Leucocyte depletion in cardiopulmonary bypass: a comparison of four strategies

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Leucocytes have been shown to play a fundamental role in the pathophysiology of inflammation. This prospective, randomized, controlled study was designed to identify the most advantageous leucocyte depletion technique in terms of reduction in systemic inflammatory response syndrome and myocardial ischaemia reperfusion injury associated with cardiopulmonary bypass (CPB). Forty consecutive patients undergoing elective coronary artery bypass graft (CABG) surgery were randomly allocated to one of four groups. The four groups consisted of a control group, a systemic leucocyte depletion (SLD) group, a cardioplegic leucocyte depletion (CLD) group and a total leucocyte depletion (TLD) group. There were 10 patients in each group. Lactoferrin (marker of neutrophil activation) and troponin-I (marker of myocardial ischaemia reperfusion injury) were measured at six time points: post induction, 5 min on CPB, 5 min before releasing the aortic crossclamp, 15 min after releasing the clamp and 1 and 24 hours after the discontinuation of CPB.

Plasma lactoferrin levels increased rapidly in every group after the commencement of CPB, subsequently reached a peak after releasing the aortic crossclamp and gradually declined after the discontinuation of CPB. The lowest lactoferrin concentration was observed in the TLD (range 2.15–141.9 ng/mL) and CLD groups (7.469–114.6 ng/mL). Regarding myocardial injury, plasma cardiac troponin-I levels did not differ significantly between groups; but troponin-I concentrations rose dramatically after releasing the aortic crossclamp in all groups. Nevertheless, the CLD group had the lowest troponin-I level (1.37–5.55 ng/mL).

In conclusion, it is believed that myocardial ischaemia is probably a major contributor to the inflammatory response. Although there is no clear statistical significance shown in this pilot study, the data tend to support the cardioplegic leucocyte depletion strategy as the optimal method for attenuating neutrophil activation and myocardial ischaemia reperfusion injury. Perfusion (2003) 18, 95–103.

Introduction

Systemic inflammatory response (SIR), an initial, nonspecific inflammatory process responsible for considerable pathophysiology, remains one of the most common adverse effects of cardiopulmonary bypass (CPB), and may manifest itself in a range of severities from subclinical presentation to marked cardiac dysfunction or multiorgan failure.¹ The most severe consequence, multiorgan dysfunction (MOD), has a high mortality rate. Therefore, in recent years, minimization of the SIR syndrome has been studied widely to improve operative outcome, especially in high-risk patients.

Activation of leucocytes, particularly the neutrophils, has been shown to play a central role in initiating SIR.²–⁴ Immediately after establishing CPB, leucocytes and endothelial cells are activated as a result of the contact of blood with the none-endothelial surface of the CPB circuit and, subsequently, the cascades of activation of the complement system, coagulation and various mediators occur. The majority of leucocytes involved in the SIR are neutrophils, as these are principally involved in destroying micro-organisms and tissues.⁵ Leucocytes are further provoked by ischaemic myocardium and endothelial cells during aortic crossclamping. It is believed that myocardial ischaemia during surgery is a major source of inflammatory mediators.⁶

Myocardial protection during the operative procedure is crucial to successful cardiac surgery. Despite the advanced methods of myocardial protection currently available, postoperative myocardial dysfunction remains a major problem. Wan et al. found that proinflammatory cytokines, including tumour necrosis factor alpha (TNFα), interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) can be detected in higher levels in the coronary sinus than in the artery during aortic crossclamp-
Activated leucocytes again play an important role in ischaemia reperfusion injury, and a number of strategies have evolved to reduce this, including leucocyte filters.

A number of therapeutic approaches have evolved to minimize the severity of undesirable consequences of myocardial ischaemia reperfusion injury and SIR. Currently, therapeutic methods include surface modification of the perfusion circuit, systemic pharmacological interventions, such as using aprotinin and steroids, and leucocyte depletion technology. These strategies have been designed either to prevent the activation of leucocytes or to reduce the severity of organ damage associated with activated neutrophils. Leucocyte depletion technique, by using leucocyte filters, is considered to be a reliable method of minimizing SIR and maximizing myocardial protection, and is now being used routinely in extracorporeal circulation. This strategy, however, has three variations: systemic leucocyte depletion (SLD), cardioplegic leucocyte depletion (CLD) and total leucocyte depletion (TLD), a combination of systemic and cardioplegic. The first technique, SLD, is effected by placing a leucocyte filter in the arterial tubing system of the CPB circuit (Figure 1). SLD has been shown to reduce inflammatory parameters and the need for postoperative ventilatory support and blood transfusion. CLD, effected by placing the filter in the cardioplegic delivery system of the CPB system (Figure 2), has no demonstrable systemic effect, but it has a confirmed myocardial protective effect, which has been demonstrated by a more favourable postoperative cardiac performance and lower levels of troponin-T and CK-MB, markers of cardiac injury. The last technique, TLD, is the combination of the other two techniques (SLD and CLD). To achieve the optimal benefit, it may be advisable to use the TLD approach, but this is more expensive than the conventional one. However, there is no clear-cut evidence indicating which approach is the most appropriate. The primary aim of this prospective, randomized, controlled study was, therefore, to determine which of these filtration approaches is the most beneficial in terms of myocardial protection and SIR.

