Aspects of leucocyte and fat filtration during cardiac surgery

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CHAPTER 2

LEUCOCYTE DEPLETION RESULTS IN IMPROVED LUNG FUNCTION
AND REDUCED INFLAMMATORY RESPONSE
AFTER CARDIAC SURGERY

Y. J. Gu, A. J. deVries, P. W. Boonstra, W. van Oeveren

ABSTRACT

Leucocyte depletion during cardiopulmonary bypass (CPB) has been demonstrated in animal experiments to improve pulmonary function. Conflicting results have been reported, however, with clinical depletion by arterial line filter of leucocytes at the beginning of CPB. In this study, we examined whether leucocyte depletion from the residual heart-lung machine blood at the end of CPB would improve lung function and reduce the postoperative inflammatory response. Thirty patients undergoing elective heart operations were randomly allocated to a leucocyte-depletion group or a control group. In the leucocyte-depletion group \((n = 20)\), all residual blood (1.2 L to 2.1 L) was filtered by leucocyte-removal filters and reinfused after CPB, whereas in the control group an identical amount of residual blood after CPB was reinfused without filtration \((n = 10)\). Leucocyte depletion removed more than 97% of leucocytes from the retransfused blood \((p < 0.01)\) and significantly reduced circulating leucocytes \((p < 0.05)\) and granulocytes \((p < 0.05)\) compared with the control group. Levels of the inflammatory mediator thromboxane \(B_2\) determined at the end of operation were significantly lower in the depletion group than the control group \((p < 0.05)\), whereas no statistical differences in interleukin-6 levels were found between the two groups. After operation, pulmonary gas exchange function (arterial oxygen tension at a fraction of inspired oxygen of 0.4) was significantly higher in the leucocyte-depletion group 1 hour after arrival to the intensive care unit \((p < 0.05)\) and after extubation \((p < 0.05)\). There were no statistical differences between the two groups with respect to postoperative circulating platelet levels and blood loss, and no infections were observed during the whole period of hospitalization. These results suggest that leucocyte depletion of the residual heart-lung machine blood improves postoperative lung gas exchange function and is safe to be used for those patients who are expected to develop severe inflammatory response after heart operations.
INTRODUCTION

Cardiopulmonary bypass (CPB) induces a whole body inflammatory response that leads to postoperative lung dysfunction. This response is largely mediated by the activation of polymorphonuclear leucocytes and by subsequent leucocyte deposition and interaction with the lung endothelium. During the initial phase of CPB, leucocytes are activated by the contact of blood with foreign materials in the extracorporeal circuit. After release of the aortic crossclamp in the late phase of CPB, when heart and lungs are reperfused, activation of leucocytes and leucocyte-endothelium interaction are intensified, leading to the impairment of lung function and the induction of a postoperative inflammatory response known as the “post-perfusion syndrome”.

Leucocyte depletion by means of filtration was originally used by blood banks to prevent transfusion complications associated with donor leucoytes. Recent animal experiments demonstrated that leucocyte depletion in different heart operation models reduces heart and lung reperfusion injury. Conflicting results have been noted, however, in reports of clinical use at the beginning of CPB of arterial line-filters with leucocyte-depleting capacities. Furthermore, there has been concern regarding the simultaneous removal of platelets during leucocyte depletion, which could influence postoperative haemostasis.

In this article, we report a study in which only the blood residual in heart-lung machine was depleted of leucocytes, because this blood contains a considerable number of activated leucocytes and is usually reinfused to patients immediately after CPB. We examined whether leucocyte depletion from the residual blood at the end of CPB would improve postoperative lung function and reduce the postoperative inflammatory response. We also examined whether such a “partial” leucocyte depletion method would minimize the major side effect in patients undergoing heart operations, reduction of circulating platelets.

PATIENTS AND METHODS

Patients

After approval by the medical ethical committee in the University Hospital in Groningen and informed consent from patients, 30 patients electively undergoing either coronary artery bypass grafting, heart valve replacement or a combined procedure were randomly allocated to a leucocyte-depletion group \((n = 20)\) or a control group \((n = 10)\). Exclusion criteria were a history of allergy or recurrent infection, reoperation, and emergency operation. The demographic data of patients in both groups are summarized in table 1.

