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Evaluation of two types of leukocyte removal filters on transfusion dependent thalassaemics

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Abstract

The advent of leukocyte filters has enabled effective removal of leukocytes from certain blood products thus avoiding many adverse effects of blood transfusion. Many different materials have been incorporated into these filters to achieve >95% leukocyte removal. In this study we evaluated the efficacy of leukocyte removal of two different filters, using actual bedside transfusion settings involving patients with transfusion dependent thalassaemia. Fifty-one transfusion events were randomised to use either a polyurethane filter or a non-woven polyester filter. We found that the two filters achieved 98.4% and 96.2% leukocyte removal respectively ($p = 0.022$). We also found no significant correlation between pre-filtration white blood cell count and the volume transfused with the efficacy of leukodepletion. No untoward events or transfusion reactions were observed during the study.

Key words: Leukocyte filters, leukodepletion, transfusion, thalassaemia

INTRODUCTION

The use of leukocyte filters is becoming increasingly popular in transfusion medicine and in some situations highly warranted to avoid the complications resulting from the presence of contaminating leukocytes in blood components.[1,2] These leukocytes are known to cause various adverse effects including non-haemolytic febrile transfusion reactions, urticaria, anaphylactic shock, refractoriness to platelet transfusions due to alloimmunisation and transfusion-associated graft versus host disease.[2,3,4] The leukocytes are also host cells for the replication of certain viruses such as cytomegalovirus, Epstein-Barr virus and human-T-cell-lymphotropic virus type I. It has been shown that the adverse reactions as well as the transmission of the aforementioned viruses may be effectively prevented by the removal of leukocytes from blood components with the use of leukocyte filters.[5] Until recently, the material adopted into these filters was non-woven polyester which selectively adsorb leukocytes. A new leukocyte filter made of porous polyurethane (Terumo Co.) which is non-adsorbent and removes leukocytes selectively by simple filtration has recently entered the market. A few studies have shown that this removal by simple filtration is more efficient than that by conventional filters.[6,7]

We tested two different types of leukocyte filters on transfusion dependent patients with thalassaemia major at the haematology day care centre, Hospital Universiti Kebangsaan Malaysia. The major objective was to assess the efficacy of leukocyte removal from packed red blood cells by these two filters at the bedside and to correlate the influence of pre-filtration white blood cell content and the volume transfused on the efficacy of leukocyte depletion by both filters.

MATERIALS AND METHODS

Patients

Patients with transfusion dependent beta-thalassaemia major or HbE-beta thalassaemia who were admitted to the day care centre for blood transfusion were included in the study. The period of study was from October 1997 to April 1998. Some of the patients were already regular users of leukocyte filters. Informed consent was taken from all patients. Those patients who insisted on using filters other than those tested in this study were excluded.

Blood products

All patients received packed red blood cells supplied by the National Blood Transfusion

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Centre, Hospital Kuala Lumpur. The packed cells were prepared by centrifugation from 450ml whole blood collection without buffy coat removal followed by resuspension in SAGM (sodium chloride, adenine, dextrose and mannitol). The volume of blood transfused was based on the requirement of each patient (15ml/kg). For patients who required more than one unit of packed red cells, analysis of leukodepletion was performed only in the first unit transfused.

Filters

Two filters were tested: (1) Imugard III-PL which is a leukocyte removal filter using porous polyurethane (Terumo Co.) and (2) a filter which uses the non-woven polyester. The filters were assigned by simple random sampling. Both filters had specifications for use on up to 2 units of packed cells and both filters were used without priming with normal saline. The haematologist (N.R.M) responsible for the manual counting of the white blood cells (WBCs) was blinded to the type of filter used for each transfusion event.

Transfusion parameters

Blood was pre-warmed using a blood warmer. The blood pack was positioned at a fixed height of 150cm from the arm with the venous cannula during transfusion. The flow rates were kept constant at 2-3 mL/minute in all cases.

