Inadequate white cell reduction by bedside filtration of red cell concentrates

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Background: White cell filtration of red cell concentrates is often performed at the bedside, in the ward, with the filter inserted in the blood administration line. The aim of this study was to evaluate the efficiency of this filtration method and compare it to filtration in the blood bank.

Study Design and Methods: One-day-old, buffy coat-reduced, hard-packed red cell concentrates in saline-adenine-glucose-mannitol solution were filtered through different filters designed for bedside or laboratory use. With filters designed for bedside use, filtration of red cells was performed under laboratory conditions at fast flow (10 min) or under bedside conditions at slow flow (2 hours). The remaining white cells were counted microscopically. Filters designed for laboratory use were evaluated at fast flow, and the number of contaminating white cells was counted by flow cytometry.

Results: With bedside filters, a significantly higher contamination of white cells was found in the units filtered at slow flow than at fast flow, regardless of the filter used. The number of units with >5 x 10⁶ white cells was 52 (78%) of 67 filtered at slow flow compared to 11 (23%) of 47 at fast flow, all filters taken together. This difference in white cell contamination was mainly due to an increase of polymorphonuclear cells in the red cell concentrates filtered at slow flow. With filters designed for laboratory use, 0 to 2 percent of units (n ~ 1448) were contaminated with >5 x 10⁶ white cells.

Conclusion: Bedside filtration for white cell reduction at slow flow is inefficient for 1-day-old, buffy coat-reduced red cell concentrates. TRANSFUSION 1994;34:765-768.

Abbreviations: PMNs = polymorphonuclear cells; WBC(s) = white cell(s).

AT BLOOD TRANSFUSION, contaminating white cells (WBCs) may be harmful to the patient in several ways. The WBCs cause nonhemolytic febrile transfusion reactions. After removal of the buffy coat, which reduces WBCs by about 65 percent, these reactions were reduced by 50 percent. Some viruses and bacteria, such as cytomegalovirus and Yersinia sp., are viable within the WBCs. Removal of the WBCs before they disintegrate, may also remove the intracellular infectious agents. The WBCs are thought to be immunosuppressive and thus they may contribute to development of postoperative infections and recurrences of cancer.

For patients requiring long-term platelet transfusion therapy, alloimmunization to HLA antigens is of major concern. Alloimmunization is triggered by HLA-DR-presenting WBCs (B-lymphocytes, monocytes, and dendritic cells) in the transfused blood. Once alloimmunization has occurred, the HLA antibodies can react with allogeneic platelets and the patient becomes refractory to random donor platelet transfusions.

The most efficient way to reduce the WBCs in a blood component is to pass it through a filter, today usually consisting of polyester fibers or cellulose acetate. The filtration is often performed at the bedside in the ward, with the filter inserted in the administration line.

The number of WBCs needed to induce HLA immunization has been disputed. It has been claimed that <10⁷ residual WBCs would prevent primary HLA immunization. When blood components containing <10⁶ WBCs per unit were used, immunization was avoided in leukemic patients. European guidelines state that filtered blood components should contain <5 x 10⁶ WBCs per transfusion of red cell concentrates and <1 x 10⁶ WBCs per transfusion of platelet concentrates.

In an attempt at quality control of the WBCs in red cell concentrates filtered at the bedside, we determined the number of WBCs after transfusion in a segment of the transfusion line situated directly under the filter. All the units (n = 28) were transfused 1 to 7 days after collection. We found a high number of WBCs (median, 386 x 10⁶/L; range, 10⁵-5 x 10⁹). The samples we examined probably contained a higher WBC concentration than the
Materials and Methods

Blood (450 mL) from healthy volunteer donors was collected into 63 mL of CPD (Optipac, Baxter S.A., La Châtre, France) and left at room temperature (20°C) for 2 to 12 hours. After centrifugation at 2500 g (at the interface) for 12 minutes, the blood was divided into plasma, buffy coat (55 mL), and red cells suspended in 100 mL of saline-adence-glucose-mannitol solution by use of a plasma expressor (Optipress, Baxter). The red cells were stored at 4°C overnight and kept at room temperature for approximately 10 minutes before filtration. We performed all filtrations of the red cells in the laboratory, either at fast flow (by gravity, approx. 10 min) or at slow flow (approx. 2 hours).