Anaesthesia was induced and maintained by intravenous infusion of sufentanil citrate \((1–3 \, \mu g/kg)\) and midazolam \((0.05–0.1 \, mg/kg)\). Muscle relaxation was achieved with pancuronium bromide \((100–140 \, \mu g/kg)\). Cefamandol 2 g and dexamethason 1 mg/kg were administered after induction. Anticoagulation was achieved by intravenous administration of bovine lung heparin at a dose of 300 IU/kg about 5 minutes before the start of bypass.
Table 1. Patient demographic information

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>Depretion (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>62 ± 13</td>
<td>60 ± 11</td>
</tr>
<tr>
<td>Sex (n)</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>173 ± 8</td>
<td>172 ± 10</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>80 ± 14</td>
<td>77 ± 11</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>197 ± 0.22</td>
<td>19.1 ± 0.16</td>
</tr>
<tr>
<td>Body surface (m²)</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>CABG (n)</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>AVR (n)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>MVR (n)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>CABG + AVR (n)</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>CABG + MVR (n)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation of the mean. CABG, coronary artery bypass grafting; AVR, aortic valve replacement; MVR, mitral valve replacement.

**CPB**

The extracorporeal circuit consisted of roller pumps (Stöckert Instrumente, Munich, Germany) and a microporous polypropylene membrane oxygenator (CML Excel, Cobe Laboratories Inc., Lakewood, CO). Within 10 minutes of CPB initiation at a flow rate at 2.4 L/min/m², the aorta was crossclamped and 1 L of St. Thomas cardioplegia solution (4°C) was infused into the aortic root to provide myocardial preservation. During CPB, moderate hypothermia was induced to maintain the nasopharyngeal temperature between 28 to 30°C. The mean arterial pressure was maintained at 50 to 60 mmHg during CPB. Anticoagulation during CPB was monitored by the celite activated clotting time (International Technidyne Co., Edison, N.J.). After CPB, heparin was neutralised by protamine chloride (3 mg/kg).

**Leucocyte depletion**

Leucocyte depletion was achieved with the use of RC400 leucocyte-removal filters (Pall Biomedical, Portsmouth, UK) designed particularly for leucocyte filtration under high flow conditions in the operating room. After the termination of CPB, a total volume of 1200 mL to 2100 mL residual blood in the extracorporeal circuit was collected into a blood transfusion bag. In the leucocyte-depletion group, the collected blood was filtered by two or three filters and reinfused before the end of operation, whereas in the control group the residual blood was reinfused through the venous transfusion line without leucocyte filtration.
Lung function

Pulmonary gas exchange was measured by the partial arterial oxygen pressure from blood samples drawn from the radial artery line and standardized at a fraction of inspired oxygen of 0.4. Pulmonary haemodynamics exemplified by mean pulmonary artery pressure (PAP) and pulmonary capillary wedge pressure (PCWP) were measured through a Swan-Ganz catheter (Edwards, Baxter Healthcare Corp, Irvine, CA) introduced percutaneously through the right internal jugular vein into the pulmonary artery. Pulmonary vascular resistance (PVR) was calculated according to the following formula: PVR (dyne.sec.cm⁻⁵) = (PAP - PCWP) / CO x 80.

Other clinical parameters

Duration of postoperative intubation was recorded during each patient’s stay in the intensive care unit. Blood loss was indicated by 24-hour chest drainage. In addition, durations of stay in the intensive care unit and of hospitalization after operation were obtained from hospital registration records.

Laboratory parameters

For laboratory haematologic tests and biochemical assays, blood samples were taken from the indwelling radial arterial catheter at the baseline before operation, at the end of CPB before transfusion of the leucocyte-depleted blood, at the end of operation during skin closure, 1 hour and 3 hours after the patient’s arrival in the intensive care unit, and at 6 am the next day in the intensive care unit. In addition, prefiltration and postfiltration samples were taken from the transfusion bags to determine the cell counts and calculate the rate of leucocyte removal.

Cell counts were determined by a cell counter (Cell-Dyn 610, Sequoia Turner, Mountain View, CA) with a dilution of 1:250 for counting leucocytes and granulocytes and of 1:25,000 for counting platelets. For the postfiltration samples, leucocytes were counted by means of the Nageotte manual counting chamber or by the cell counter with a dilution of 1:100.

