Aspects of leucocyte and fat filtration during cardiac surgery

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CHAPTER 8

THE CLINICAL APPLICATION OF A FAT REMOVAL FILTER: BIOCHEMICAL RESULTS AND CELL COUNTS.

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Submitted
ABSTRACT

Retransfusion of cardiectomy suction blood during cardiac surgery is associated with cerebral microemboli and an inflammatory reaction. Recently, a fat removal filter has been introduced, but little is known about the clinical effects. To assess the effects of this filter we measured biochemical markers and cell counts in patients.

Randomized prospective study in elective cardiac surgical patients (n = 28). During cardiopulmonary bypass the cardiectomy suction blood was filtered with a fat removal filter and retransfused in 14 patients (filter). In 14 patients the cardiectomy suction blood was discarded (waste). Triglyceride and glycerol, and neuron-specific enolase (NSE) and S-100β as brain injury markers, and circulating total white blood cell and granulocyte counts and interleukin-6 as inflammatory markers were measured.

The filter removed triglycerides (0.9 ± 0.08 mmol.L⁻¹ v. 0.63 ± 0.08 mmol.L⁻¹, p = 0.004), leucocytes (4.3 ± 0.8 x 10⁹.L⁻¹ v. 2.3 ± 0.6 x 10⁹.L⁻¹, p = 0.03) and platelets (116 ± 26 x 10⁹.L⁻¹ v. 75 ± 21 x 10⁹.L⁻¹, p = 0.003, paired t-test) from the cardiectomy blood. Apart from a transient increase in S-100β and NSE values in the filter group, there was no difference between the groups on the first postoperative day (S-100β 0.28 ± 0.05 µg.L⁻¹ filter vs. 0.29 ± 0.04 µg.L⁻¹ waste; NSE 16.7 ± 1.5 ng.L⁻¹ filter vs. 14.9 ± 0.9 ng.L⁻¹ waste). Triglyceride levels on the first postoperative day were similar. Total white blood cell (p = 0.009) and granulocyte counts (p = 0.01, both repeated measurements ANOVA) were higher in the filter group.

The filtration related transient increase in brain markers and the higher white blood cell and granulocyte counts in the filter group suggest that the filter efficacy should be improved.
INTRODUCTION

Retransfusion of cardiotomy suction blood during cardiopulmonary bypass (CPB) is used in cardiac surgery as a cost effective way to reduce the number of allogenic blood transfusions. However, this practice may be questioned for at least two reasons. In the first place, cardiotomy suction blood that is retransfused, contains many fat emboli. The use of cardiotomy suction is associated with an increase in cerebral emboli. These emboli are largely responsible for the postoperative neurocognitive dysfunction that affects up to 30% of the patients 3 months after cardiac surgery.

A second problem with retransfusion of wound blood is an inflammatory reaction in the patient. The activation of cardiotomy blood in the presence of fat and tissue factor from the pericardium leads to a high concentration of platelet- and leucocyte-derived microparticles which are involved in the systemic inflammatory response after CPB. Increased concentrations of the pro-inflammatory agent interleukin (IL)-6 in wound blood have also been related to febrile reactions after retransfusion.

Recently, a fat removal filter has been developed that is suitable for retransfusion of wound blood. This is a high flow polyester screen filter, based on a leucocyte removal filter. During cardiac surgery a beneficial effect from the application of this filter was suggested, and a moderate clinical effect was observed in orthopaedic surgery. For this study we hypothesized that the application of a fat removal filter for the cardiotomy suction blood would have a positive effect on brain injury and the inflammatory response after CPB as assessed by biochemical markers and cell counts.

Serum levels of the brain injury markers neuron specific enolase (NSE) and S-100β can be measured, but S-100β has been demonstrated in cardiotomy suction blood. However, NSE and S-100β can still be used as markers for brain dysfunction if extracerebral sources are controlled for. The best option for control is to discard the cardiotomy suction blood completely. This has the additional advantage that the systemic inflammatory response after CPB also will be minimized. Therefore, we compared in this study a group of patients in which we filtered and retransfused the cardiotomy suction blood during CPB, with a control group of patients in which we discarded the cardiotomy suction blood completely. Use of an effective fat removal filter would result in similar postoperative values for markers of brain injury and inflammation in both groups. As markers for brain injury we measured the serum levels of NSE and S-100β, triglycerides and glycerol, and as inflammatory markers total white blood cell, granulocyte and platelet counts and IL-6 levels.

