Evaluation of particulate material in stored units of New Zealand re-suspended red cells

Katie R Anderson and James M Faed
University of Otago, Dunedin

ABSTRACT

Objective: To evaluate the amount and nature of aggregated protein material in units of New Zealand re-suspended red cells (RRC).

Methods: Eleven units of RRC were passed through filtered intravenous administration sets that contained a mesh filter, using a method similar to a standard patient transfusion protocol. The filters were then flushed gently with saline to remove free red cells, inspected for aggregates and agitated gently at 37°C to dissolve trapped proteins. Samples of the flushing saline were collected both before and after the 37°C incubation. All samples were tested for total protein; the sample with the highest protein concentration was run on mass spectrometry to determine the composition of the protein.

Results: Negligible visible aggregated material was observed or detected in the protein assays of flushing solutions. Mass spectrometry identified that the major protein was haemoglobin. This protein is likely to have arisen from residual red cells in an area of the filter which could not be flushed thoroughly.

Conclusions: There was no aggregated material found in this experiment and it can be presumed that current storage procedures and processing to remove leucocytes and coincidentally platelets, satisfactorily prevents formation of aggregated particulates of leucocytes, platelets and fibrinogen. The methods used in this study were not able to confirm whether filter clogging by aggregates remains a bedside issue, although anecdotal information states that a problem still exists. An alternative method that can detect loosely aggregated material unable to withstand the shearing forces of flushing and which may not even withstand rapid transfusion will be required, together with direct study of administration set filters immediately after bedside transfusions.

Keywords: red blood cells, total protein, particulate, protein aggregate, leucodepletion.


INTRODUCTION

Patients suffering from acute or ongoing haemorrhage, or clinically symptomatic anaemia are transfused with re-suspended red cells (RRC) (1). Preparation of this clinical blood component involves leucocyte depletion by high-efficiency filtration in an adsorption depth filter, followed by a centrifugation step and separation of the red cells and plasma (1). The red cells are then resuspended in saline, adenine, glucose and mannitol (SAGM) additive solution with only a minimal volume of plasma. SAGM maintains the viability of the red cells during 4°C storage and dilutes the red cells allowing minimal volume of plasma. SAGM maintains the viability of the red cells during 4°C storage and dilutes the red cells allowing them to flow freely through the intravenous (IV) administration sets used to connect the blood component to the patient (2). The specification for residual leucocyte count is less than 1 x10⁶ /unit. The specification for filter mesh size in the transfusion administration set is 170-200 μm (3).

Prior to the introduction of pre-storage leucocyte depletion, the presence of aggregated material in stored red cell units would frequently result in clogging of the infusion set after several units had been transfused (4). Studies of aggregated material in unfiltered units of blood have indicated that platelets, granulocytes and monocytes, together with small amounts of fibrin, are the principal constituents (5-9). Maximal formation of aggregates occurs after 2-3 weeks storage (7,10), but the mechanisms for formation has not been confirmed at the molecular level. The role of platelets and leucocytes are thought to be essential for formation of the aggregates but it is suspected that thrombin, fibrinogen and globulins are also contributors (11-13).

Early studies on microaggregates started from an apparent association with post transfusion acute lung injury (14,15). However, subsequent studies that used improved clinical support showed that microaggregates in transfused blood were not the major cause for acute respiratory distress syndrome (ARDS). However, quantitative differences in amounts of aggregated material were considered by some authors to explain the discrepancy in findings.

Other factors, such as sepsis, were the major factor for development of ARDS in many patients. Nevertheless, concern existed over the association of aggregated material with febrile transfusion reactions, transmission of cell-associated cytomegalovirus, immunisation by transfused leucocytes resulting in HLA antibodies and refractoriness to platelet transfusion, post-transfusion thrombocytopenia, immunomodulation and potentially other adverse effects (2,16,17).

This study aimed to determine if visible aggregate material were present in stored New Zealand red cell units and whether these could potentially be trapped in the 170-200 micron administration set filters after the passage of one unit of RRC. The study also assessed the composition of any material trapped and later eluted from the filters.