For biochemical assays, plasma was obtained by centrifugation of whole blood at 1100 g and stored at -80°C until further determinations. Thromboxane was determined by enzyme immunoassay (Cayman Chemical Company, Ann Arbor, Mich) in plasma anticoagulated with citrate and indomethacin. Interleukin-2 and interleukin-6 were determined by enzyme immunoassay (Quantikine, R&D Systems Europe, Abingdon, UK) from citrated plasma.

Statistics

Data processing as well as statistical tests were performed with the StatView software (Brain-power Inc, Calabasas, CA). Data are expressed as mean plus or minus standard error of the mean unless otherwise indicated. A repeated-measures analysis of variance was used to determine the difference between the two groups. Student’s t test or Mann-Whitney test was used for analysis of difference between the two groups at each sampling or recording time point. A p-value less than 0.05 was considered statistically significant.
RESULTS

There were no significant difference between the leucocyte-depletion group and the control group with respect to duration of CPB and aortic crossclamp time. All patients recovered uneventfully after operation.

Leucocyte reduction in residual machine blood

The average leucocyte count determined from the residual machine blood before filtration was 5.76±0.44 x 10^9/L. After filtration, the count was 0.152±0.01 x 10^9/L. More than 97% of leucocytes were removed from the residual blood in the leucocyte-depletion group. The average platelet count from the machine blood before filtration was 107±6 x 10^9/L, after filtration, it was 43±2 x 10^9/L. About 60% of the platelets in the machine blood were removed by the filters in the leucocyte-depletion group.
Circulating leucocytes and platelets

Circulating leucocyte and granulocyte counts at the end of operation were significantly less in the leucocyte-depletion group than in the control group (p < 0.05). There were no significant differences in circulating lymphocyte and platelet counts between the two groups (figure 1).

Inflammatory mediators

Thromboxane B₂ levels were significantly lower in the leucocyte-depletion group than in the control group at the end of operation (p < 0.05; table 2). Interleukin-6 levels increased in both the leucocyte-depletion and control groups during the early postoperative period. No significant difference was found between the two groups. Interleukin-2 was not detectable in any of the samples.

Lung function

Pulmonary gas exchange, measured by partial oxygen pressure, was significantly higher in the leucocyte-depletion group than that in the control group both at one hour after arrival in the intensive care unit (118 ± 10 mmHg versus 86 ± 10 mmHg, p < 0.05) and immediately after extubation (120 ± 8 mmHg versus 89 ± 10 mmHg, p < 0.05, figure 2). PAP was somewhat lower in patients receiving leucocyte depletion than in the control group, but this difference in PAP was not significant. Similarly, there were no statistical differences in PCWP and PVR between the two groups (table 3).

Other clinical outcomes

There was no significant difference between the two groups with respect to postoperative blood loss recorded from the chest drainage until the first postoperative morning. Duration of intubation after operation was slightly shorter in the leucocyte-depletion group than in the control group, but this difference was not statistically significant. Similarly, no statistical difference was found between the two groups regarding the duration of intensive care unit and hospital stay (table 4).

Table 2. Inflammatory mediators before and after operation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before CPB</th>
<th>End CPB</th>
<th>End operation</th>
<th>ICU 1 hr</th>
<th>ICU 3 hr</th>
<th>POD 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromboxane (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depletion</td>
<td>ND</td>
<td>48 ± 15</td>
<td>48 ± 9*</td>
<td>33 ± 7</td>
<td>23 ± 29</td>
<td>19 ± 26</td>
</tr>
<tr>
<td>Control</td>
<td>ND</td>
<td>62 ± 96</td>
<td>127 ± 63</td>
<td>48 ± 15</td>
<td>59 ± 56</td>
<td>21 ± 34</td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depletion</td>
<td>36 ± 14</td>
<td>126 ± 96</td>
<td>ND</td>
<td>393 ± 116</td>
<td>344 ± 90</td>
<td>125 ± 46</td>
</tr>
<tr>
<td>Control</td>
<td>20 ± 24</td>
<td>197 ± 246</td>
<td>ND</td>
<td>208 ± 103</td>
<td>260 ± 38</td>
<td>155 ± 29</td>
</tr>
<tr>
<td>Interleukin-2 (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depletion</td>
<td>UD</td>
<td>UD</td>
<td>ND</td>
<td>UD</td>
<td>UD</td>
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</tr>
<tr>
<td>Control</td>
<td>UD</td>
<td>UD</td>
<td>ND</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
</tbody>
</table>

Values are expressed as the geometric mean and the standard error of the mean. ICU, intensive care unit; POD, postoperative day; ND, not determined; UD, undetectable (below the lowest detectable level stated by the manufacturer).