MATERIAL AND METHODS

Patients

After institutional human investigation committee approval and patient consent 28 consecutive patients scheduled for elective coronary artery bypass grafting were prospectively studied. They were on the operating day with a computer generated randomization table allocated to either a filter group (n = 14), in which cardiotomy suction blood throughout the CPB period was filtered and retransfused, or a waste group (n = 14), in which the cardiotomy suction blood throughout the operation was discarded. This number of patients was calculated as follows. We estimated that a
A difference of one standard deviation between the S-100β and NSE values after induction of anaesthesia and on the morning of the first postoperative day would be clinically relevant. It was therefore estimated that 14 patients in each group would be required to have a power of 0.8 at an α of 0.05 in order to detect a significant difference among the groups. Patients with redo-operations, with pre-existing cerebral disease or with a recent (<1 month) myocardial infarction were excluded.

**Anaesthesia and perfusion**

Anaesthesia was induced and maintained according to an established protocol and consisted of infusion of midazolam (0.1 mg.kg⁻¹) and sufentanil (1.5 μg.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 Co., Edison, N.J., 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 Instrumente, München, Germany), a hollow fibre oxygenator (Sarns Turbo, 3M, St. Paul, Minn., USA) and an arterial line filter (Affinity 38μ, Medtronic, Minneapolis, Minn., USA). The priming consisted of 500 mL hydroxyethylstarch 10% (Haes 10%, Fresenius, Bad Homburg, Germany) and 1000 mL lactated Ringer's solution. Pump flow was adjusted to 2.4 l.m⁻².min⁻¹. Nasopharyngeal temperature during CPB was maintained at 32°C and α-stat pH-management was used.

**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 wound suction blood passed under gravity through a fat removal filter (LipiGuard, Pall Biomedical, Portsmouth, GB) into the cardiotomy reservoir of the CPB circuit. After each 600 mL of suction blood a new filter was used in order not to exceed the recommended filter capacity.

In the waste group, the cardiotomy suction blood was aspirated with the hospital wall suction system and discarded.

After CPB, the residual blood in the heart-lung machine was collected in a transfusion bag and in both groups retransfused to the patients. Postoperative shed mediastinal blood was not retransfused. Postoperative transfusion of homologous blood products was according to our hospital guidelines. The staff of the intensive care unit (ICU) was blinded to the study groups.

**Measurements**

For all laboratory tests and biochemical assays EDTA and citrate anticoagulated blood was drawn from the patients’ radial artery catheter. Blood samples were drawn (1) after induction of anaesthesia, before the start of CPB, (2) at the end of the operation, (3) after three hours in the ICU and (4) on the morning of the first postoperative day. For biochemical assays, plasma was obtained by centrifugation of whole blood at 1000g and immediately stored at –80°C for further determinations. Serum levels of S-100β and neuron specific enolase (NSE) were both determined using enzyme immunoassays (Sangtec Medical, Bromma, Sweden). Interleukin-6 was determined using an enzyme immunoassay (Quantikine, R&D Systems, Minneapolis,
Minn, USA). Plasma levels of glycerol and triglyceride were both determined by routine biochemical methods (Sigma, St. Louis, MO, USA). Haemoglobin, haematocrit and platelet, total white blood cell and granulocyte counts were determined by an electronic cell counter (Cell-Dyn 610, Abbott, Santa Clara, CA, USA). Levels of triglycerides, and leucocyte and platelet counts were measured in addition from EDTA and citrate anticoagulated samples taken simultaneously before and after the filter to assess the efficacy of the fat removal filter.

Statistics

All data are presented uncorrected for haemodilution and expressed as mean ± standard error. 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 the paired Student’s t-test was used. To identify group, time, and group-time interactions two way analysis of variance (ANOVA) for repeated measurements was used. To allow for multiple comparisons the Bonferroni adjustment was applied. Correlations were calculated by means of the Pearson correlation coefficient. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

All patients that were included completed the study. The demographic data are summarized in table 1, which shows that both groups were similar. The postoperative clinical data are summarized in table 2, and indicate that there were no differences between both groups.

