removal efficiency of 99.9 percent and fourth generation filters by 4 $\log_{10}$ with a removal efficiency of 99.99 percent per blood unit[2].

American Association Blood Banks (AABB) proposed that WBC-reduced RBCs must contain fewer than 5×10^6 WBCs per unit to prevent alloimmunization[11]. This degree of WBC reduction can be achieved by filtration of RBCs and platelet concentrates through the third and fourth generation bedside or laboratory-type WBC filters[1,2].

In our Thalassemia Center, bedside filtration has been the standard method of transfusion since 1991. However, we doubted the efficacy of bedside filters in decreasing WBCs when whole blood was used because we could not find any study on this subject in the literature. The aim of this study, therefore, was to compare six different commercially available bedside white cell-reduction filters for leukocyte removal efficiency from the whole blood.

Material and Methods

This study was carried out with six different white cell reduction polyester fiber filters of third and fourth generation at the patients' bedsides in the Thalassemia Center of Social Security Tepecik Teaching Hospital. A total of 430 whole blood units filtered through six different white cell filters were analyzed before and after filtration by an automated counter; postfiltration leukocyte count was obtained by Negeottę chamber. The filters were compared in terms of leukocyte removal efficiency, log reduction and residual leukocyte count per unit of blood.

Filters

The third and fourth generation filters used and the number of the filters from each filter type are given below:

Third generation filters (n = 196).

1. Bio R-01 (Biofil, Medolla, Italy), n = 92
2. Leucostop 4LT-1 (Miramed, Italy), n = 72
3. Pall RC 50 (Pall Corp. Glen Cove, NY), n = 32

Fourth generation filters (n = 234)

1. Bio R-01 Plus (Biofil, Medolla, Italy), n = 90
2. Pall RC 400 (Pall Corp., Glen Cove, NY), n = 54
3. Pall RC XL-1 (Pall Corp., Glen Cove NY), n = 90

Blood Units

Whole blood units that were provided from our hospital's blood bank and drawn from healthy donors were used for filtration. Each unit of blood was 400 ± 25 ml in 63 ml CPDA. The age of blood units were one to 28 days and the storing temperature was 4 °C.

Filtration Procedure

Bedside filtration was carried out by two experienced nurses of the Thalassemia Center in the transfusion room. The blood units were kept at room temperature until the transfusion was started (30 to 45 minutes after the unit's removal from the refrigerator).

The filtration procedure followed the manufacturer's instructions. After the filters were connected to the plastic blood bags, all, except Bio R-01 Plus, were primed with blood by a soft manual squeezing of the bag, whereas Bio R-01 Plus was primed spontaneously by its own "auto-venting system". The filtration procedure was then left to spontaneous gravity and the transfusion was started. The transfusion of each unit was accomplished in an interval ranging from 50 to 90 minutes. A new filter was used for each unit of blood.

Evaluation

Both prefiltration and postfiltration blood samples were analyzed for complete blood count by an automated counter (T 660, Coulter Electronics Inc., Hialeah, Florida, USA). The postfiltration specimens taken from the distal end of the connecting tube of the filter, with very low concentrations of WBCs, were also counted manually using a hemocytometer (Negeotte chamber) with a light microscope, according to the method described by Masse et al.[12] One hundred µl of the sample was mixed with 900 µl of Turk's solution (1 ml 1% Gention Violet plus 3 ml glacial acetic acid) in a test tube to prepare 1:10 dilution. The diluted sample was thoroughly mixed and left to rest for 10 minutes at room temperature, to allow the WBCs to stain. We then placed 100 µl of this sample on the Nageotte chamber (Saaringia, West Germany) and allowed it to rest again for 10 minutes before counting by a light microscope (40 x objective). A 50 µl grid (an area of 40 rectangles) was than counted. Each sample was counted by two different pediatricians and the average of the two was taken into consideration. The filters were tested at random and the doctors were not informed about the filter used.