* p < 0.05 compared with control.
Figure 2. Arterial partial O₂ pressure (PaO₂) determined after arriving in the intensive care unit (ICU) and after extubation in patients receiving leucocyte depletion (Depletion, n = 20) and in patients without receiving depletion (Control, n = 10). The fraction of inspired oxygen was standardized at 40%. (*p < 0.05 between the two groups)

Table 3. Pulmonary haemodynamics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before CPB</th>
<th>End CPB</th>
<th>AP</th>
<th>End operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depletion</td>
<td>16 ± 1.1</td>
<td>17 ± 1.0</td>
<td>14 ± 1.4</td>
<td>17 ± 1.1</td>
</tr>
<tr>
<td>Control</td>
<td>17 ± 0.9</td>
<td>20 ± 0.7</td>
<td>19 ± 0.7</td>
<td>21 ± 1.0</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depletion</td>
<td>10 ± 0.9</td>
<td>12 ± 1.4</td>
<td>10 ± 1.5</td>
<td>10 ± 1.2</td>
</tr>
<tr>
<td>Control</td>
<td>12 ± 0.7</td>
<td>13 ± 1.1</td>
<td>11 ± 0.9</td>
<td>14 ± 1.3</td>
</tr>
<tr>
<td>PVR (dyne.sec⁻¹ .cm⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depletion</td>
<td>156 ± 34</td>
<td>129 ± 32</td>
<td>120 ± 23</td>
<td>173 ± 25</td>
</tr>
<tr>
<td>Control</td>
<td>145 ± 18</td>
<td>116 ± 22</td>
<td>103 ± 15</td>
<td>125 ± 14</td>
</tr>
</tbody>
</table>

AP, after protamine. Values expressed as mean ± standard error of the mean.

Table 4. Perioperative and postoperative data

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>Depletion (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPB time (min)</td>
<td>100 ± 24</td>
<td>94 ± 38</td>
</tr>
<tr>
<td>Crosclamp time (min)</td>
<td>67 ± 19</td>
<td>63 ± 29</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>685 ± 79</td>
<td>587 ± 87</td>
</tr>
<tr>
<td>Intubation time (hr)</td>
<td>13.8 ± 1.3</td>
<td>11.7 ± 0.9</td>
</tr>
<tr>
<td>ICU stay (days)</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>8.7 ± 2.1</td>
<td>7.3 ± 0.5</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation of the mean. CU, intensive care unit.
DISCUSSION

Leucocyte depletion from systemic circulation during cardiopulmonary bypass has been reported to reduce free radical-mediated lung injury and granulocyte-mediated ventricular dysfunction in animal experiments. Clinically, however, leucocyte depletion using an arterial line filter at the beginning of CPB has not achieved the goals of reducing intraoperative and postoperative leucocytosis and of improving lung function after heart operations. In this study, we have demonstrated that leucocyte depletion of only 1.2 to 2.1 litres residual heart-lung machine blood significantly attenuates postoperative leucocytosis and improves pulmonary gas exchange function in patients undergoing heart operations. Of more importance, because this blood is usually transfused through the venous line without any substantial filtration, leucocyte depletion in this setting may have provided a local protective effect for the lungs, even if the amount is relatively small.

There are at least two reasons why leucocyte depletion of the residual heart-lung machine blood may protect the lung. First, we observed in a recent study that the residual heart-lung machine blood contained higher levels of leucocyte release products than seen in the systemic circulation, suggesting that the leucocytes remaining in the heart-lung machine are highly activated. Second, it is known that the heart-lung machine blood contains a number of foreign substances as well as microaggregates formed mainly by platelets and leucocytes. During CPB, the blood is returned to patients from the arterial side of the heart-lung machine, where an arterial line filter removes microaggregates. After CPB, however, the residual heart-lung machine blood is reinfused to patients via intravenous transfusion without any substantial filtration (usually only a clot filter with large pore size is used). Because the lung is anatomically located to receive all the reinfused blood from venous side, lung injury may occur as a result of the pulmonary accumulation of microaggregates mediated by trapped platelets and leucocytes.

