Aspects of leucocyte and fat filtration during cardiac surgery

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CHAPTER 7

CLINICAL EVALUATION OF A NEW FAT REMOVAL FILTER DURING CARDIAC SURGERY.


ABSTRACT

Fat microemboli are generated during cardiac surgery that are associated with postoperative organ injury. Recently, a fat removal filter has been developed, based on a polyester leucocyte depletion filter. However, the efficacy of such a filter in a clinical setting is unknown. In this study we tested the efficacy of this filter.

Coronary artery bypass patients were randomly divided in two groups. Group I: filtration of cardiotomy suction blood during cardiopulmonary bypass with a fat removal filter (n = 14). Group II: control patients without filtration (n = 14). Filter efficacy was evaluated in group I using biochemical assays and thin layer chromatography of blood samples taken simultaneously before and after the filter. In addition, clinical and biochemical markers for organ injury were determined in both groups.

We found that the fat filter removed triglycerides (0.9 ± 0.08 mmol.L\(^{-1}\) vs. 0.63 ± 0.08 mmol.L\(^{-1}\), \(p = 0.004\), paired \(t\)-test), leucocytes (4.3 ± 0.8 x 10\(^9\) vs.2.3 ± 0.6 x 10\(^9\) .L\(^{-1}\), \(p = 0.03\)), and platelets (116 ± 26 x 10\(^9\) vs. 75 ± 21 x 10\(^9\) .L\(^{-1}\), \(p = 0.003\)) from the blood samples taken before and after the filter. Chromatography showed a significant reduction in free fatty acids, phospholipids and triglycerides. Clinically, leucocyte counts were similar, but platelet counts were higher (181 ± 14 x 10\(^9\).L\(^{-1}\) v. 117 ± 8.6 x 10\(^9\).L\(^{-1}\) control, \(p < 0.001\)) in group I on the first postoperative day.

This study shows that the fat filter removed 40% fat, leucocytes and platelets from cardiotomy suction blood during cardiac surgery. A larger scale study is necessary to determine clinical effects on organ damage.
INTRODUCTION

Recently, fat microemboli have been demonstrated in brain tissue after cardiopulmonary bypass (CPB).\(^1\) These were related to retransfusion of cardiotomy suction blood,\(^2\) and associated with postoperative neurocognitive dysfunction.\(^3\) Therefore, attention is again focussed on the adverse effects of retransfusion of cardiotomy suction blood during cardiac surgery. In addition, the role of fat on organ damage may have been underestimated, because fat microemboli have not only been demonstrated in brain tissue after CPB, but also in lung tissue.\(^4\) In animal experiments, the injection of free fatty acids, particular oleic acid, consistently produces the development of an acute respiratory distress syndrome.\(^5\) Finally, fat deposits have been demonstrated in kidney tissue\(^6\) as well as in urine after CPB.\(^7\)

Several strategies are used to prevent retransfusion of cardiotomy suction blood. Off-pump revascularization is increasingly performed, but is not suitable for intra-cardiac surgery. In some centers the cardiotomy suction blood is completely discarded,\(^8\) but this may lead to increased allogenic transfusion requirements. Cell savers are used to wash the wound suction blood, but their use is expensive, and the quality of the processed blood is questioned.\(^9\) Retransfusion of cardiotomy suction blood, however, is still used during CPB, and thus a novel approach with a simple and inexpensive filter for the removal of fat and debris from cardiotomy suction blood may be an alternative. Such a fat removal filter has been developed. This is a polyester 40 \(\mu\)m screen filter, which is based on a leucocyte removal filter and allows high flow transfusions. In a laboratory experiment this filter removed fat from reconstituted blood.\(^10\) In patients however, little is known about the performance of a fat removal filter. The aim of this study was therefore to assess the efficacy of a fat removal filter in a clinical setting by filtering cardiotomy suction blood during CPB in patients undergoing coronary artery bypass grafting (CABG). As this study also served as a pilot study for cardiotomy blood filtration on postoperative organ injury, we evaluated some of the possible filter effects on lung, kidney and heart with clinical and biochemical markers, using an unfiltered group of CABG patients as controls.

