Hemolysis During Leukocyte-Reduction Filtration of Stored Red Blood Cells

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Abstract. Hemolysis has been reported in red blood cells (RBCs) that have undergone leukocyte-reduction filtration. This study investigated whether the age of RBCs or the filter type affected hemolysis. One hundred eighty units of RBCs (adenine-saline added) were leukocyte-reduced by filtration. At each of the 6 weeks of shelf life, 10 units were filtered with the "BPF4" filter, 10 units with the "Purecell RCQ" filter, and 10 units with the "Sepacell" filter. Filtration was performed with strict adherence to the manufacturers' directions. Pre- and post-filtration samples were assayed for plasma hemoglobin by measuring the plasma absorbances at 578 nm and 562 nm. The increase of plasma hemoglobin concentration following filtration was significantly greater (p < 0.05) in older units, compared to fresher units, when the Sepacell and BPF4 filters were used. For example, the increase of plasma hemoglobin at week 6 (83.47 mg/dl: Sepacell, 128.93 mg/dl BPF4) was significantly greater than at week 1 (7.07 mg/dl Sepacell, 4.77 mg/dl BPF4) (Sepacell: p=0.008; BPF4: p=0.006). For units stored 1, 2, 4, 5, or 6 weeks, the increase of plasma hemoglobin concentration post-filtration was significantly greater with the BPF4 filter, compared to the Purecell RCQ filter (p <0.045); for units stored 5 weeks, the increase in plasma hemoglobin concentration post-filtration was significantly greater with the BPF4 filter compared to the Sepacell filter (p = 0.009). Mean filtration times were significantly longer in older units compared to fresh units. This study shows that increased storage duration of RBCs (adenine-saline added) is attended by greater hemolysis during leukocyte-reduction filtration and by prolongation of the filtration time. In addition, the amount of hemolysis may be influenced by the type of filter.

Keywords: Blood transfusion, leukocyte reduction filtration, hemolysis, blood storage, blood filtration time

Introduction

More than 90% of adverse reactions to transfusions are associated with the presence of white blood cells (WBCs) in blood components [1,2,3]. These include febrile nonhemolytic transfusion reactions, HLA alloimmunization with subsequent refractoriness to platelet transfusions, graft-versus-host disease, transfusion-related acute lung injury, transfusion-related immunomodulation, and transfusion of leukotropic viruses, such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human T-cell lymphotrophic virus (HTLV) [1,4].

Red blood cells (RBCs) that have not been leukoreduced contain approximately $10^8$-$10^9$ leukocytes per component [3]. At present there are no definitive data on the level of WBC reduction necessary to avoid the aforementioned transfusion complications, although the United States Food and Drug Administration and the Standards of The American Association of Blood Banks state that each transfusion of leukocyte-reduced RBCs should contain fewer than 5 x $10^6$ leukocytes [1,5,6].

Filtration is employed currently as the standard technique for leukocyte reduction of RBCs. Leukocyte reduction filters have the capacity for approximately
4-5 log10 reduction in WBCs [1-4,7,8]. These filters are designed so that blood encounters a large surface area of interlaced synthetic microfibers with a pore size as small as 4 μm [3]. A variety of factors can affect filter performance, including input number of leukocytes, flow rate, pressure, priming, rinsing, temperature, blood viscosity, holding time between blood collection and filtration, erythrocyte and leukocyte deformability, and plasma content of the cell suspension [3,8]. In addition, there are reports of increased hemolysis in stored red blood cells [9-11].

This study compared three leukocyte reduction filters: the Purecell™ RCQ filter (Pall Biomedical Products Co.); the BPF4R filter (Pall Biomedical Products Co.); and the Sepacell™ filter (Baxter Healthcare Corp.). The study determined whether the age of RBCs subjected to filtration, and/or the filter type, affects hemolysis, and whether the age of RBCs affects the time required for filtration.

