FINE SCREEN FILTRATION OF BANKED BLOOD:
EFFECT ON RED CELL SURVIVAL

J. D. BROWNING, I. C. M. GRAY AND I. MCA. LEDINGHAM

SUMMARY

Two fine screen mesh microfilters and the Baxter blood administration set commonly used in U.K. hospitals were compared for their effect on the red cell survival of stored autologous blood in six healthy males. The effect of the filtered transfusions on pulmonary gas exchange and on blood coagulation was measured. No significant difference was found between either microfilter and the Baxter giving-set for the red cell survival of transfused blood. Pulmonary gas exchange and coagulation were unaffected by transfusion of one unit of whole blood. Post-transfusion bilirubin concentrations were significantly less with one of the microfilters compared with the Baxter giving-set, possibly because of trapping of effete red cells within the filter. An unsuspected finding was the effect of transfusion in increasing the peripheral blood neutrophil count.

Microfilters are used routinely in many centres during cardiopulmonary by-pass procedures and less frequently for massive transfusion of stored banked blood in an attempt to reduce the frequency of post-bypass or post-transfusion pulmonary insufficiency. Occlusion of pulmonary capillaries is thought to be caused by the micro-aggregates of debris known to be present in stored banked blood, or by released vasoconstrictor substances. A number of questions remain unanswered regarding the effect of microfilters on the blood traversing them. Does the relatively small pore size of the filter cause "invisible damage" to red cells, resulting in their early removal by the reticulo-endothelial system thus reducing the effectiveness of the transfusion? Does the large surface area of the microfilter produce activation of coagulation factors with the possible risk of inducing a consumptive coagulopathy?

Studies performed on patients undergoing bypass procedures or receiving massive blood transfusion are often difficult to interpret because of the large number of factors which may influence red cell survival, lung function and blood coagulation. We examined two fine screen mesh microfilters, the Pall Ultipor and the Biotest MF 10, and the Baxter blood administration set normally used in U.K. hospitals for their effect on the red cell survival of blood transfused through them and the effect of these transfusions on pulmonary gas exchange and blood coagulation in healthy male volunteers.

METHODS

Six healthy male volunteers (age range 22–50 yr) were studied; routine haematological and biochemical examination was performed to exclude any blood-losing conditions. By venesection 500 ml of blood was withdrawn with the assistance of the Glasgow and West of Scotland Blood Transfusion Service, the blood being collected into plastic double-pack blood storage bags containing CPD anticoagulant (Fenwal double pack FKR 1997). The blood was stored at 4 °C for 28 days, following which an aliquot was removed, labelled with chromium-51 and returned to the original unit of blood immediately before transfusion.

Transfusion was performed through one or other of the microfilters (both of which had integral administration sets) or through the Baxter blood administration set, in each case using an 18-gauge "Abbocath"-T cannula (Abbott Laboratories 4535–18) with gravity flow over 20–30 min. Following each transfusion the filter with attached giving set was removed from the empty blood pack and the residual blood allowed to transfuse under continuous supervision until the filter chamber had emptied. During each of the three phases of the study two filters of each type were used. Venous and arterial samples...
were taken before and after transfusion to allow measurement of red cell survival, serum bilirubin, blood-gas tensions, coagulation studies, platelet count and full blood count.

The whole procedure, including venesection, transfusion and sampling, was performed on each volunteer on three separate occasions with 3 months between each phase, until all had received a transfusion through each of the three filters.

**Filters**

The Pall Ultipor is a fine screen mesh filter with a 40-μm pore size. The type used had an integral administration set (Pall Biomedical Limited SQ 40 KL).

The Biotest MF 10 is a multiple-layer fine screen mesh filter the pore sizes of the four different layers being 200, 50, 20 and 10 μm. This filter also had an integral administration set (Biotest Folex Ltd MF-10 B Luer).

The Baxter administration set for blood and blood derivatives is widely used in hospitals throughout the U.K. and incorporates a filter with a 170-μm pore size (Travenol Laboratories Limited FKC 2055).

**Red cell survival**

After 28 days an aliquot of the stored blood was transferred, following thorough mixing, to the satellite pack of the Fenwal double pack which was separated after clipping. Twenty millilitre of this aliquot was labelled with 25 μCi of chromium-51 (Gray and Sterling, 1950) then returned to the original blood pack immediately before transfusion. Venous samples were taken from the volunteer at 5 min, 1, 2, 4 and 24 h after transfusion and then every 2nd or 3rd day for 3 weeks. The dose of radioactive label was reduced to 25 μCi in view of the measurement being performed three times on the same individual. To compensate for the reduced labelling, dose counting times were increased to 10 min. In the second and third phases of the study unlabelled samples from the units of stored blood and pre-transfusion samples from each volunteer were checked for residual activity from the previous phase.

