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Evaluation of blood filters for the exclusion of leukocytes from red cell transfusions

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Running head: Evaluation of leukocyte filters
Abstract

Filtration is a useful method for the exclusion of leukocytes from red cell transfusions, primarily for the prevention of febrile nonhemolytic reactions. We experimentally evaluated five commercially-available leukocyte filters with respect to red cell recovery and leukocyte exclusion in filtration of packed red cells. The results of published evaluations and clinical studies of these filters, including the individual characteristics of the filters, are presented as they relate to their clinical use. We have determined that among the available blood filters the Sepacell filter offers the best combination of leukocyte exclusion, red cell recovery and cost-effectiveness in a clinical setting.
Introduction

Leukocytes in donated blood are unwanted in transfusion therapy and frequently cause alloimmunization that is etiologic of febrile reactions\(^1\). Since leukocytes harbor latent and persistent viral infections, it may be desirable to exclude them from donated blood. Filtration of blood to exclude the transfusion of leukocytes in red cell therapy has recently attracted considerable interest and has been extensively reviewed\(^2,3\). As compared to other means of leukocyte removal, it offers numerous potential advantages including lower cost, lack of sophisticated equipment, and in some cases longer storage life or greater leukocyte removal. We report the experimental evaluation of five FDA-approved filters for the exclusion of leukocytes from red cell transfusions.
Materials and methods

Filters were provided by the respective manufacturers, and their characteristics are shown in Table 1. All units tested were packed red cell masses stored in Adsol (Fenwal Laboratories, Deerfield, IL) or CPDA-1 (citrate-phosphate-dextrose-adenine) preservative solutions. These units were collected from volunteer donors and intended for clinical transfusion, but were excluded on the basis of an elevated serum alanine aminotransferase (>40 IU/mL) or an antibody to the hepatitis B core antigen, or both. All units were maintained at 4 °C. until the start of filtration. The age of the units at testing, as defined by the days elapsed between collection and filtration, is shown in Table 2. Red cell and leukocyte counts were determined before and after filtration using samples drawn from the donor bag or the recipient transfer bag, and were run in duplicate on a Coulter S automated cell counter (Coulter Electronics, Hialeah, FL). For those filters which depend on the formation of microaggregates prior to filtration (Fenwal and Pall), a modification of the spin and cool method of Parravicini et al. was used. After obtaining samples for prefiltration counts, units were centrifuged at 5000g for 20 minutes in the upright position in a swinging-bucket rotor, using a Beckmann J-6M/E centrifuge (Beckmann Instruments, Palo Alto, CA), carefully transferred to a refrigerator so as not to disturb the buffy coat, and left undisturbed at 4 °C. for at least 4 hours. For those filters which require "priming" to remove air, pyrogens, and/or acids from the filtration column (Imugard and Erypur), at least 400 mL of 0.9% saline solution was filtered and discarded. All units were filtered and collected in a manner to simulate
actual clinical transfusion, i.e. suspended 50-150 cm above the collection bag and allowed to filter at a rate of 4 mL/min, except for those filters intended for blood bank use prior to actual transfusion (Imugard and Erypur). Initial instillation of saline into the donor bag, wetting of the filter, and use of transfusion sets were in accordance with the respective manufacturer's directions and standard transfusion practice. All but the Pall filters were flushed with saline after filtration of the unit until clear, to improve red cell recovery. When multiple units were successively filtered through the same filter, similar flushing until clear was used between units, except for the Pall filter. Hemolysis due to filtration was excluded by visual inspection of the plasma in at least one unit from all filters. Volumes of blood were calculated based on their weights and a specific gravity of 1.135.
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Results

A comparative summary of published results and our results is shown in Table 2. Leukocyte removal is shown both by the percentage of original leukocytes removed, and by the more relevant statistic of the absolute number remaining after filtration. Red cell recovery is defined simply by the percentage of the original number. "Second" units refers to those units filtered after one unit had already been passed through the same filter. In filtration of single units, there was no significant difference in red cell recovery between the various filters (Student's t, p>.05). In leukocyte exclusion, the Pall filter was significantly less effective than the Erypur and Sepacell filters (p<.01), based on the absolute number of leukocytes remaining. The Pall filter was not significantly different from the Imugard filter on the basis of the absolute number of leukocytes remaining, but was significantly different on the basis of the percentage excluded (p<.05). On the basis of absolute leukocytes remaining, there was no significant difference between the Sepacell and Erypur filters, or the Erypur and Imugard filters; there was a significant difference between the Sepacell and Imugard filters (p<.05). The Fenwal filter was not significantly different from the other filters in either red cell recovery or leukocyte exclusion, despite the apparently large difference in the sample means; this was probably due to the small number of units filtered with this filter. In comparing the mean performance of filters in single vs. second units, there was no significant difference in red cell recovery for either the Pall or Fenwal filters. Based on the absolute number of leukocytes removed, the Fenwal filter performed significantly worse on the second units (p<.05), whereas there was no significant difference for the Pall filter.
Discussion