Materials and Methods

One hundred eighty units of RBCs (adenine-saline added) were leukocyte-reduced by filtration. For each of the 6 weeks of shelf life, 10 units were filtered with the BPF4R filter (catalog no. PBF4C, Pall Biomedical Products, Co., East Hills, NY), 10 units were filtered with the Purecell™RCQ filter (catalog no. RCQT, Pall Biomedical Products, Co., and 10 units were filtered with the Sepacell™ filter (catalog no. 4C2481, Baxter Healthcare Corp., Fenwall Division, Deerfield, IL). Filtration was performed with strict adherence to the manufacturers’ directions, as printed on the package inserts.

Prior to filtration, the RBCs were gently mixed (30-120 sec). Filtration was performed at 4°C with the BPF4 and Sepacell filters, simulating the temperatures used by our blood bank when performing filtration. Filtration was performed at 20-24°C with the Purecell RCQ filter, simulating the temperatures that occur during bedside filtration.

A sterile connection device allowed sampling of 15 ml of post-filtration RBCs to a 150 ml transfer bag (Terumo, Tokyo, Japan) without altering the RBCs’ outdate.

Plasma hemoglobin concentrations were measured in specimens obtained pre- and post-filtration. Each sample was initially centrifuged at 1050 x g for 5 min (DYNAC II centrifuge, Clay Adams Co., Parsippany, NJ). To ensure red cell removal, the plasma was centrifuged a second time at 2000 x g for 2 min (Clay Adams Serofuge II). Absorbances of plasma at 578 nm and 562 nm were measured using a model DU 64 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA), and plasma hemoglobin concentrations were calculated as described by Blakney and Dinwoodie [12].

Results

For each filter, mean weekly post- minus pre-filtration plasma hemoglobin concentrations were compared by a one-way ANOVA with Scheffe’s post-hoc comparison. The increase in plasma hemoglobin concentration after filtration was significantly greater (p < 0.05) in older units compared to fresher units when the Sepacell and BPF4 filters were used (Table 1). For the Sepacell filter, the increase of plasma hemoglobin concentration observed at week 6 (83.47 mg/dl) was significantly greater than at week 1 (7.07 mg/dl) (p=0.008). For the BPF4 filter, the increase of plasma hemoglobin concentration observed at week 6 (128.93 mg/dl) was significantly greater than at week 1 (4.77 mg/dl) (p=0.006). Also, for the BPF4 filter, the increase

<table>
<thead>
<tr>
<th>Week of storage</th>
<th>Mean plasma hemoglobin conc. (mg/dl) (post-filtration minus pre-filtration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.07</td>
</tr>
<tr>
<td>2</td>
<td>5.63</td>
</tr>
<tr>
<td>3</td>
<td>22.91</td>
</tr>
<tr>
<td>4</td>
<td>38.95</td>
</tr>
<tr>
<td>5</td>
<td>53.34</td>
</tr>
<tr>
<td>6</td>
<td>83.47*</td>
</tr>
</tbody>
</table>

*p wk 6 > wk 1 (p = 0.008) and wk 2 (p = 0.007)
†wk 5 > wk 1 (p = 0.012)
‡wk 6 > wk 1 (p = 0.006)
Hemolysis after leukocyte-reduction filtration of stored red blood cells

Compared with the Purecell RCQ filter, although a trend toward increased post-filtration plasma hemoglobin with increased storage was evident.

Table 2. Mean post-filtration minus pre-filtration plasma hemoglobin concentration in RBC units that were filtered after 1 to 6 weeks of storage.

<table>
<thead>
<tr>
<th>Week of storage</th>
<th>BPF4 vs Purecell RCQ</th>
<th>BPF4 vs Sepacell</th>
<th>Purecell RCQ vs Sepacell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.030</td>
<td>0.607</td>
<td>0.121</td>
</tr>
<tr>
<td>2</td>
<td>0.045</td>
<td>0.062</td>
<td>0.339</td>
</tr>
<tr>
<td>3</td>
<td>0.078</td>
<td>0.071</td>
<td>0.937</td>
</tr>
<tr>
<td>4</td>
<td>0.006</td>
<td>0.066</td>
<td>0.250</td>
</tr>
<tr>
<td>5</td>
<td>0.006</td>
<td>0.009</td>
<td>0.566</td>
</tr>
<tr>
<td>6</td>
<td>0.031</td>
<td>0.267</td>
<td>0.142</td>
</tr>
</tbody>
</table>

* Statistically significant p values (p < 0.05) in bold type.