**Serum bilirubin concentration**

This was measured before and 4 h after transfusion using a standard technique (Gambino and Schreiber, 1964).

**Pulmonary gas exchange studies**

All subjects lay supine for at least 20 min before arterial sampling. After infiltration of local anaesthetic immediately before and 1 h after transfusion arterial blood was sampled under anaerobic conditions into heparinized plastic syringes from the radial artery.

The samples were analysed immediately for \( P_{O_2} \), \( P_{CO_2} \), pH and base excess using a Radiometer ABL 1 blood-gas analyser. The measurements were corrected for the difference between the temperature of the electrodes and the subject's sublingual temperature, which was measured periodically. Alveolar-arterial \( P_{O_2} \) difference was calculated:

\[
(P_{A_{O_2}} - P_{A_{O_2}}) = [P_{T_{O_2}} - (P_{A_{CO_2}}/0.8)] - P_{A_{O_2}}
\]

**Coagulation studies**

The Prothrombin Time (Dacie and Lewis 1975a) and the Partial Thromboplastin Time (Dacie and Lewis, 1975b) were measured on citrated samples obtained immediately before and at 5 min and 1 h after transfusion. The Partial Thromboplastin Time was measured also on a 50/50 mixture of test plasma and normal plasma.

**Full blood count**

These were performed using a Coulter Model S (Coulter Electronics Ltd) with differential counts performed manually on a 400 cell count. Platelets were counted using a modified Coulter ZF6 particle counter.

**Statistics**

Statistical analysis was performed using a two-way analysis of variance.

**RESULTS**

Red cell survival studies showed no significant difference between any of the filters used (table I). Measurements made during the first 24 h in order to detect any increased immediate red cell destruction showed a wide range of values for all three filters; no consistent differences were detectable between them. Serum bilirubin concentrations showed a marked and significant increase in all cases at 4 h after transfusion, but the increase was significantly less with the Biotest MF 10 filter than with the Baxter filter (table II).

There was no significant difference between the microfiltered and the non-microfiltered transfusion for \( (P_{AO_2} - P_{A_{O_2}}) \) difference (table III). Coagulation studies and platelet counts remained unaltered by the transfusions.
TABLE I. Red cell half-life of transfused blood (days)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pall Ultipor</th>
<th>Baxter FKC 2055</th>
<th>Biotest MF 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>26.5</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>28</td>
<td>20</td>
</tr>
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<td>3</td>
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<td>23</td>
</tr>
<tr>
<td>4</td>
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<td>24</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>17</td>
<td>23</td>
</tr>
</tbody>
</table>

\(P > 0.1\)

TABLE II. Increase in serum bilirubin 4 h after transfusion (\(\mu mol \text{ litre}^{-1}\))

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pall Ultipor</th>
<th>Baxter FKC 2055</th>
<th>Biotest MF 10</th>
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<td>24</td>
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<tr>
<td>6</td>
<td>55</td>
<td>63</td>
<td>50</td>
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\(0.05 > P > 0.01\)

TABLE III. \((P_{A1} - P_{A2}) \text{ (mm Hg)}\) before (A) and after (B) transfusion

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Smoking (S)</th>
<th>Pall Ultipor</th>
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<th>Biotest MF 10</th>
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<tr>
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<td>—</td>
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<td>9.5</td>
<td>0.6</td>
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<tr>
<td>2</td>
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<td>5.5</td>
<td>9.5</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>—</td>
<td>2.0</td>
<td>10.1</td>
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<tr>
<td>4</td>
<td>50</td>
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<td>22</td>
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<td>3.9</td>
</tr>
<tr>
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<td>42</td>
<td>—</td>
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<td>11.6</td>
<td>10.9</td>
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</table>

\(P > 0.1\) \(P > 0.1\) \(P > 0.1\)

Fig. 1. Effect of transfusion of 500 ml of 4-week-old autologous blood on the peripheral white blood cell count (WBC). Mean and observed range for WBC as a percentage of pre-transfusion count shown against hours from the end of transfusion.
The white blood cell count was found to increase markedly immediately after transfusion in all volunteers in all three stages. From a value immediately after transfusion of approximately 2.5 times values before transfusion, the white cell count returned to normal over the ensuing 12 h (fig. 1).