In fact, current leucocyte-depleting filters remove not only leucocytes but also other particulates less than 5 µm in diameter. It has been reported recently that a similar type of blood transfusion filter was able to remove the microfibrillar collagen haemostat from the wound blood harvested from the surgical field. Particulate microaggregates are continuously generated during CPB; this is particularly evident in the cardiotomy returning line. These microaggregates are mostly smaller than 30 µm in diameter and are not always caught by the cardiotomy filter which usually has a pore size between 20 to 40 µm. Because the residual machine blood collected at the end of CPB contains a large portion of blood from the cardiotomy reservoir, filtration with a leucocyte-removal filter may prevent any particulates larger than 5 µm from being retransfused to patient, thereby reducing lung injury.

Although a direct comparison of our results with results obtained from arterial line leucocyte depletion is unjustified, it does appear that leucocyte depletion of the residual machine blood is more likely to have a local effect on protecting the lungs. In addition, leucocyte depletion with transfusion filters may have other advantages in clinical application. The procedure is easy to handle because the filter can be installed at any time before use without flush or priming. Moreover, it could serve as an optional intervention method that can be added at the end of CPB according to patient’s clinical condition, particularly for patients with a longer duration of CPB and a predicted strong postoperative inflammatory response. One potential disadvantage
of this method, however, is the limited blood volume available for filtration, which depends on the volume of residual blood in the heart-lung machine.

The inflammatory mediator thromboxane $\text{B}_2$ is usually increased during and after CPB in patients undergoing heart operations. In this study, we observed a significantly reduction of plasma thromboxane $\text{B}_2$ at the end of operation in the leucocyte depletion group compared with the control group; this difference can be explained by the removal of activated leucocytes and the simultaneous removal of platelets after the end of CPB. We also measured interleukin-6 and interleukin-2; the former is a marker of acute-phase response produced by mononuclear phagocytes and the latter is mainly produced by lymphocytes. We confirmed that the peak release of interleukin-6 occurred about 1 hour after arrival in the intensive care unit, as reported by other groups. No significant difference was found between the depletion and the control groups, however, which suggest that leucocyte depletion in this setting has no effect on the release of interleukin-6 during the early postoperative period. Interleukin-2 was not detectable in any samples, indicating that there was no lymphocyte-associated release of cytokines in these patients. This is in agreement with a recent report that interleukin-2 could be detected only occasionally after heart operations.

One of the concerns regarding leucocyte depletion during heart operations is that the simultaneous removal of platelets might affect postoperative haemostasis. In this study, little influence on circulating platelet count was observed in patients receiving leucocyte depletion, although considerable numbers of platelets were removed from the reinfused heart-lung machine blood. Consistently, there was no significant difference between the two groups with respect to the postoperative blood loss. On the other hand, it remains to be elucidated whether removal of platelets from the residual heart-lung machine blood contributed to improved postoperative lung function. It is known that the platelets may deposit in the myocardium during reperfusion, leading to myocardial reperfusion injury. Moreover, release products from platelets such as platelet activating factor and platelet associated adhesive molecules may further activate leucocytes and promote leucocyte adhesion to the endothelium. This mechanism may also operate in initiating lung injury because platelet deposition occurred similarly during lung reperfusion in the lung microvasculature.

In conclusion, leucocyte depletion from residual heart-lung machine blood at the end of CPB improves postoperative lung gas exchange function and reduces postoperative leucocytosis. Furthermore, leucocyte depletion in this setting did not result in any postoperative complications with respect to haemostasis and infection. Further investigations should be carried out to compare the different leucocyte depletion methods with respect to their clinical benefits against costs, and to determine which patient populations can profit most from this intervention.

ACKNOWLEDGEMENT

We thank the perfusion team and the intensive care unit nursing staff in the Thorax Center, University Hospital Groningen for collecting the clinical data and J. Haan in the Blood Interaction Research Lab for performing the biochemical assays.
REFERENCES