MATERIAL AND METHODS

Patients

After institutional human investigation committee approval and patient consent 28 patients scheduled for CABG were prospectively randomized to Group I: fat filtration of cardiotomy suction blood during CPB (\(n = 14\)), or Group II: control patients without filtration (\(n = 14\)). Exclusion criteria were pre-existing lung disease, cerebral vascular disease, diabetes mellitus, emergency operation and re-operation. Blood samples were drawn from the radial artery (1) after induction of anaesthesia, (2) at the end of the operation, (3) after three hours in the ICU, and (4) on the morning of the first postoperative day. From blood samples taken pre-operatively and postoperatively on day 1, 2 and 6, the creatinin clearance was calculated according to the Cockcroft formulae.\(^11\)
Anaesthesia and perfusion

Anaesthesia was induced and maintained by intravenous infusion of midazolam (0.1 mg.kg⁻¹) and sufentanil (1.5 µg.kg⁻¹), as previously described.¹² Pancuronium (0.1 mg.kg⁻¹) was used for muscle relaxation. Dexamethasone (1 mg.kg⁻¹) was given after induction. Ventilatory management was aimed at normocapnia throughout the operation and in the intensive care unit (ICU), with an inspiratory oxygen fraction of 0.4, a positive end-expiratory pressure of 6 cm H₂O and a tidal volume of 6-8 mL.kg⁻¹. Bovine lung heparin (300 IU.kg⁻¹) was used for anticoagulation. This was monitored by the celite activated clotting time (ACT) (International Technidyne, Edison, NJ, USA) and maintained at a value ≥ 400 s. After CPB, heparin was neutralized by protamine (300 IU.kg⁻¹).

The extracorporeal circuit consisted of roller pumps (Stöckert, München, Germany), a hollow fibre oxygenator (Sarns Turbo, 3M, St. Paul, MN, USA) and a standard arterial line filter (Affinity 38µ Medtronic, Minneapolis, MN, USA). The priming consisted of 500 mL hydroxyethylstarch 10% (Haes, Fresenius, Bad Homburg, Germany) and 1000 mL lactated Ringer’s solution. Pump flow was adjusted to 2.4 L.m⁻² per min. Nasopharyngeal temperature during CPB was maintained at 32°C.

Filtration procedure

In the filter group the cardiotomy suction blood was collected in a separate cardiotomy reservoir (ATR120, Fresenius, Bad Homburg, Germany) from the moment that the ACT was ≥ 400 s. After aortic cross clamp release this cardiotomy blood passed under gravity through a fat removal filter (LipiGuard, Pall, Portsmouth, UK) into the cardiotomy reservoir of the CPB circuit. After each 600 mL of cardiotomy blood a new filter was used. In the control group the cardiotomy suction blood was collected directly in the cardiotomy reservoir of the CPB circuit from the moment that the ACT was ≥ 400 s.

In both groups, the residual blood in the extracorporeal circuit after CPB was collected in a transfusion bag and transfused into the patient using a standard transfusion system.

Measurements

When 200 mL blood had passed through the filter, samples were taken simultaneously before and after the filter. From EDTA-anticoagulated blood, hematocrit, platelet and total white blood cell counts were determined by an electronic cell counter (Cell-Dyn 610, Abbott, Santa Clara, CA, USA). Triglyceride levels were determined with a biochemical assay (Sigma, St. Louis, MO, USA).

To assess the capacity of the filters blood samples were taken from 4 additional filters in separate patients after 50 mL, 200 mL and 600 mL of blood had passed through the filter. From these samples platelet and total white blood cell counts, and triglyceride levels were measured as before.