METHODS AND MATERIALS

Re-suspended red cell (RRC) units that had passed their expiry date were evaluated. Units were kept at room temperature for 30 minutes prior to filtration, to replicate transport and handling time prior to transfusion of red cells. All units were between 35 and 42 days from the date of collection. The transfusion administration sets used were Baxter Clearlink FNC3110 (20 drops per mL) with a 200 μm filter and a roller clamp to regulate flow. The administration sets were primed with phosphate buffered saline (PBS), before each unit was filtered.
A predetermined drip rate was calculated from the volume of each unit and the intended filtration time (180-240 minutes) and controlled by the roller clamp. At all stages, the procedure mimicked those of standard transfusion protocols. Once filtered, the administration set spike was removed from the unit and the line flushed at 3 mL/min with PBS, until visibly clear of red cells. Flushing volumes were recorded. The IV tubing was sealed at two points: 20 cm and 5 cm below the filter, using a microwave tubing welder. A 15 cm tubing segment was collected as the ‘pre’ sample for residual protein concentration in the flushing solution, whilst the filter remained full of PBS. A photograph of the filter was taken at this point to record any visible particulate material. The ‘pre’ samples were frozen at -35°C until testing was carried out.

The filters were sealed and agitated in a 37°C water bath for 2 hours to dissolve any cold-insoluble protein (cryoglobulins or other protein with low solubility) (1). Immediately after removal from the water bath, the remaining saline was collected as the ‘post’ sample by forward flushing and also stored frozen. Another photograph was taken. As no visible aggregates were evident no further steps were taken to solubilise proteins.

All samples were thawed at room temperature for 9 hours prior to testing. Total protein was tested at Canterbury Health Laboratories (CHL) biochemistry using the Biuret method. The highest of these results was used for mass spectrometry as this was the most likely to provide qualitative results. Liquid phase chromatography was used to separate the sample into its component parts and then time of flight mass spectrometry was used to determine the mass characteristics of the components, using equipment at CHL endocrinology.

### RESULTS

Specific details and statistical analysis of the filters used in this experiment are shown in Table 1. The samples had very little protein present and this finding is concordant with the photographs, showing no visible particulates in most filters. A ‘post’ photograph of the filter with the highest total protein result can be seen in Figure 1.

#### Table 1. Specific details for each filter and statistical analysis.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Volume (mL)</th>
<th>Age of RRC at filtration (days)</th>
<th>Time to filter (minutes)</th>
<th>Minimum saline to flush (mL)</th>
<th>‘Pre’ total protein (mg/mL)</th>
<th>‘Post’ total protein (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>293</td>
<td>45</td>
<td>192</td>
<td>43.6</td>
<td>0.00</td>
<td>0.49</td>
</tr>
<tr>
<td>2</td>
<td>256</td>
<td>45</td>
<td>201</td>
<td>53</td>
<td>0.04</td>
<td>0.29</td>
</tr>
<tr>
<td>3</td>
<td>292</td>
<td>46</td>
<td>224</td>
<td>56.8</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>276</td>
<td>41</td>
<td>190</td>
<td>54.6</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>5</td>
<td>262</td>
<td>48</td>
<td>189</td>
<td>41.1</td>
<td>0.02</td>
<td>0.17</td>
</tr>
<tr>
<td>6</td>
<td>294</td>
<td>48</td>
<td>227</td>
<td>70</td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>7</td>
<td>289</td>
<td>48</td>
<td>191</td>
<td>56.2</td>
<td>0.00</td>
<td>0.09</td>
</tr>
<tr>
<td>8</td>
<td>273</td>
<td>47</td>
<td>188</td>
<td>47.8</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>9</td>
<td>242</td>
<td>48</td>
<td>199</td>
<td>78.9</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>10</td>
<td>250</td>
<td>47</td>
<td>197</td>
<td>92.8</td>
<td>0.00</td>
<td>0.13</td>
</tr>
<tr>
<td>11</td>
<td>334</td>
<td>47</td>
<td>236</td>
<td>70.3</td>
<td>0.00</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean</td>
<td>278.27</td>
<td>46.36</td>
<td>203.09</td>
<td>60.46</td>
<td>0.03</td>
<td>0.16</td>
</tr>
<tr>
<td>SD</td>
<td>24.83</td>
<td>2.01</td>
<td>16.56</td>
<td>15.11</td>
<td>0.04</td>
<td>0.12</td>
</tr>
<tr>
<td>Median</td>
<td>276</td>
<td>47</td>
<td>197</td>
<td>56.2</td>
<td>0.03</td>
<td>0.1</td>
</tr>
<tr>
<td>IQR 1</td>
<td>259</td>
<td>45.5</td>
<td>190.5</td>
<td>50.4</td>
<td>0.00</td>
<td>0.09</td>
</tr>
<tr>
<td>IQR 3</td>
<td>292.5</td>
<td>48</td>
<td>212.5</td>
<td>70.15</td>
<td>0.04</td>
<td>0.17</td>
</tr>
</tbody>
</table>