Monitoring

The routine monitoring for patients undergoing blood transfusion was performed. The presence of transfusion reactions were looked for and recorded. In the presence of such reactions, the transfusion was terminated and the standard procedures for transfusion reactions were performed.

Blood sampling

Care was taken to mix the contents of the blood pack evenly before a pre-filtration blood sample for counting was taken. Following this, post-filtration blood samples were then taken from the distal end of the transfusion tubing kit, at 15, 30 and 45 minutes after commencement of transfusion. These 3 samples were then pooled together and evenly mixed before taking an aliquot for leukocyte counting.

Leukocyre counting

The white blood cell (WBC) count before filtration was determined by an automated blood cell counter, the cytometer Coulter Counter SKTS (Coulter Co.). The WBC counts after filtration were determined both by the automated and manual methods. The diluting fluid used for manual counting of white cells was Turk's solution (2% acetic acid v/v with a few drops of gentian violet). The manual leukocyte counting was performed by light microscopy using a Neubauer chamber following a 1:20 dilution with the diluting solution. To allow lysis of the red cells, the diluted sample was left undisturbed for at least 2 minutes, then mixed, whereafter both grids of the Neubauer chamber were filled. The cells were allowed to settle for at least I minute after which leukocytes were counted in duplicate over the nine lmm^2 areas. The counting was performed by one haematologist (N.R.M.) who was blinded to the type of filter used.

Calculation:
WBC count (per litre)

$$= \frac{\text{Number of cells counted}}{\text{Volume counted } (\mu L)} \times \text{Dilution} \times 10^6$$

Efficacy of leukocyte removal

The efficacy of leukocyte removal was assessed by calculating the percentage of leukocyte removed using the following formula:-

% leukodepletion

$$= \frac{(\text{Pre-filtration WBC counts} - \text{Post-filtration WBC counts})}{\text{Pre-filtration WBC counts}} \times 100$$

Statistical analysis

Statistical analysis was performed using the SPSS software version 9.0. The mean and standard deviation (SD) were used to report the results in a simple and concise manner. The t-test was used to test for equality of means and significance. Correlation coefficient was calculated to ascertain the correlation between pre-transfusion WBC counts and volume of blood transfused with the percentage of leukodepletion. A p value of <0.05 was taken as of statistical significance.

RESULTS

The age of packed cells used ranged between 5-30 days (mean 14.39 days, SD 5.83 days). The volume of packed cells filtered through each filter ranged from 300-430mls (mean 368.7mls, SD 32.4mls).

The total white blood cell count of the packed cell units used ranged from 1.5-13.8 x 10^9/L, with a mean of 5.55 x 10^9/L (SD=2.88). Out of a total of fifty-one transfusion events which were randomised, 24 involved the use of the Imugard III filters whilst the other 27 cases used the non-woven polyester filters. The pre- and post-filtration white blood cell counts using both filters are shown in Table 1A and Table 1B. Overall, the mean percentage of leukodepletion using both filters was 97.3%. The Imugard filters achieved a marginally higher percentage of leukodepletion (98.4% versus 96.2%) as compared to the non-woven polyester filter ($p=0.002$ for automated counting of the WBC, $p=0.022$ for manual counting of the WBC) (Table 2). The volume of blood transfused and the initial WBC counts did not influence the efficiency of the leukodepletion (Correlation coefficient = 0.149 and 0.414 respectively, $p=0.26$). None of the patients using either filter developed transfusion reactions.

DISCUSSION

The results of this study showed that both the filters investigated achieved mean leukocyte depletion rates of 96.2-98.4%. With an average pre-filtration content of 5.55 x 10^9 leukocytes per liter, this represented a 1-2 log leukocyte reduction. Previous studies showed a 2-3 log leukocyte reduction although the pre-filtration leukocyte content in those studies were lower.' Both filters removed leukocytes effectively although the Imugard filters showed a significantly higher percentage of leukodepletion as compared to the non-woven polyester filter ($p=0.022$).