For every filter used in the study, the number of leukocytes per µl, lukocyte removal efficiency (LRE), residual lukocyte count per unit (RLc) and log reduction capability were calculated as follows:

$$\text{Leukocyte/µl (Negeotte)} = \frac{\text{number of counted cells}}{\text{volume counted (µl)}}$$

$$\text{LRE (\%)} = \left(1 - \frac{\text{Postfiltration (effluent) leukocytes/µl}}{\text{Prefiltration (influent) leukocytes/µl}}\right) \times 100$$

$$RLc = Leukocyte/\mu l \times 1000 \times unit\ volume\ (ml)$$

$$Log\ reduction = \log_{10} \frac{Prefiltration\ (influent)\ leukocytes/\mu l}{Postfiltration\ (effluent)\ leukocytes/\mu l}$$

Statistical Analysis

To compare the six different filters, one-factor analysis of variance (ANOVA) was used for measured continuous values and chi-square test for nominal data. When the F statistics were found significant ($p < 0.05$), repeated t tests were used. Correlation analysis was performed to investigate any relationship between the parameters.

Results

Prefiltration and postfiltration WBC counts, hemoglobin levels, filtration time and age of blood in the filter groups are shown in Table I. There were no significant differences among the six groups in terms of filtration time, age of blood and prefiltration and postfiltration hemoglobin values by ANOVA. A comparison of the leukocyte depletion performances of the filters is shown Table II. Median leukocyte removal efficiency is highest in the filter group of Pall RC XL-1 (99.3%) and lowest in the group of Bio R-01 (97.45%).

Table I: Prefiltration and Postfiltration Data on the Six Different Filters*

	Bio R-01 (n = 92)	Leucostop 4LT-1 (n = 72)	Pall RC 50 (n = 32)	Bio R-01 Plus (n = 90)	Pall RC 400 (n = 54)	Pall RC XL-1 (n = 90)
Hemoglobin (g/dl), mean ± SD						
Prefiltration	12.3 ± 1.4	12.5 ± 1.5	12.5 ± 1.5	13.0 ± 2.0	13.1 ± 2.2	11.8 ± 1.6
Postfiltration	12.1 ± 1.4	12.3 ± 1.4	12.5 ± 1.2	12.9 ± 2.1	13.0 ± 2.1	12.0 ± 1.9
WBc (coulter), x $10^3/\mu l$						
Prefiltration	5.8 (3.2 - 11.0)	6.6 (2.1-9.9)	5.5 (3.1 - 11.0)	6.5 (2.6 - 12.2)	7.3 (3.6 - 19)	6.0 (2.7-9.9)
WBCs/µl (Negeotte)						
Postfiltration	148 (2 - 980)	46 (1 - 580)	41 (8 - 492)	10 (1 - 280)	10 (1 - 50)	4 (1 - 30)
Filtration time (mins), mean ± SD	72 ± 10	71 ± 9	70 ± 8	72 ± 10	70 ± 9	68 ± 10
Age of blood (days), mean ± SD	10.7 ± 7.4	9.7 ± 7.9	10.8 ± 7.8	10.1 ± 7.7	10.0 ± 8.0	9.6 ± 8.1

* Values are presented as median (range) unless otherwise noted.

Table II: A Comparison of the Leukocyte Depletion Performances of the Filters

Performance	Bio R-01 (n = 92)	Leucostop 4LT-1 (n = 72)	Pall RC 50 (n = 32)	Bio R-01 Plus (n = 90)	Pall RC 400 (n = 54)	Pall RC XL-1 (n = 90)
Leukocyte removal efficiency (%)*	97.45 (89.30 - 99.97)	99.30 (90.80 - 99.97)	99.25 (90.90 - 99.97)	99.85 (97.40 - 99.99)	99.86 (99.40 - 99.99)	99.93 (99.40 - 99.99)
WBC reduction (log_{10})	1.59	2.16	2.13	2.82	2.86	3.18
Residual leukocyte count/unit*	5.9×10^7	1.8×10^7	1.6×10^7	4×10^6	4×10^6	1.6×10^6
Residual WBC count $< 5 \times 10^6$ per unit, n (%)**	8 (9)	20 (28)	2 (6)	52 (58)	28 (52)	82 (91)

* Median (range).
** $P < .0001$.