IQR= inter quartile range.

As any protein present was expected to be predominantly haemoglobin (Hb), a Hb control was used for the mass spectrometry. Results presented in Figure 2 show the control in black with the upper (blue) spectrum showing the results of the test sample. The test sample matched very closely the control results with no other proteins of significance detected by mass spectrometry. The 15126 Da is the peak for the alpha chains of haemoglobin and 15867 Da the beta chains of haemoglobin. Smaller peaks indicated by the callipers are alpha and beta chains with other molecules covalently bonded. The two smaller peaks 162 Da to the right of each chain indicated the presence of HbA1c. The widest callipers (613 Da mass difference) identify alpha chains with extra haem groups abnormally bound. The beta chains with 305 Da added are the result of oxidative damage with binding of glutathione (2).
DISCUSSION

This study was performed to investigate quantitatively and qualitatively the presence of aggregated material trapped in the mesh filters of administration sets after passage of one unit of RRC. Published literature is limited despite strong prevalent opinions, that aggregates are a problem among experienced nurses and current transfusion bedside practice guidelines.

Outdated units were used in the study to avoid the use of expensive viable units and also because these have the highest chance of aggregates at or above the expected maximum amount of aggregates (2). Blood banks have refined their operations to minimise wastage of blood components and as a result, few RRC units now reach expiry. A sample number that is larger than the 11 units tested in this study might detect more low frequency events and be more representative of the bedside issues reported by nurses.

Standard transfusion practices for nurses provide rules for changing administration sets. These comply with the guidelines of the Australian and New Zealand Society of Blood Transfusion (ANZSBT) which specify that administration sets must be changed at the end of each transfusion or every 12 hours, whichever comes first (3). The main reason for changing the sets is to minimise the risk of bacterial growth after accidental contamination during connections. This reduces the risk for sepsis in a patient (2).

Trapping of particulate material in the administration sets alters the flow of each unit, and may lead to a blocked filter. Historically, this issue was a well-known cause for slowing of transfusions and difficulty with maintaining flow rates (4,11,12,18). New Zealand Blood Service (NZBS) now employs universal leucodepletion which reduces the content of leucocytes in each unit to $10^3$ to $10^4$ of the content in donated whole blood units (1). This practice removes almost all leucocytes from blood components, and reduces the risk of adverse reactions. The most frequent adverse reactions are febrile non-jaeolytic transfusion reactions which were reduced by almost 50% after introduction of leucocyte depletion (2).

Prior to leucodepletion, units found to completely clog transfusion set mesh filters (17-200 microns) excessively would frequently be sent back to the blood bank for investigation (3). A loose fibrin clot was most commonly at fault in these units. (Faed JM, oral communication, 11 Feb 2016). Degeneration of granulocytes (particularly neutrophils) and monocytes is believed to contribute to particulate development through the release of proteases. Some free proteases are able to activate prothrombin to thrombin and both thrombin and other proteases are able to cleave fibrinopeptides from fibrinogen to form fibrin monomer and fibrin aggregates during storage (1,2).