In addition, to assess the qualitative effects of filtration, modified thin layer chromatography according Folch¹³ was performed on samples before and after the filter and on a patient blood sample before CPB, as well as on the blood samples that were taken for the assessment of the filter capacity. Briefly, plasma was extracted with a chloroform-methanol mixture. The extract was mixed with butylated hydroxytoluene to avoid oxidation and after drying solved in chloroform. On a silica plate 10 µL
samples were applied. This was run with a mixture of n-hexane-diethylether-acetic acid and developed with copper sulphate in fosforic acid. Five bands were discerned: cholesteryl esters, triglycerides, free fatty acids, cholesterol and phospholipids. For a semiquantitative evaluation of the chromatography, the bands were scanned by computer and the intensity of the bands was attributed a score from 0, being totally white to 100, being totally black. The values given in table 3 are these computerized density scores.

As we found a significant preservation of platelet counts after filtration including significant higher postoperative circulating platelet counts in the filtration group, we retrospectively analyzed the adsorption of the filter material of platelet activating factor. Therefore, we incubated 40 mg pieces of filter material with 0.05 mM purified platelet activating factor (PAF) C-18 (Sigma, St Louis, Mi, USA) which was then added to fresh platelet rich plasma from healthy volunteer blood. Platelet aggregation was compared between PAF C-18 with or without pre-incubation with filter material by means of optical aggregometry (Chronolog, Havertown, PA, USA).

Statistics

The sample size for this study was calculated on the assumption that the fat filter would remove at least 50% of the fat from the surgical cardiotomy suction blood. Twelve patients would therefore be needed with an power of 0.8 and an $\alpha$ of 0.05. All data are presented uncorrected for haemodilution and expressed as mean ± standard error unless otherwise stated. For comparison of single data between the groups a two-tailed Student’s $t$-test was used. For comparison of the measurements before and after the filter a paired Student’s $t$-test was used. Two way analysis of variance (ANOVA) for repeated measurements was used to determine the effects of time, group and interaction over the different time points. In case of multisample sphericity Greenhouse-Geisser ($\epsilon$) adjustments were made. To allow for multiple comparisons the results were corrected using the least square difference method. A $p$-value $\leq 0.05$ was considered statistically significant.

RESULTS

Both groups were similar with respect to age, sex, length, weight, haematocrit ($p = 0.16$), creatinin clearance, grafts and CPB time ($p = 0.2$). The demographic data are summarized in table 1.

Filter characteristics

The amount of cardiotomy suction blood was $1104 \pm 152$ mL with a haematocrit of $19 \pm 1.4\%$. Baseline plasma triglyceride level in the patients was $1.02 \pm 0.15$ mmol. L$^{-1}$. The filter removed 30% of the triglycerides and reduced leucocytes by 47% and platelets by 35% (table 2). Thin layer chromatography revealed that after filtration, free fatty acids (FFA), triglycerides and phospholipids were reduced (table 2). The efficacy of the filter decreased slightly during the 600 mL of blood that passed through the filter. After 600 mL of blood the filter removed 13% of the triglycerides and reduced leucocytes by 34% and platelets by 31%. Thin layer chromatography of
Table 1. Demographics

<table>
<thead>
<tr>
<th>Group</th>
<th>Filter</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>62 ± 2.5</td>
<td>63 ± 3.2</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>176 ± 2.6</td>
<td>172 ± 2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81 ± 2.5</td>
<td>77 ± 2</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>33.5 ± 0.9</td>
<td>35.8 ± 1.3</td>
</tr>
<tr>
<td>Creatinin Cl (mL.kg⁻¹ per min)</td>
<td>76 ± 4</td>
<td>72 ± 7</td>
</tr>
<tr>
<td>CPB (min)</td>
<td>97 ± 9.2</td>
<td>83 ± 7.1</td>
</tr>
<tr>
<td>Grafts</td>
<td>arterial (n)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>venous (n)</td>
<td>20</td>
</tr>
</tbody>
</table>

Creatinin Cl, creatinin clearance according to the Cockcroft formulae; CPB, cardiopulmonary bypass.