There were no complications requiring a prolonged hospital stay. The fat filter removed triglycerides (0.9 ± 0.08 mmol.L⁻¹ v. 0.63 ± 0.08 mmol.L⁻¹, p = 0.004), glycerol (5.7 ± 0.37 mmol.L⁻¹ v. 4.5 ± 0.44 mmol.L⁻¹, p = 0.05), leucocytes (4.3 ± 0.8 x 10⁹.L⁻¹ v. 2.3 ± 0.6 x 10⁹.L⁻¹, p = 0.03) and platelets (116 ± 26 x 10⁹.L⁻¹ v. 75 ± 21 x 10⁹.L⁻¹, p = 0.003) from the cardiotomy suction blood (1103 ± 154 mL). Compared to the baseline preoperative serum levels in the patients (figures 1 and 2), leucocyte and platelet counts were lower in the cardiotomy suction blood, the triglyceride levels were similar, but the glycerol levels were higher.

The release pattern over the time of the brain marker S-100β was different in both groups. A significant interaction between group and time was observed (p = 0.006, figure 1). This was due to a transient peak at the end of the operation in the filter group. On the first postoperative day however, S-100β serum levels were similar again (0.28 ± 0.05 µg.L⁻¹ filter vs. 0.29 ± 0.04 µg.L⁻¹ waste, figure 1). The NSE serum levels also showed a transient peak in the filter group, but there was no significant interaction between group and time (p = 0.07, figure 1). This peak was about 3 hours postoperatively. However, repeated measurements ANOVA revealed that the two groups were not different (p = 0.37, figure 1). The NSE serum levels in both groups on the first postoperative day were higher than the preoperative serum levels (figure 1), but this was not the case for the S-100β levels. The NSE serum levels at the end of the operation showed a weak positive correlation with the S-100β serum levels at the end of the operation (r = 0.4, p = 0.04).
Analysis of the glycerol levels over the time revealed a difference between the two groups \((p = 0.04,\) figure 1), but this was not the case for the triglyceride levels \((p = 0.49,\) figure 1). The glycerol and triglyceride serum levels on the first postoperative day were not different between the groups (triglycerides \(0.63 \pm 0.07\) mmol.L\(^{-1}\) filter vs. \(0.73 \pm 0.11\) mmol.L\(^{-1}\) waste; glycerol \(1.27 \pm 0.18\) mmol.L\(^{-1}\) filter vs. \(1.11 \pm 0.2\) mmol.L\(^{-1}\) waste, figure 1).

The total white blood cell counts increased during the study period \((p < 0.001)\) and were different between the groups \((p = 0.009,\) figure 2). This resulted in higher total white blood cell counts in the filter group on the first postoperative day. Similarly, the granulocyte counts, as the more reactive part of the white blood cells, increased during the study period \((p < 0.001)\) and were higher in the filter group \((p = 0.01,\) figure 2). A positive correlation between the granulocyte counts and the CPB time was present in the waste group \((r = 0.69, p = 0.007),\) but this was not the case in the filter group \((r = 0.21, p = 0.48).\)

Although the IL-6 levels were higher in the waste group on the first postoperative day \((33.6 \pm 5.9\) ng.L\(^{-1}\)) than in the filter group \((15.0 \pm 5.7\) ng.L\(^{-1}\)), there was no difference between the two groups over the time \((p = 0.43,\) figure 2).
DISCUSSION

In this study in cardiac surgical patients, we did not observe different serum levels of NSE and S-100β on the first postoperative day, whether we retransfused the cardiotomy suction blood after passage through a fat removal filter, or completely discarded this blood. This finding suggests that the application of a fat removal filter is effective. However, we also observed a transient increase in serum levels of NSE and S-100β in the filter group. The serum levels of S-100β had a peak at the end of the operation, whereas the serum levels of NSE had a peak about 3 hours later. In addition, the glycerol levels and the total white blood cell and granulocyte counts, as inflammatory markers, were higher in the filter group. These findings suggest that the efficacy of the filter should be improved.