DISCUSSION

Microaggregates of debris are found in stored banked blood (Swank, 1961; Solis et al., 1974). The clinical significance of this observation remains controversial. Many groups have suggested these microaggregates may cause problems with lung function and cerebral function if transfused in large numbers and that this problem can be reduced by removal of these aggregates by filtration (Jenevein and Weiss, 1964; Connell and Swank, 1973; Reul et al., 1973; Branthwaite, 1975). Virgilio and others (1977) showed no pulmonary problems following transfusion of stored blood with or without a microfilter. Whatever the truth of this controversy, filters are widely used.

Of the six filters at present available three are "depth" filters and utilize the fact that microaggregates are sticky and will adhere to filter elements made of dacron wool, polyurethane foam or polyester fibres. By having the blood pass through a considerable depth of element, these filters encourage multiple contacts of the aggregates with the surface in the hope that they will adhere and thus be removed (Guidoin, Gaylor and Borsanyi, 1976; Guidoin and Gilchrist, 1976; Marshall et al., 1978). Two of the six available filters, the Pall Ultipor and the Biotest MF 10, use fine screen mesh elements which trap aggregates mechanically, having a mesh with a pore size of suitable calibre. The remaining filter incorporates both depth and fine screen mesh elements (Marshall et al., 1976).

Many publications indicate that microfilters do not damage the elements of transfused blood. These reports have generally derived their results from in vitro or animal studies as determined by changes in plasma haemoglobin concentration (Dunbar, Price and Cannarella, 1974; Cullen and Ferrara, 1975). We attempted to show if, in the absence of damage to red cells causing haemolysis, there might be "invisible" damage resulting in their early removal by the reticulo-endothelial system. We are not aware of the sensitive technique of red cell survival being used previously in a study of this nature. Blood stored for 4 weeks was used as this duration of storage would increase the rigidity of the red cells (Mollison, 1974) and further increase the sensitivity of the investigation. Our results indicate that, under rapid gravity flow, no shortening of red cell survival could be demonstrated following transfusion through either microfilter although it should be noted that the blood transfused in our study was the first through each filter and these results may not hold for larger quantities. Furthermore, since both filters used in our study were of the fine screen mesh type no conclusions regarding the effect of the various depth filter elements can be drawn.

The initial decrease in radioactivity over the first 24 h showed a wide scatter of results and was not felt to be satisfactory in assessing the amount of red cell destruction occurring immediately after transfusion. However, the increase in serum bilirubin 4 h after transfusion gave figures related to the amount of haemoglobin metabolized immediately following transfusion (Mollison, 1974). The results show a significantly smaller increase in serum bilirubin following transfusion through the Biotest MF 10 filter than through the Baxter FKC 2055 filter, although differences between the Baxter and Pall filters and between the Pall and Biotest filters were not significant at the 5% level. We suggest this may be a result of mechanical trapping within the Biotest filter of effete red cells which, on storage, have "ballooned" to more than 10 μm diameter and which would normally be removed by the reticulo-endothelial system immediately after transfusion. The Pall Ultipor filter and the filter on the Baxter blood administration set have pore sizes well in excess of 10 μm diameter, and would not trap these cells so readily. With transfusion under pressure, however, these cells may be liable to break down in the filter (Haas, Blümell and Gathof, 1977).

It has been suggested that lung function may be compromised by massive transfusion of stored blood and protected by removal of aggregate debris with a microfilter (Ruel et al., 1973; Dawidson et al., 1975). Our results did not show any significant difference between values before and 1 h after transfusion for \( (P_{A_{O_2}} - P_{A_{O_2}}) \) with any of the filters tested.

Thrombus formation has been demonstrated on the elements of microfilters after transfusion of stored blood (Connell and Webb, 1975; Guidoin, 1976) and in view of the large surface area of the filter elements it is possible that activation of coagulation factors may occur even in the presence of an anticoagulant. Infusion of activated coagulation factors to a patient on bypass or receiving massive transfusion could
possibly comprise an already active coagulation system with initiation of an intravascular coagulopathy. In vitro studies have not demonstrated any effect on coagulation (Gervin et al., 1973). Our studies were performed in an attempt to detect any coagulation abnormality induced by the transfusion or the filters. The 50/50 mixture of test and normal plasma was used to screen for early coagulation abnormalities where factor deficiencies are balanced by factor activation giving a normal partial thromboplastin time (PTT) result, but a shortened PTT when factor deficiencies are corrected by addition of normal plasma. No abnormalities were detected following transfusion through any of the filters used. The platelet count often decreases as an early indicator of a consumptive coagulopathy (Milligan et al., 1974) but in this study no significant decrease of the platelet count, even within the normal range, was found.