Table 2. Filter performance

<table>
<thead>
<tr>
<th>Samples</th>
<th>before the filter</th>
<th>after the filter</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Biochemical assays</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol.L⁻¹)</td>
<td>0.9 ± 0.09</td>
<td>0.63 ± 0.07</td>
<td>0.003</td>
</tr>
<tr>
<td>Leucocytes (x10⁶.L⁻¹)</td>
<td>4.3 ± 0.8</td>
<td>2.3 ± 0.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Platelets (x10⁹.L⁻¹)</td>
<td>116 ± 26</td>
<td>75 ± 21</td>
<td>0.003</td>
</tr>
<tr>
<td><em>Chromatography</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>7.6 ± 1.1</td>
<td>4.1 ± 0.8</td>
<td>0.005</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>6.4 ± 1.0</td>
<td>3.4 ± 1.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>49.3 ± 3.4</td>
<td>45.3 ± 3.8</td>
<td>0.04</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>35.1 ± 2.8</td>
<td>31.9 ± 2.7</td>
<td>0.11</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>9.8 ± 1.4</td>
<td>9.7 ± 1.7</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Values given for the chromatography are the computerized density scores. See text for explanation. The p-values reflect the statistical analysis of the samples taken before and after the filter by one-way Student t-test.

Table 3. Clinical results on the first postoperative day and hospital stay

<table>
<thead>
<tr>
<th>Group</th>
<th>Filter</th>
<th>Control</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr Cl (mL.kg⁻¹ per min)</td>
<td>101 ± 6.5</td>
<td>79 ± 7.9</td>
<td>0.04</td>
</tr>
<tr>
<td>Fluid in (mL)</td>
<td>4040 ± 262</td>
<td>4072 ± 291</td>
<td>0.94</td>
</tr>
<tr>
<td>Blood loss (mL)</td>
<td>928 ± 126</td>
<td>753 ± 99</td>
<td>0.29</td>
</tr>
<tr>
<td>Diuresis (mL)</td>
<td>2920 ± 215</td>
<td>3183 ± 308</td>
<td>0.49</td>
</tr>
<tr>
<td>CK-enzymes (IU.L⁻¹)</td>
<td>236 ± 56</td>
<td>169 ± 27</td>
<td>0.32</td>
</tr>
<tr>
<td>CK-MB (IU.L⁻¹)</td>
<td>12 ± 6.4</td>
<td>6 ± 1.8</td>
<td>0.44</td>
</tr>
<tr>
<td>Platelets (x10⁹.L⁻¹)</td>
<td>181 ± 14</td>
<td>117 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leucocytes (x10⁶.L⁻¹)</td>
<td>15.6 ± 0.9</td>
<td>13.5 ± 1</td>
<td>0.13</td>
</tr>
<tr>
<td>Hospital stay (day)</td>
<td>7.2 ± 0.8</td>
<td>10.8 ± 1.5</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Cr Cl, renal creatinin clearance according to the Cockcroft formula; CK, creatinin kinase; MB, myocardial band creatinin iso-enzyme.
these four filters after 600 mL revealed the same pattern as after 200 mL, with reductions in FFA, triglycerides and phospholipids. The time needed to pass 200 mL of blood under gravity at a height of 90 cm was 2 min 40s ± 13s, and for 600 mL this time was 7 min ± 20s. We found no adsorption of (hydrophobic) platelet activating factor (PAF) on the filter material, excluding its effect on the preservation of the blood platelets.

Clinical effects

The calculated creatinin clearance was higher in the filter group on the first postoperative day (p = 0.04) (tables 1 and 3). The two groups were similar with respect to fluid intake, diuresis, blood loss, lung function and myocardial injury (table 3). In the control group, one patient had a myocardial infarction (defined as new Q-wave on the ECG and CK > 180 U/L with CK-MB > 10% of total), one patient had major blood loss and one patient developed renal function disturbances with a serum creatinin level of 231 mmol.L⁻¹. Overall hospital stay was slightly shorter in the filter group (table 3). It is noted that the attending ICU and hospital staff were blinded to the study groups.

The PaO₂ showed a time effect (p = 0.001), but there was no difference between the groups (p = 0.25) (figure 1). The A-a gradients showed a time effect (p < 0.001), but no difference between the groups (p = 0.25) (figure 1).