Bedside filters: filtration at fast and slow flow

We used five filters in this study (RC50 and RC100, Pall Corporation, Glen Cove, NY; Sepacell R200 and R500, Asahi Medical Co., Tokyo, Japan; and ErypurOptima b, Organon Teknika B.V., Boxtel, the Netherlands). We filtered 1 unit of red cells through each filter, although the RC100, the R500, and ErypurOptima b were originally designed for 2 units of red cells. The priming of the filters was done as recommended by the manufacturers. No priming or rinsing with saline was done, except for the ErypurOptima b, which was rinsed with 50 mL of saline to reduce the loss of red cells (approx. 100 mL without rinsing, compared to 30-60 mL for the other filters).

We counted the number of WBCs in a Bürker hemocytometer (0.9 µL, prefiltration samples) and in a Nageotte hemocytometer (25 µL, postfiltration samples) at a dilution of 1-in-10 in a WBC-staining solution (Plaxan, Laboratoire Sobioda, Grenoble, France; or Türk’s solution). If ≤10 cells were found in 25 µL after filtration, an additional 25 µL was counted. We performed a differential count of polymorphonuclear cells (PMNs) and mononuclear cells.

Laboratory filters: filtration at fast flow

This study was performed with filters designed to be used under laboratory conditions. Units of red cells were filtered by gravity with the following filters: BP4 (Pall), RS200 (Asahi), and BioR1 plus (Biofil, Modena, Italy). All filters were primed as recommended by the manufacturers. No filters were rinsed with saline after filtration. We counted the number of contaminating WBCs after filtration by flow cytometry (Epics Profile I, Hialeah, FL) using our standard method, which is a modification of Dzik et al. In brief, 100 µL of the sample was added to 500 µL of propidium iodide solution (propidium iodide 5 mg, citric acid 71 mg, sodium chloride 60 mg, Triton X-100 1 mL, diluted to 100 mL with water). After incubation for 30 minutes, a 15-mW argon laser was used for fluorescence excitation at 488 nm. We set a gate for WBCs and counted the number of cells exhibiting red fluorescence at a constant flow rate for 130 seconds. This method has a detection level of 0.1 x 10⁶ per L in our setting.

Statistics

All results are expressed as median and range, if not stated otherwise. Wilcoxon’s signed-rank test was used to assess the significance of differences. A p value of <0.05 was considered significant.

Results

Prior to filtration, a unit of red cells contained 638 x 10⁶ ± 41 WBCs in a volume of 276 ± 2 mL (mean ± SD; n = 60). The percentage of PMNs was 95.0 ± 0.47 (mean ± SD; n = 52).

Bedside filters: comparison of fast and slow flow

The red cells filtered at slow flow contained a significantly higher postfiltration number of WBCs than the red cells filtered at fast flow, regardless of the filter used (Table 1). The number of units filtered at fast flow with a postfiltration contamination of >5 x 10⁵ WBCs per unit was 6 (22%) of 27 filtered with RC50, 2 (20%) of 10 with R200, and 3 (33%) of 10 with ErypurOptima b. When the filtration time was 2 hours, almost all units contained >5 x 10⁶ WBCs when filters designed for 1 unit of red cells were used (RC50: 89% [n = 27]; and R200: 100% [n = 10]). Using filters designed for 2 units of red cells but in this study used for 1 unit, 50 percent or more of the units had a contamination of >5 x 10⁶ WBCs per unit (5/10 units for RC100 and R500; 8/10 units for ErypurOptima b).

Using ErypurOptima b, the decrease in red cell volume caused by the filtration was about 100 mL, and therefore we considered rinsing with isotonic saline solution necessary. After such rinsing with 50 mL of saline, as recommended by the manufacturer, the number of WBCs per unit was significantly higher in the units filtered at fast flow (before rinsing: 2.1 x 10⁹, range, 0.13-11.0; after rinsing: 2.7 x 10⁶; range, 0.3-13.7 [p = 0.003]) as well as in the units filtered at slow flow (before rinsing: 3.6 x 10⁶; range, 0.7-6.4 x 10⁶ and after rinsing: 8.6 x 10⁶; range, 1.7-14.6 [p = 0.003]).