Patients and methods

Between 1 February and 30 April 2001, 40 consecutive patients undergoing elective coronary artery bypass graft (CABG) surgery participated in this prospective, randomized, controlled trial with the approval of the local Ethical Committee.

Inclusion and exclusion criteria

All patients were informed and consented to the procedure. We included 40 consecutive eligible patients without valvular lesion in order to exclude confounding effects of additional procedures. Emergency CABG patients were also ineligible because the circumstance itself contributed risks to the outcome. However, neither acute myocardial infarction nor unstable angina patients were excluded unless myocardial function was markedly deteriorated. Aspirin was discontinued at least seven days before operation.
prior to the operation, and other anticoagulants were stopped at least five days before the operation.

The exclusion criteria included severe myocardial impairment [left ventricular ejection fraction (LVEF) less than 0.30], preoperative coagulopathy and severe preoperative renal dysfunction. Those patients with underlying autoimmune and inflammatory diseases were also excluded. Patients of an extreme age, more than 75 years old, were also excluded from the study.

**Randomization method**

Every patient was allocated to a study group by the perfusionist team, using block randomization, before undergoing the operation. Other staff were unaware into which group each patient was placed. At the completion of all laboratory work, the randomization code was broken and the results analysed.

**Intraoperative management**

The same premedication scheme was used for all patients. Two hours before surgery, they received 10 mg morphine and 0.6 mg atropine. Anaesthesia was induced using fentanyl 0.01–0.02 mg/kg, midazolam 1–2 mg and pancuronium 0.1 mg/kg. All patients underwent general anaesthesia.

All patients underwent the operation under standard cardiopulmonary support using the Stockert roller-pump system (Stockert, Munich, Germany). During the operation, circulation was supported by pulsatile flow at a cardiac index of 2.4 L/m\(^2\)/min. A standard arterial filter was routinely used in the control and cardioplegic groups. The extracorporeal oxygenator was the Biocor model 200 HIS, high performance oxygenator with integrated hardshell venous reservoir (Minntech Corporation, Minnesota, USA). The priming volume was 1700 mL in all cases. Myocardial preservation was achieved using antegrade cold blood cardioplegia (4°C; 1:4 ratio of blood to cardioplegic solution) infused at a rate of 250 mL/min through the ascending aorta at 30-min intervals in every patient. Furthermore, systemic hypothermia by core temperature cooling was between 32 and 34°C.

Patients in the leucocyte filtration groups were exposed to one of the leucocyte depletion regimes: 1) this involved a systemic leucocyte filter (Pall LG6, Pall Biomedical, Portsmouth, UK), which was placed in the arterial tubing system between the oxygenator and the patient (Figures 1 and 3); 2) the second had a cardioplegic leucocyte filter (Pall BC1B, Pall Biomedical, Portsmouth, UK) applied in the blood cardioplegic delivery system (Figures 2 and 4); 3) the third was a combination of the two previously mentioned methods.

**Sample collection**

Five millilitres of blood was collected at six time points from an indwelling venous catheter or the venous reservoir. The first sample was obtained immediately after anaesthetic induction as the baseline specimen throughout the study; hence, the proinflammatory effects of anaesthetic techniques can be excluded. The second sample, at 5 min after the onset of CPB, represented an initial step of inflammatory response. Subsequently, 5 min prior to releasing the aortic crossclamp, the third sample was gathered after a period of myocardial ischaemia, and just before myocardial reperfusion in order to measure myocardial necrosis and systemic inflammatory parameters. This time point was also the time of the commencement of rewarming. At 15 min after releasing the aortic crossclamp, the fourth sample was collected. This sample provided the
immediate amounts of myocardial necrosis parameters released after myocardial reperfusion. We took the next sample at 1 hour after the termination of CPB. It provided the level of the early period of myocardial reperfusion, and we could follow up any trend in the levels of myocardial and inflammatory parameters afterwards. Finally, the last blood sample was obtained at 24 hours after the termination of CPB.

Each blood sample was stored in its heparinized syringe submerged in ice. Subsequently, the study samples were centrifuged at 3000 rpm for 10 min to fractionate into plasma and blood cells. The plasma was kept frozen at a temperature between −70 and −75°C until all samples from the 40 patients had been collected. Laboratory analyses were performed after all samples had been collected in order to attain complete double-blinded randomization at the measurement phase of the study.

**Enzyme-linked immunosorbent assay**
We applied enzyme-linked immunosorbent assay (ELISA) kits for measuring biochemical outcomes, including lactoferrin ELISA kit (Bioxytech Lactof-EIA, OXIS International Inc., Portland, OR, USA) and cardiac-specific troponin-I ELISA kit (TIEL-01, Life Diagnostics Inc., Philadelphia, PA, USA). The concentrations of lactoferrin and troponin-I are measured by spectrophotometry at 420 nm and 450 nm, respectively.