Table 3. Comparison of mean filtration times for RBC units that were filtered after 1 to 6 weeks of storage.

<table>
<thead>
<tr>
<th>Week of storage</th>
<th>Sepacell filter</th>
<th>BPF4 filter</th>
<th>Purecell RCQ filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.57</td>
<td>9.64</td>
<td>8.71</td>
</tr>
<tr>
<td>2</td>
<td>10.06</td>
<td>11.01</td>
<td>10.65</td>
</tr>
<tr>
<td>3</td>
<td>17.36</td>
<td>24.51</td>
<td>11.22</td>
</tr>
<tr>
<td>4</td>
<td>14.43</td>
<td>29.72</td>
<td>18.27</td>
</tr>
<tr>
<td>5</td>
<td>21.05</td>
<td>19.02</td>
<td>22.39</td>
</tr>
<tr>
<td>6</td>
<td>37.11*</td>
<td>53.26†</td>
<td>37.47‡</td>
</tr>
</tbody>
</table>

* wk 6 > wk 1 (p=0.003), wk 6 > wk 2 (p=0.007), wk 6 > wk 4 (p=0.038)
† wk 6 > wk 1 (p=0.001), wk 6 > wk 2 (p=0.002), wk 6 > wk 5 (p=0.022)
‡ wk 6 > wk 1, wk 2, wk 3, and wk 4 (all p=0.001), wk 6 > wk 5 (p=0.017)
∞ wk 5 > wk 1 (p=0.041)

Donor leukocytes in RBCs and platelet concentrates are associated with adverse transfusion outcomes (e.g., transmission of CMV and other leukotropic viruses, HLA immunization, and febrile nonhemolytic transfusion reactions) [1,4,7,8].

Studies suggest that pre-storage filtration by the blood bank may be more effective than bedside filtration in reducing some of the adverse effects of transfusion [3,8]. However, many hospitals have elected to use bedside filtration owing to its lower cost. Addressing these issues, we studied two leukocyte reduction filters which are designed for pre-storage or during-storage filtration (BPF4 and Sepacell), as well as one designed for bedside use (Purecell RCQ). Since RBCs are transfused at varying ages, our study examined the effects of leukocyte-reduction filtration throughout their 6-week shelf life.
There are several reports of hemolysis after leukocyte-reduction filtration [9-11]. Schmidt et al [11] described hemolysis associated with a woven stainless steel micropore filter. These authors ascribed the hemolysis to mechanical damage caused by the filters and stated that the damage was sufficient to cause hemolysis with blood as fresh as 1 day old [11]. Carson et al [9] noted increased hemolysis in leukocyte-reduced filtered units which were 2-3 weeks old [9].

Current leukocyte reduction filters are designed so that blood is distributed over a large surface area of the filter, the volume of blood retained by the filter is minimal, and the filter fits tightly enough with the housing so that blood entering the device cannot bypass the filtration media [3]. The leukocyte reduction filter is designed so that the pore size of the filter media is large enough to allow passage of the deformable red blood cells and platelets, but small enough to impede passage of the more rigid leukocytes [3]. To achieve the small pore size required, the filtration media within the housing consists of nonwoven webs of synthetic microfibers with a diameter of 0.3-3.0 µm [3]. Synthetics such as cellulose acetate or polyester need to be used, since natural fibers do not exist at diameters that are this small [3,13,14]. These fibers are interlaced in a series of layers from coarse to fine so that at the upstream end the pore size is large, but as blood passes through the layers of the filter, the pore size decreases to 4 µm [3,13]. Variables which may affect the efficiency of filtration include charge of the filter fibers and biologic forces (eg, leukocytes adhere to activated platelets, which adhere to the filter fibers, increasing the efficiency of the filtration) [3].