This step was introduced widely in Europe and later New Zealand after the observation by Aguzzi (reviewed by Turner and Ironside, and by Ponte) that the potential for transmission of variant Creutzfeldt-Jakob disease (vCJD) by leucocytes, and in particular B cells, could be substantially reduced by leucocyte depletion (19,20). Other benefits also exist, notably reduced risk for transmission of cell associated viruses such as cytomegalovirus, and reduced risk of HLA immunisation which may cause refractoriness to platelet transfusion (2). However, leucocyte depletion is thought to be not sufficiently effective to eliminate the risk from graft versus host disease (2).

The leucodepletion procedure involves passing blood through a filter containing tightly compressed fine plastic fibres that have specific chemical epitopes to assist adsorption binding of leucocytes (2). The type of filtration used is termed depth filtration as it relies on a thick layer of fibres over which the cells percolate, rather than screen filtration which has a single membrane or mesh with defined pore size. Aggregates of proteins and cell material are thought to form if leucocytes and platelets remain in stored red cell units and this event may be worst in the buffy coat of leucocytes and platelets that settle on the red cells. Degenerating leucocytes release enzymes that may slowly produce low-grade activation of coagulation and also damage red cells or plasma protein (1,2). This particular type of filtration will eliminate any larger clots from RRC units.

Figure 2. Mass spectrum of 5944539 (upper) and control haemoglobin (lower).
LIMITATIONS OF THE STUDY

This study had access only to expired (outdated) RRCs but this issue is likely to have led to maximization of the production of aggregates over time, although formation and subsequent dissolving of aggregates either enzymatically or as a result of shearing forces during filtration cannot be excluded. Better control of mixing of blood during collection from the donor and of storage and transport conditions (such as temperature) by the Blood Service may now play a role in reducing the activation of residual leucocytes and formation of particulate material (1).

The use of PBS instead of intravenous grade saline should not have had any significant effect since PBS is isotonic and does not contain ions that may activate coagulation or cells. This is regarded as a negligible difference from standard transfusion protocols (2).

Alternative methods for future studies

Patients often receive multiple red cell units through the same administration set. Comparison of quantities of particulate material after a series of units (e.g. 10 units) that have been passed through a single transfusion set may be more clinically relevant (1,3). Increasing the number of units tested would provide a more representative picture of potential problems with particulates, especially since they now appear to be uncommon following the introduction of leucodepletion (1).

A final recommendation to address several of these problems would be to carry out a retrospective study; collecting used filters from the hospital wards, with relevant information such as number of units transfused and time taken to transfuse. Scientists would then be able to actively assess and investigate issues reported by nurses. This would avoid the potential for laboratory-induced artefacts and differences in the study reported here, when compared with bedside transfusion practice. A retrospective study would require immediate access to the used transfusion sets to evaluate the administration set filters as soon as each transfusion was finished. It would take considerably longer to carry out. Ethics approval would be required if the study involved a change in clinical and bedside practices to facilitate access to administration sets.

CONCLUSION

No particulate material was observed in any of the samples tested in this experiment. This suggests that the procedures in place to reduce the development of aggregates are successful. Nursing staff still believe this is a real problem due to observations with patients. For this reason, further testing is appropriate and should use some of the suggested alterations to the study.

ACKNOWLEDGEMENTS

I would like to thank all those I was involved with over my time as a part of the New Zealand Blood Service during my fourth year BMSc placement, particularly those who assisted me with this study; Dunedin Blood Bank, NZBS Christchurch Processing Laboratory, Canterbury Healthcare Ltd (CHL) Endocrinology Laboratory, and especially Professor Stephen Brennan.

AUTHOR INFORMATION

Katie R Anderson, BMSc, Medical Laboratory Scientist1
James M Faed, BMedSci MB ChB FRCPA, Senior Lecturer2

1Southern Community Laboratories, Dunedin
2Department of Pathology, University of Otago, Dunedin

Author for correspondence: Katie Anderson. Histology, SCL, Plunket House, 472 George Street, Dunedin.
Email: k.anders92@gmail.com

REFERENCES

4. Faed JM. Personal observation.
12. Reiss RF, Katz AJ. Microaggregate content and flow rates of packed red blood cells. Transfusion 1977; 17: 484-489.