![Figure 1](image)

**Figure 1.** Arterial oxygen tension (PaO₂) and alveolar-arterial (A-a) oxygen gradients in the fat filter group and in the unfiltered control group preoperatively (pre-op), at the end of operation (end-op), after 3 hours in the intensive care unit (3h ICU) and on the morning of the first postoperative day (day 1). Values shown are the means, estimated by the repeated measurement model with standard error. The PaO₂ showed a time effect (p = 0.009), but no difference between the groups. The A-a gradients showed a time effect (p < 0.001), but no difference between the groups.
The postoperative platelet counts on the first postoperative day were higher in the filter group than in the control group (figure 2, table 3). There was a time effect ($p < 0.001$) and a difference between the groups ($p = 0.04$). The postoperative circulating leucocyte counts were similar in both groups. There was a time effect ($p < 0.001$), but there was no difference between the groups ($p = 0.08$) (figure 2).

**DISCUSSION**

This study showed that the application of a fat removal filter reduced the fat content of cardiotomy suction blood in cardiac surgical patients. The filter removed 46% of the free fatty acids and 30% of the triglycerides as shown by thin layer chromatography and plasma samples.

The mechanism for fat removal is not clear. The filter consists of tightly packed fibers with a porous structure of about 40 μm. This may mechanically stop the larger fat globules. Such a view is supported by a recent study on cardiotomy suction blood.\textsuperscript{14} Fat microemboli were divided in large (> 50 μm) and small (10-50 μm) size emboli. In a subset of 6 patients an additional filter was placed after the cardiotomy reservoir. No large emboli were detected after the filter. In our filter the removal of the various fat subgroups was highly variable. This may be explained by a difference in electrostatic
adhesion to the filter material. The thin layer chromatography supports this view, because the more polarized substances as the free fatty acids were removed more effectively. One could therefore speculate on filter improvement by coating of the fibres to increase the removal of the other subgroups, but clinically the free fatty acids appear to be the most important. Increased levels of free fatty acids have documented effects. In pancreatic tissue \( \beta \) cells are damaged.\(^{15}\) In kidney tissue tubulointerstitial damage is aggravated.\(^{16}\) In lung tissue free fatty acids are associated with the development of an acute respiratory distress syndrome.\(^{5}\) In endothelial cells free fatty acids cause vasoconstriction and granulocytes are activated through surface expression and activity of CD11b.\(^{17}\)

We found a lower overall efficacy of the filter in the clinical setting of our study than previously reported in a laboratory setting with reconstituted blood.\(^{10}\) It has recently been shown that the composition of the cardiotomy suction blood is different, and that a low temperature increases filter efficacy.\(^{18}\) This could explain our results and is supported by another clinical study that also showed a moderate efficacy of this filter in 3 orthopaedic patients.\(^{19}\) Free fatty acids are bound to albumin. Plasma albumin is reduced by haemodilution after CPB. For this reason we did not use a prime with albumin, but instead used hydroxyethylstarch, which is not known to interfere with binding of free fatty acids.

With about 85 mL/min the filter appeared to have a high flow during transfusion under gravity. However, a high flow reduces the contact time between blood and filter medium and thus may result in a lower filter efficiency.\(^{20}\) Thus, filter efficiency may be improved by coating the fibers, or alternatively by packing more filter materials in the housing. This latter option would reduce the flow over the filter. However, a flow of 30 mL/min should be sufficient to filter 1.5 L, which equals the amount of cardiotomy suction blood, during a cross clamp time of 45 min. For widespread use the fat removal filter will need a larger capacity, as our results indicated that the filter became saturated after 600 mL, requiring to change it.

We did not measure lipoprotein levels in this study. Lipoproteins consist of a layer of phospholipids which covers triglycerides and cholesterol esters. These complexes are necessary to facilitate lipid transport through the plasma compartment. The objective of the identification of the several subgroups of lipoproteins lies in their contribution to the atherosclerotic risk profile. That was not the purpose of this study. Moreover, we speculated that fat release during the operation would mainly result from mechanical damage through surgical manipulation and shear forces. This would result in a direct release of the triglycerides and free fatty acids, which we measured.