RBCs undergo a variety of storage lesions as they age, which include decreased pH, increased concentrations of potassium, phosphate, and ammonia, and ultimately changes in red cell deformability [15]. The complexity of current leukocyte reduction filters and the variety of surfaces that red cells come in contact with during filtration may result in filtration-induced hemolysis, which may increase with storage duration. Statistically significant increases (p < 0.05) in hemolysis with storage age were seen post-filtration with the Sepacell and BPF4 filters, but not with the Purecell RCQ filter.

Filtration time may be shortened, and efficiency increased, by applying pressure to the RBC container. In fact, the manufacturer's insert for the Sepacell and BPF4 filters recommend squeezing the RBC container during the initial stages of filtration. A certain amount of pressure may be advantageous in priming the filter by reducing "dead spaces," thus placing the filtrate in contact with a greater surface area of the filter. However, Gambino et al [10] showed that too much pressure (100-150 mm/Hg) may result in increased hemolysis. The importance of reducing "dead space" in filtration has been shown in donors with hemoglobin AS, in which there was gelation of the hemoglobin, resulting in obstruction and channeling of the filter and causing inadequate removal of the WBCs (>5 x 10⁶/unit) in 75% of units studied [16].

Regardless of the cause of hemolysis, the fact that it may occur during leukocyte-reduction filtration illustrates the importance of pre-shipment and pre-transfusion inspection of leukocyte-reduced blood components. To prevent inadvertent transfusion of a grossly hemolyzed unit, the components should be stored in a manner that allows inspection of the plasma-red cell interface [9]. The ability to perform such an inspection after leukocyte reduction filtration is one advantage of performing this procedure prior to transfusion.

Because blood banks and transfusion services may use a variety of leukocyte reduction filters, we compared the mean weekly post- minus pre-filtration plasma hemoglobin concentrations of the three filters used in our study. Significant differences were seen comparing the BPF4 and the Purecell RCQ filters in all weeks except week 3 (all p < 0.05). Comparing the BPF4 to the Sepacell filters, significant difference was seen only at week 5 (p < 0.01). No statistically significant difference in hemolysis was noted between the Purecell RCQ and Sepacell filters. Since a relatively small number of units of RBCs were tested, the present comparisons of hemolysis with different filters require confirmation. However, the transfusion laboratory must be aware that various leukocyte reduction filters may be associated with differing degrees of hemolysis.

Filtration times increased as the RBCs increased in age. All three types of filter gave significant increases in mean filtration time at week 6, compared to weeks 1 and 2. The increase in filtration times may be due to increased microaggregates of cells and fibrin, which have been shown to collect on filter fibers and may
obstruct filter pores [13,16]. Of more concern, however, are reports that slower rates of filtration are associated with poorer efficiency of leukocyte reduction [17]. Ledent et al [17] showed that units with slow filtration (filtration time approximately 2 hr) had a highly significant increase in the number of polymorphonuclear leukocytes after filtration compared to those subjected to fast filtration (10 min). Thus, older RBCs, which had longer filtration times, may, in addition to having increased mean post-minus pre-filtration plasma hemoglobin levels, contain increased numbers of post-filtration WBCs.

Conclusions

The increased post-filtration plasma hemoglobin concentrations that were seen in RBC (adenine-saline added) components depended upon the type of filter used as well as the age of the RBCs. Increasing age of the RBCs (adenine-saline added) also resulted in slower filtration rates, which may reduce the efficiency of filtration [17]. Regardless of the age of the RBCs (adenine-saline added), visual inspection of the post-filtration component at the red blood cell/plasma interface may determine if there is a significant degree of hemolysis. This inspection is not feasible with bedside leukocyte reduction filtration. Leukocyte reduction filters provide significant benefits, but blood bank staff need to be mindful of the possibility of causing hemolysis, particularly in older units of RBCs.

References