There were considerably great statistical differences ($P < .0001$) among the six filter groups in decreasing WBC count below the critical antigenic load (5×10^6), If Pall RC XL-1 were excluded from the analysis, the other two fourth generation filters were comparable in this regard (58% and 52% for Bio R-01 Plus and Pall RC 400, respectively).

Third generation filters generally showed a median of approximately 2 log_{10} WBC removal and the fourth generation filters about 3 log_{10} WBC depletion. The most efficient was Pall RC XL-1 with a 3.18 log_{10} WBC removal. There was no statistically significant correlation between the filtration time and postfiltration WBC number ($P > .05$).

Discussion

Leukocytes are largely responsible for adverse transfusion reactions, such as HLA alloimmunization and, in turn, development of nonhemolytic febrile reactions and refractoriness to platelet transfusions. Reduction of WBCs in blood components by filters before transfusion with about 3 log_{10} depletion can prevent these adverse reactions. For a higher degree of purity, new generation filters with a 4 log_{10} WBC depletion are now available[1, 2, 13, 14].

In several comparative studies, different results have been reported on the efficacy of various filters for WBC removal[1, 2, 15-17]. In the study of Sirchia et al[10], they tested only one filter (Pall RC 100) and found a median of 20×10^6 residual WBC count per 2 units of RBC and better results per one unit of RBC. Bontadini et al.[2] compared six different white cell reduction filters and found that two of the third generation filters demonstrated an efficacy of about 3 log_{10} WBC reduction. But they performed only five experiments for each filter type. Koerner

et al.[15] also compared five different filters for leukocyte removal from red cell concentrates and they determined median WBC removal efficiencies between 98.1 and 99.9 percent similar to our results.

The Thalassemia Center in our hospital is very busy, with about 50 patients attending each week for blood transfusion. For this reason, our blood bank sometimes cannot meet the requirements of WBC-filtered packed RBCs for thalassemic children. Since 1991, therefore, we have also been using bedside WBC-reduction filters in the filtration of whole blood as well as in that of packed RBCs. However, we could not find any study in the literature that investigated the effectiveness of white cell filters for whole blood.

Our results showed that all the filters generally reduced WBCs about 1 $\log_{10}$ less than the manufacturer's claim. It may have occurred because samples postfiltration for WBC counts were taken at the end of the filtration process. However, as can be expected, the fourth generation filters were capable of greater leukocyte removal from whole blood and, subsequently had lower residual leukocyte counts than the third generation filters ($P < .001$). But, there was no uniformity for WBC reduction even among the same generation filters. For example, of the third generation filters, Leucostop 4LT-1 and Pall RC 50 demonstrated median WBC reductions slightly higher than 2 $\log_{10}$ (2.16 $\log_{10}$ and 2.13 $\log_{10}$, respectively), while Bio R-01 decreased WBCs less than 2 $\log_{10}$ (1.59 $\log_{10}$). Likewise, among the fourth generation filters, only Pall RC XL-1 removed WBCs from whole blood more than 3 $\log_{10}$ (a median of 3.18 $\log_{10}$). This filter was also succesful in 91 percent of the filtrations in decreasing residual WBCs less than 5×10^6, the limit for residual leukocytes per unit, proposed by the American Association of Blood Banks to prevent alloimmunization[11]. Wenz[18] has termed the concentration of leukocytes necessary to cause clinical symptoms of transfusion reactions as critical antigenic leukocyte load (CALL), and the concentration of WBCs necessary to cause sensitization as critical immunogenic leukocyte load (CILL). This requires the administration of approximately 10^6 to 10^7 WBCs. Thus, even with 10^7 WBCs, polytransfused patients are largely saved from adverse transfusion reactions. In our study, a total of six febrile transfusion reactions were observed only in the filter groups of Bio R-01 (4 cases) and Leucostop 4LT-1 (a cases). These filter groups also had the highest residual WBC counts. However, there was no statistically significant difference ($p > .05$) among the groups regarding the number of febrile reactions.