The greatest interest in removing leukocytes from red cell transfusions has been in preventing febrile nonhemolytic reactions. The likelihood of such a reaction has been correlated with the absolute number of leukocytes transfused. However, there is considerable variation between patients (and perhaps donors) in the minimum number of leukocytes necessary to produce a reaction, ranging from $0.25 \times 10^9$ to $2.5 \times 10^9$. Furthermore, the number of leukocytes may be insufficient to produce a detectable reaction in a patient, but sufficient to cause or enhance alloimmunization. Therefore, it seems desirable, when practical, to reduce the number of transfused leukocytes to the lowest possible level. Further incentive for reducing leukocyte transfusion exists in the possibility that leukocytes are a major vector in the transmission of viruses: CMV, HBV, non-A non-B hepatitis, EBV, and HIV. The potential exists for removal of leukocytes, and thus any viral agents contained therein, by filtration incorporated into the donor collection system; this would have certain practical advantages, and prevent the problem of viral release due to any leukocyte lysis during storage. Removal of platelets may also be desirable, as these are usually nonfunctional in packed red cell products but may cause alloimmunization. The filters listed here are reported to achieve varying degrees of platelet removal (Table 2).

The spin-cool-filter method to remove leukocytes contained as microaggregates can produce what might be termed a leukocyte-poor red cell unit, with a total number of $0.25$ to $0.5 \times 10^9$ leukocytes. Studies using this method in chronically transfused patients have shown a dramatic reduction in the incidence of febrile nonhemolytic reactions; however, the effect on alloimmunization is uncertain. The adsorption column filters (Erypur
and Imugard) can produce what has been termed a leukocyte-free red cell unit, with a total number of less than $0.1 \times 10^9$ leukocytes. In several of the units we filtered with these, the concentration of leukocytes after filtration was at or below the limit of reliable detection with the automated cell counter (100/microliter), so that the total number of leukocytes may even have been less than the number calculated. Our results show that the Sepacell filter is also capable of delivering a relatively leukocyte-free product; in fact, it had the greatest absolute leukocyte exclusion of the filters tested. Studies using adsorption column filters have shown virtual elimination of febrile non-hemolytic reactions, as well as prevention and even regression of alloimmunization, as evidenced by the disappearance of anti-lymphocyte antibodies. Limited studies of the Sepacell filter have also reported no febrile reactions.

If filtration is chosen by a blood bank and/or hospital to reduce leukocyte transfusion, a number of factors should be considered in selecting the type and model of filter. These include: cost of the filter, as well as additional equipment and solutions; open vs. closed systems, i.e. whether the storage life is shortened by processing; demands on blood bank personnel; speed of filtration and the capacity for multiple units, if use during surgery or rapid transfusion is desired; efficiency of red cell recovery; platelet exclusion; and whether a leukocyte-poor, as opposed to a leukocyte-free, product is needed. Microaggregate filters have the advantages of low cost per filter and unchanged storage life; however, they require the use of a centrifuge and blood bank personnel in spinning and cooling, and leukocyte
removal is only sufficient for a leukocyte-poor product. In addition, formation of microaggregates (and thus leukocyte removal) increases with the age of the unit; depending on the degree of leukocyte exclusion desired, the age before which the unit cannot be filtered may leave only a limited remaining storage life, despite the system being closed. The adsorption column filters provide excellent red cell recovery and leukocyte exclusion, and thus can provide a leukocyte-free product; they do not require a centrifuge or advanced preparation of the unit. However, the cost per filter is higher, solutions are required, the storage life is limited, and blood bank personnel time is required.