A significantly higher percentage of PMNs was found in the units filtered at slow flow than at fast flow (Table 2). This result was consistent with all filters tested at both flow rates. The median number of mononuclear cells per unit was also significantly higher after filtration at slow flow than after that at fast flow with the RC50 (2.9 x 10⁹; range, 0.4-20.4; and 1.6 x 10⁶; range, 0.5-4.2 [p = 0.036]) and R200 (5.4 x 10⁶; range, 2.9-9.1; and 1.1 x 10⁶; range, 0.4-3.9 [p = 0.003]). There was no significant difference between the numbers of mononuclear

<table>
<thead>
<tr>
<th>Filter</th>
<th>Fast flow</th>
<th>Slow flow</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>R200 (n = 10)</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ErypurOptima b (n = 10)</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RC100 (n = 10)</td>
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<td>NT</td>
<td></td>
</tr>
<tr>
<td>R500 (n = 10)</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
</tbody>
</table>

* Values expressed as median WBCs x 10⁶ per unit.
† Range.
‡ Not tested.
cells obtained at slow and fast flow using ErypurOptima b after
the filter was rinsed with 50 mL of saline.

**Laboratory filters: fast flow**

In units filtered at fast flow, with filters designed for labora-
tory use, the number with a WBC contamination below the de-
tection limit of our standard method (0.1 x 10^6/L) was 598 (46%) of 1300 filtered units for BPF4, 8 (9%) of 94 for RS200, and 24 (44%) of 54 for BioRO1plus.

After filtration with RS200, we found 2 red cell concen-
trates containing >5 x 10^6 WBCs per unit (2%). With BPF4 or
BioRO1plus, we did not find any units with a contamination
>5 x 10^6 WBCs per unit (Table 3).

**Discussion**

In this study we have chosen to mimic the bedside
situation by filtering red cells at slow flow (2 hours). We
consider this to be an adequate flow rate, as patients re-
ceiving filtered blood components often are fragile, suf-
ferring from malignant hematologic diseases. According
to Pikul et al., a filtration time of 2 hours (slow flow)
as compared to fast flow will not affect the WBC-reduc-
tion ability of the filter (RS500, Sepacell). On the contrary,
we found, using several filters designed for bedside use,
a significantly higher number of WBCs in red cells fil-
tered at slow flow than at fast flow. The discrepancy could
be due to the fact that Pikul et al. used non-buffy coat-
reduced red cells, while we useduffy coat-reduced red
cells in our study. It is evident that non-buffy coat-reduced
red cells contain a higher number of platelets than do
hard-packed, buffy coat-reduced red cells. It has recently
been suggested by Steneker et al. that platelets play

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**Table 2. Percentage of PMNs in red cells after filtration
at fast or slow flow**

<table>
<thead>
<tr>
<th>Filter</th>
<th>Flow rate</th>
<th>PMNs* (%)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>R200 (n = 10)</td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>ErypurOptima b (n = 10)</td>
<td>Fast</td>
<td>41 (23-62)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Slow</td>
<td>57 (42-70)</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 3. Number of residual WBCs after filtration at fast flow of
1-day-old buffy coat-reduced units of red cells using filters
designed for laboratory use**

<table>
<thead>
<tr>
<th>Filter</th>
<th>Number of units tested</th>
<th>Residual WBCs (x 10^6/unit)</th>
<th>Units with &gt;5 x 10^6 WBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS200</td>
<td>94</td>
<td>0.173</td>
<td>2</td>
</tr>
<tr>
<td>BioRO1plus</td>
<td>54</td>
<td>0.021</td>
<td>0</td>
</tr>
</tbody>
</table>

* Median (range)
cells as well as for the filter designed for 2 units tested at both flow rates (ErypurOptima b). Using filters designed for 1 unit of red cells, we also found a significantly higher number of mononuclear cells in units filtered at slow flow than in those at fast flow. A high number of mononuclear cells after filtration increases the incidence of HLA immunization, but a high number of PMNs might also be deleterious to the patient, since viruses such as cytomegalovirus and bacteria such as Yersinia sp. can be harbored within and transmitted by these cells.

New generations of filters are constantly under development. The efficiency of the filtration not only is due to the flow rate and the manual technique used but also is influenced by factors such as the composition and the temperature of the blood component. National guidelines and standard operational procedures decide what criteria a unit of red cells should meet. Even if the same standard procedures are used by several blood banks, there might be local variations in the composition of the red cells, due to centrifugation and other aspects of blood processing. When choosing a filter, it is important to ensure that the filter has a good performance with the type of red cell preparation used and under all conditions that will prevail. Quality control of the filtration process should be performed at a routine basis. This is more conveniently done after blood bank filtration than at the bedside.

References