From the data presented here, we conclude that fourth generation filters, preferably Pall RC XL-1, can be used to reduce the level of residual WBCs in whole blood (when packed RBC is not available) below the threshold that is thought to result in alloimmunization.

REFERENCES

1. Sprogoe-Jacobsen U, Saetre AM, Georgsen G. Preparation of white-cell reduced red cells by filtration: comparison of a bedside filter and two blood bank filter systems. Transfusion 1995: 35: 421-426.
2. Bontadini A, Fruet F, Tazzari PL, Conte R. Comparative analysis of six different white cell-reduction filters for packed red cells. Transfusion 1994; 34: 531-535.
3. Sniecinsky I, O'Donnel MR, Nowicki B, Hill LR. Prevention of refractoriness and HLA-alloimmunization using filtered blood products. Blood 1988; 71: 1402-1407.
4. Blumberg N, Triulzi DJ, Heal JM. Transfusion-induced immunomodulation and its clinical consequences. Transfus Med Rev 1990: 4 (Suppl): 24-25.
5. Oksanen K, Elonen E. Impact of leukocyte-depleted blood components on the hematological recovery and prognosis of patients with acute myeloid leukemia. Finnish Leukemia Group. Br J Haematol 1993; 84: 639-647.
6. Rawal BD, Busch MP, Endow R, et al. Reduction of human immuno-deficiency virus-infected cells from donor blood by leukocyte filtration. Transfusion 1989; 29: 460-462.
7. Parravicini A, Rebulla P, Apuzzo J, et al. The preparation of leukocyte-poor red cells for transfusion by a simple cost effective technique. Transfusion 1984; 24: 508-509.
8. Wenz B, Gurtlinger KF, O'Toole AM, Dugan EP. Preparation of granulocyte-poor red blood cells by microaggregate filtration. a simplified method to minimize febrile transfusion reactions. Vox Sang 1980; 39: 282-287.
9. Meryman HT, Hornblower M. The preparation of red cells depleted of leukocytes. Review and evaluation. Transfusion 1986; 26: 101-106.
10. Sirchia G, Wenz B, Rubella P, et al. Removal of white cells from red cells by transfusion through a new filter. Transfusion 1990; 30: 30-33.
11. Windmann FK, ed. Standards for Blood Banks and Transfusion Services (14th ed). Arlington: American Association of Blood Banks; 1991.
12. Masse M, Naegelen C, Pellegrini N, et al. Validation of a simple method to count vey low white cell concentrations in filtered red cells or platelets. Transfusion 1992; 32: 565-571.
13. Rebulla P, Bertolini F, Parravicini A, Sirchia G. Leukocyte-poor blood components: a purer and safer transfusion product for recipients? Transfus Med Rev 1990; 4 (Suppl): 19-23.
14. Lane TA, Anderson KC, Goodnough LT, et al. Leukocyte reduction in blood component therapy. Ann Intern Med 1992; 117: 151-162.
15. Koerner K, Sahlmen P, Zimmermann B, Kubonek B. Preparation of leukocyte-poor red cell concentrates: comparison of five different filters. Vox Sang 1991; 60: 61-62.
16. Pietersz RN, Steneker I, Reesink HW, et al. Comparison of five different filters for the removal of leukocytes from red cell concentrates. Vox Sang 1992; 62: 76-81.
17. Sirchia G, Rubella P, Parravicini A, et al. Quality control of red cell filtration at the patient's bedside. Transfusion 1994; 34: 26-30.
18. Wenz B. Clinical and laboratory precautions that reduce the adverse reactions, alloimmunization, infectivity, and possibly immunomodulation associated with homolog transfusions. Transfus Med Rev 1990; 4 (Suppl): 3-7.