It was noted during the initial phase of the study that the peripheral blood white cell count immediately after transfusion was increased in all volunteers to about 2.5 times the value before transfusion. This was an unexpected finding although it has been noted previously (Huck, 1919). During subsequent phases of the study the same phenomenon was observed in all subjects with counts in some cases increasing to 20,000 white cells per µl and more. At no time during or immediately after transfusion did any of the volunteers show an increase in sublingual temperature. The leucocytosis was a result of an increase in neutrophils and settled to normal over the ensuing 12 h. In view of the significance often attached to an increased white blood cell count, we feel this observation is worthy of note. The explanation for the increase in white cell count is not clear but may represent the effect of a relatively rapid increase in circulating volume on the margination of neutrophils.

We feel that further observation of the effect of blood transfusion and microfilters on lung function and blood coagulation is indicated and that clarification of the effect of infusion on the white cell count would be of value.

ACKNOWLEDGEMENTS

We gratefully acknowledge the help of Dr A. W. Hutcheon in providing red cell survival results, the Glasgow and West of Scotland Blood Transfusion Service for their assistance with venesection, Mrs N. Haddow for secretarial help and Pall Biomedical Limited and Biotest Folex Limited for financial support.


FILTRATION AU TAMIS FIN DE SANG
PROVENANT DE BANQUES DU SANG:
EFFET SUR LA SURVIVANCE DES GLOBULES ROUGES

RESUME
On a compare deux microfiltres ayant des tamis à fines mailles et l'appareil de Baxter pour l'administration du sang que l'on utilise couramment dans les hopitaux du Royaume-Uni, afin de déterminer leurs effets sur la survivance des globules rouges du sang autologue entreposé et transfusé à six sujets mâles en bonne santé. On a mesuré les effets des transfusions filtrées sur l'échange de gaz pulmonaire et sur la coagulation du sang. On n'a trouvé aucune différence importante entre le microfiltre et l'appareil de Baxter du point de vue survivance des globules rouges du sang transfusé. L'échange de gaz pulmonaire et la coagulation n'ont pas été affectés par la transfusion d'une unité de sang complet. Après la transfusion, les concentrations de bilirubine ont été nettement moindres avec l'un des microfiltres qu'avec l'appareil de Baxter, probablement à cause de la rétention à l'intérieur du filtre des globules rouges épuisés. Une découverte inattendue a été l'effet de la transfusion sur l'augmentation de la numération des neutrophiles du sang périphérique.

BRITISH JOURNAL OF ANAESTHESIA

FEINSIEBFLTRIERUNG VON GESPEICHERTEM BLUT: AUSWIRKUNG AUF DAS ÜBERLEBEN DER ROTEN ZELLEN

ZUSAMMENFASSUNG

FILTRACION POR PANTALLA FINA DE SANGRE ALMACENADA EN BANCO: EFECTO SOBRE LA SUPERVIVENCIA DE LOS GLOBULOS ROJOS

SUMMARIO
Se llevó a cabo una comparación de dos microfiltros a malla de pantalla fina y del equipo de administración de sangre Baxter usado comúnmente en los hospitales del R.U. respecto de sus efectos en la supervivencia de los glóbulos rojos de sangre autóloga almacenada transfundida en seis hombres sanos. El efecto de las transfusiones filtradas sobre el intercambio de gas pulmonar y la coagulación de la sangre fue medido. No hubo ninguna diferencia significativa entre la supervivencia de los glóbulos rojos de transfusión provenientes ya sea de los microfiltros ya sea del equipo Baxter. La transfusión de una unidad de sangre completa no afectó el intercambio de gas pulmonar ni tampoco la coagulación. Las concentraciones post-transfusión de bilirrubina fueron significativamente menores con uno de los microfiltros en comparación con el equipo Baxter, posiblemente por causa de la retención de los glóbulos rojos gastados dentro del filtro. Un resultado imprevisto lo constituyó el efecto de la transfusión al aumentar la numeração de neutrófilos en la sangre periférica.