To obtain a more accurate assessment of the leukocyte content, we compared the manual counting with the automated method for the

Table 1A: Pre- and post-filtration total white blood cell count (TWBC) [automated counting] using polyurethane filters

Sample number	Pre-filtration TWBC (x 10^9/L)	Post-filtration TWBC (x 10^9/L)	Percentage depletion
1	8.6	0.1	98.84
2	2.8	0.1	94.23
3	4.2	0.0	100.00
4	12.2	0.1	99.18
5	3.8	0.1	97.37
6	2.7	0.1	96.30
7	13.0	0.1	99.23
8	5.4	0.1	98.15
9	6.2	0.1	98.39
10	4.7	0.1	97.87
11	7.1	0.1	98.59
12	3.4	0.0	100.00
13	3.5	0.1	97.14
14	4.1	0.0	100.00
15	3.8	0.0	100.00
16	4.4	0.1	97.73
17	6.1	0.1	98.36
18	5.8	0.1	98.28
19	2.5	0.0	100.00
20	9.4	0.1	98.94
21	3.9	0.0	100.00
22	1.5	0.1	93.33
23	1.9	0.1	94.74
24	7.9	0.1	98.73

Table 1B: Pre- and post-filtration total white blood cell count (TWBC) [automated counting] using non-woven polyester filters

Sample number	Pre-filtration TWBC (x 10^9/L)	Post-filtration TWBC (x 10^9/L)	Percentage depletion
1	5.2	0.2	96.15
2	5.9	0.1	98.31
3	4.0	0.7	82.50
4	9.6	0.7	92.71
5	10.8	0.2	98.15
6	13.8	0.1	99.28
7	7.6	0.3	96.05
8	4.2	0.2	95.24
9	6.5	0.2	96.92
10	6.5	0.1	98.46
11	4.5	0.1	97.78
12	2.3	0.2	91.30
13	2.6	0.2	92.31
14	7.0	0.0	100.00
15	6.7	1.2	82.09
16	3.2	0.1	96.88
17	2.1	0.1	95.24
18	6.2	0.2	96.77
19	3.0	0.1	96.67
20	2.8	0.1	96.43
21	2.4	0.4	83.33
22	5.8	0.2	96.55
23	8.2	0.3	96.34
24	5.0	0.1	98.00
25	7.7	0.2	97.40
26	4.2	0.5	88.10
27	6.4	0.2	96.88

TABLE 2: Mean percentages of leukodepletion achieved using 2 different leukocyte filters and two different leukocyte counting methods

Method of Leukocyte counting	Type of filter used	n	Mean	Standard Deviation
Manual	Porous polyurethane[a]	24	98.41	0.86
	Non-woven polyester	27	96.17	4.56
Automated	Porous polyurethane[b]	24	98.14	1.87
	Non-woven polyester	27	94.66	5.02

[a]p=0.022 vs non-woven polyester
[b]p=0.002 vs non-woven polyester

post-filtration counts. We found that the manual counting method is more useful for the assessment of low leukocyte numbers especially in those below 0.1 x 10^9/L. The lowest level for counting the TWBC using the Coulter counter is 0.1 x 10^9/L and anything below this level would just be recorded as zero. Previous studies have utilised similar approaches for the assessment of leukodepletion efficacy of leukocyte filters and in some of these studies the Burker haemocytometer was used for the manual method of counting.[7,8] The data obtained from these studies using the Burker haemocytometer was similar to our study.

The influence of the transfusion volume and the initial WBC counts on leukodepletion was also analysed. However we found no correlation between these two parameters and the efficacy of leukodepletion ($p>0.05$).

In conclusion, the current generation of leukocyte filters used have the capability to remove >95% of the leukocytes from packed red blood cells and hence are useful in avoiding transfusion related complications resulting from the presence of contaminating leukocytes. To our knowledge, this is the first local study on the evaluation of efficacy of leukocyte removal filters performed in actual bedside transfusion settings.

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