The new Sepacell filter appears to have the most advantages of the filters we tested. There is no decrease in storage life, delay due to unit preparation, or extra work for blood bank personnel. It has been reported that the Sepacell filter permits rapid filtration; in filtration of single units, it was the fastest of the filters we tested. In filtration of second units, the results were comparable to those of the single units, but some filtered too slowly to be practical; therefore, multiple-unit capacity cannot be claimed for the Sepacell filter. A leukocyte-free product is produced, which is also substantially depleted of platelets. The only significant disadvantage is a greater cost per filter than for the microaggregate filters, which may be offset by other savings.
Acknowledgements

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References


Table 1: Characteristics of five leukocyte filters tested in vitro

**Pall:** Ultipor SQ40S (Pall Biomedical Products Corp., East Hills, NY). This is a screen filter of polyester to remove microaggregates; the estimated pore size is 40 microns. It is designed for use during transfusion, so the unit storage life is not reduced. Formation of microaggregates is enhanced by spinning and cooling the unit, thus blood bank personnel time is required. Multiple units may be filtered through one filter. Approximate cost: $5.

**Fenwal:** 4C 2423 (Fenwal Laboratories, Deerfield, IL). This is similar in use to the Pall filter, but is a depth/screen filter with a pore size of 20 microns. Single unit capacity. Approximate cost: $6.

**Imugard:** Imugard IG500 (Terumo Corporation, Piscataway, NJ). This is a depth/adsorption column filter composed of cotton wool. The filter must be primed with 300-500 mL of saline, and pressure must be used when filtering blood. Thus it is designed for use in the blood bank, and the unit storage life is limited. Single unit capacity. Approximate cost: $10.

**Erypur:** Erypur g-0 (Organon Teknika Corp., Oklahoma City, OK). This is similar to the Imugard filter, except that it is composed of cellulose acetate, and blood may be filtered without pressure. Approximate cost: $15.

**Sepacell:** Sepacell R-500 (Asahi Medical Co. Ltd., Tokyo, Japan; distributed in the U.S. by Fenwal Laboratories). This is an in-line adsorption filter of polyester, with an estimated pore size of 20 microns. It is designed for use during transfusion, so there is no limitation in unit storage life or unit preparation by the blood bank. Single unit capacity. Approximate cost $14.
Table 2: Comparison of in vitro performance of five leukocyte filters

<table>
<thead>
<tr>
<th>Filter</th>
<th>Pall</th>
<th>Fenwal</th>
<th>Imugard (References)</th>
<th>Erypur (References)</th>
<th>Sepacell (References)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(4)</td>
<td>(2*)</td>
<td>(2,3,6-11)</td>
</tr>
<tr>
<td>Reduc. WBC (%)</td>
<td>84.4</td>
<td>65</td>
<td>90-98.3</td>
<td>97.9-99</td>
<td>99.7, 99.1</td>
</tr>
<tr>
<td>Abs. WBC x 10⁹</td>
<td>0.36</td>
<td>0.55</td>
<td>0.02-0.27</td>
<td>0.02-0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Recov. RBC (%)</td>
<td>92.7</td>
<td>73</td>
<td>85.9-97.1</td>
<td>89.4-100+</td>
<td>89.8, 91.1</td>
</tr>
<tr>
<td>Platelets % removed</td>
<td>44.8³</td>
<td>63.5³</td>
<td>51.0⁸, 68.4³</td>
<td>87.9⁸, 80.9³</td>
<td>96.4¹², 83.1³³</td>
</tr>
</tbody>
</table>

Our results

|          |      |        |        |        | |
| Single units, number tested | 19 | 2 | 4 | 5 | 10 |
| Reduc. WBC (%) | 55.0±23.2 | 66.0±8.5 | 89.7±9.9 | 98.1±0.9 | 97.9±1.9 |
| Abs. WBC x 10⁹ | 1.03±.60 | 0.34±.12 | 0.51±0.7 | 0.05±.03 | 0.03±.02 |
| Recov. RBC (%) | 92.0±9.8 | 60.5±16.2 | 92.6±14.0 | 91.5±8.0 | 93.1±6.3 |
| Second units, number tested | 5 | 2 | 1 | 1² | 2² |
| Reduc. WBC (%) | 47.0±30.6 | 26.5±14.8 | 98 | 90 | 97 |
| Abs. WBC x 10⁹ | 1.35±1.17 | 1.15±.12 | 0.05 | 0.37 | 0.07±.03 |
| Recov. RBC (%) | 91.2±17.3 | 74.5±13.4 | 96 | 86 | 85.0±1.4 |

Age at testing in days, range (mean) | 1-32 (4.8) | 5-9 | 3-7 | 3-7 | 6-18 (9.4)

Values shown are the mean ± the standard deviation

*Spun without cooling

²Unable to complete all units tested
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