NSE and S-100β are both sensitive and early markers for brain injury.\textsuperscript{16,17} NSE levels increase by cell destruction in the gray matter. An increase in serum NSE on the first postoperative day is associated with neuropsychological dysfunction.\textsuperscript{18} In contrast, S-100β is released from cells in the white matter. Increased S-100β levels on the first

Figure 1. Serum S-100β, neuron-specific enolase, triglyceride and glycerol values in a group of patients where the wound suction blood was filtered and retransfused (filter) and a group of patients where the wound suction blood was discarded (waste), measured before cardiopulmonary bypass (pre CPB), at the end of the operation (end op), after 3 hrs in the intensive care unit (3h-ICU) and on the first postoperative day (day 1). Analysis of variance for repeated measurements indicates a difference between the groups for the S-100β values ($p = 0.006$) and for the glycerol values ($p = 0.04$)
postoperative day are associated with cerebral injury, but may be difficult to evaluate after cardiac surgery due to extracerebral S-100β sources. Thus, depending on the type of cell damage, the combination of the NSE and S-100β release may be more specific for brain injury, and we determined therefore both NSE and S-100β serum levels.

The clinical efficacy of the fat removal filter appeared insufficient for several reasons, despite the significant reduction in triglycerides, glycerol, leucocytes and platelets in the retransfused cardiectomy blood, and despite the similar NSE and S-100β serum levels on the first postoperative day in both groups. In the first place, the postoperative increase in NSE suggests at least some brain injury in the filter group, because it has been shown that NSE did not increase after abdominal surgery. A second argument for an insufficient clinical efficacy of the fat filter, is the higher total white blood cell and granulocyte counts in the filter group. Moreover, in the waste group the postoperative total white blood cell and granulocyte counts correlated with the CPB time as was expected. This was not the case in the filter group, and suggests that retransfusion of the filtered cardiectomy suction blood had a more profound effect on the inflammatory parameters than CPB itself.
A low filter capacity might explain the insufficient filter efficacy, but this is less likely as we frequently changed the filter according to the manufacturer’s instructions. Others however, also felt that the filter efficacy should be improved. After orthopaedic surgery, postoperative wound blood was passed through a fat reducing filter which was found to be inferior to a leucocyte depletion filter.\textsuperscript{11} This finding is supported by a laboratory study with reconstituted blood and soya oil, in which a leucocyte depletion filter was also more effective than the fat removal filter.\textsuperscript{23}

We confined ourselves to the assessment of biochemical markers because of the small scale of this study. We also did not estimate the number of fat microemboli, but instead used triglyceride and glycerol measurements for several reasons. It has already been demonstrated that the application of a filter before retransfusion of cardiotomy blood prevented the passage of fat emboli larger than 50 μm.\textsuperscript{24} This is most likely based on the mechanical removal of the fat emboli. Moreover, the assessment of the number and size of the microemboli reflects only neutral fat, which is biologically not active. During surgery however, triglycerides break down in glycerol and free fatty acids. Especially the free fatty acids are associated with organ damage, for example in the lungs.\textsuperscript{25} These slightly polarised substances are also removed by the filter through the electrical charge of the fibers.\textsuperscript{10} Therefore the glycerol and triglyceride measurements may more accurately reflect organ damage.

The neurological effects of clinically applied filtration techniques have not been assessed before. Only Kincaid et al. processed cardiotomy suction blood in dogs with a cell saver and passed this blood through a leucocyte depleting filter in a subset of 6 dogs. They found no difference in cerebral microemboli compared to processed, but unfiltered blood.\textsuperscript{26} Their findings support our results with respect to the NSE and S-100\textsubscript{B} serum levels on the first postoperative day. However, serum levels of brain injury markers may not directly be related to clinical impairment, as a deficit in one region of the brain may have more pronounced clinical effects than that same deficit in another region of the brain. Consequently, a larger scale study is necessary to demonstrate clinical important differences.

In conclusion, the results of this study do not demonstrate a difference in S-100\textsubscript{B} and NSE values on the first postoperative day. However, the filtration related transient increase in brain injury markers and the higher leucocyte and granulocyte counts suggest that the filter efficacy should be improved. A larger scale clinical study is therefore necessary before widespread application of a fat removal filter can be recommended.

REFERENCES