Several clinical findings in this small pilot study suggest a beneficial effect of the filter. First, the higher calculated creatinin clearance in the filter group on the first postoperative day in view of a similar postoperative fluid balance. Fat emboli have been demonstrated in the kidney after CPB,\(^{6}\) and also after experimental fat embolism syndrome.\(^{21}\) The second is the higher postoperative platelet counts in the filter group. Platelets and leucocytes in the cardiotomy suction blood are activated in the presence of fat and tissue factor from the pericardium.\(^{22}\) Thus, removal of platelets and leucocytes by the filter may be advantageous and protective against the systemic inflammatory response and thrombus formation.
It has been reported that activated platelets do not remain in the circulation but are actively cleared. This may explain the higher postoperative circulating platelet counts in the filter group, suggesting that the platelets were less activated than in the control group. Direct adsorption of platelet activating factor by the filter was not shown as a mechanism of higher circulating platelet counts after filtration. We have not determined β-thromboglobulin levels, as the effects of the filter on the circulating platelet counts were not expected. Measurement of leucocyte activation, for example by determination of CD11/CD18, could have clarified the slightly higher postoperative circulating leucocyte counts in the filter group, because it is known that free fatty acids result in surface expression and activity of CD11b on human neutrophils.

Third is postoperative oxygenation. Although not significant different in itself due to the small sample size, the fact that the postoperative A-a gradients were smaller, and the postoperative PaO₂ values were higher in the filter group suggest that in the filter group less pulmonary injury occurred. This may be explained by the fact that the filter significantly reduced free fatty acids, known for their deleterious effects on lung function. In addition, the filter also removed a significant part of the leucocytes from the suction blood. We have previously shown that filtration of leucocytes improved postoperative lung function.

Several other possibilities for the management of the cardiotomy suction blood exist. Cell savers are increasingly used, but these devices might be less than ideal for several reasons. First, fat is not completely removed by cell savers. Thus, as a consequence, even cell saver blood may benefit from the application of a fat removal filter before retransfusion. Second, their use is expensive and requires attention and time to process. In contrast, fat removal filters are cheaper, about 25% of the cost of a cell saver, they are very easy to operate and processed blood is immediately available. Kaza found cell savers not more effective than the application of a filter after the cardiotomy reservoir for the elimination of small and large fat emboli. Third, processed cell saver blood contains increased levels of interleukin-1 and activated leucocytes, which may aggravate the inflammatory reaction associated with CPB.

There are shortcomings in this study. It was underpowered to detect clinical differences between the groups. Based on our results, at least 35 patients in each group had to be included to demonstrate clinical differences with a power of 0.8 and an α of 0.05. However, our results suggest that it would be worth to perform such a study. Further, we use routinely dexamethasone for all our patients to reduce the inflammatory reaction after CPB. The incidence of the fat embolism syndrome was decreased in a prospective randomized clinical trial, where steroids were given to prevent the effects of the fat embolism syndrome. Therefore, the effects of the fat removal filter on organ damage could be more pronounced than demonstrated in this study. Third, we did not use a separate cardiotomy reservoir in the control group. Instead, the cardiotomy blood was gradually mixed with the patients’ blood during the whole CPB period as usual. This gradual mixing may have reduced the effects of the transfusion of cardiotomy blood in the control group.

In conclusion, our results demonstrate that the fat removal filter removed approximately 40% of fat, leucocytes and platelets from cardiotomy suction blood. The efficiency and capacity of the filter should be improved and a prospective study of the effects on postoperative organ damage should be performed. The application of a fat filter however, is not the ultimate answer to a reduction of microemboli. It is estimated
that 60% of the emboli during surgery are caused by surgical manipulation. However, the presence of cerebral fat microemboli justify that every effort is done to reduce the fat load for the patient.

REFERENCES