**Statistical analysis**
All demographic data on the 40 patients were recorded prospectively. New York Heart Association (NYHA) functional class, the number of involved vessels and the number of grafts are presented in median and interquartile range. Therefore, the Kruskal–Wallis test was used to analyse the difference in these characteristic parameters between groups.16 A p value of less than 0.05 inferred a significant statistical difference. At the same time, other continuous data, including age, aortic cross-clamp time, total CPB time, dosage of inotropic drug use, blood loss from thoracostomy tube, non-red blood cell volume replacement and intensive care and hospital stay are shown as mean and standard deviation. The analysis of variance (ANOVA) was applied for testing the difference in the four groups altogether. Comparison between two individual groups was analysed using Student’s t-test.

Two principal outcomes of this study, lactoferrin and troponin-I, are shown as mean and standard deviation, and were tested for normality before drawing further statistical analyses in the evaluation of significant difference. Afterwards, ANOVA was applied for testing the difference, correlation analysis was employed to demonstrate whether there was a relationship between two parameters and multiple linear regression analysis was applied for comparing the effects of each type of filter on the biochemical outcomes.

All calculations of statistical tests were performed on statistical software, STATA (Stata Corporation, College Station, TX, USA) and PRISM 3 (GraphPad Software Inc., San Diego, CA, USA).

**Results**

**Demographic data**
There were no statistically significant differences between the groups with regard to preoperative data (Table 1). Age and gender were similar among the groups (p = 0.44). Types of disease were also analogous – the majority of the patients, more than 75%, had triple vessel disease. Similarly, body weight, height and body surface area of the patients were comparable (p = 0.9, 0.87 and 0.98, respectively). NYHA functional class was also the same in each group (p = 0.48).

<table>
<thead>
<tr>
<th>Categories</th>
<th>Control (n = 10)</th>
<th>SLD (n = 10)</th>
<th>CLD (n = 10)</th>
<th>TLD (n = 10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.70±6.5</td>
<td>61.80±6.5</td>
<td>65.50±5.48</td>
<td>63.10±10.0</td>
<td>0.44</td>
</tr>
<tr>
<td>Gender</td>
<td>Male (7)</td>
<td>Male (8)</td>
<td>Male (8)</td>
<td>Male (10)</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Female (3)</td>
<td>Female (2)</td>
<td>Female (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td>76.08±18.24</td>
<td>79.98±18.61</td>
<td>76.14±7.2</td>
<td>78.77±8.55</td>
<td>0.90</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.2±10.94</td>
<td>168.2±9.37</td>
<td>167.5±8.43</td>
<td>169.1±12.4</td>
<td>0.87</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.87±0.26</td>
<td>1.89±0.23</td>
<td>1.91±0.13</td>
<td>1.88±0.17</td>
<td>0.98</td>
</tr>
<tr>
<td>No. of involved vessels</td>
<td>2.6</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>NYHA Functional class b</td>
<td>1.5 (1–3)</td>
<td>2 (1–4)</td>
<td>1 (1–3)</td>
<td>1.5 (1–2)</td>
<td>0.48</td>
</tr>
<tr>
<td>Smokers</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>5</td>
<td>0.36</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>0.65</td>
</tr>
</tbody>
</table>

*p value is from a comparison using ANOVA; NA, not available; NS, not statistically significant.

16 Age, bodyweight, height and body surface area are shown as mean ± SD.

b NYHA functional class is shown as median and range.
Intraoperative data

Intraoperative data, including the number of grafts, flow rate generated by CPB, aortic crossclamp time and total bypass time, were not statistically different among the groups (Table 2). The internal mammary artery was used in all patients except one in the SLD group, while a radial arterial graft was used in three patients in the control group, three in the SLD group and six in the TLD group. Furthermore, 13 saphenous vein grafts were implanted in the patients in the control group, 20 in the SLD, 20 in the CLD and 14 in the TLD groups. There was no statistically significant difference in type or number of grafts performed among four groups (p = 0.08).

Expression of SIR and myocardial injury

Both lactoferrin and cardiac troponin-I were quantified by ELISA. Since haemodilution is an essential state of CPB, lactoferrin and troponin-I are shown in concentrations before and after correction for haemodilution calculated using the formula shown below. The diagnostic cut-off level of troponin-I for acute myocardial infarction is 2.0 ng/mL.

\[ V_{\text{corr}} = V_{\text{obs}} \times \frac{\text{Hct}_{\text{ind}}}{\text{Hct}_{\text{obs}}} \]

where \( V_{\text{corr}} \) = corrected value of lactoferrin or troponin-I, \( V_{\text{obs}} \) = observed value of lactoferrin or troponin-I at a specific time point, \( \text{Hct}_{\text{ind}} \) = haematocrit immediate postinduction period, \( \text{Hct}_{\text{obs}} \) = observed haematocrit at a specific time point.

Lactoferrin

At baseline, the mean lactoferrin level in the TLD group was the lowest (2.15 ng/mL), whereas the control group level was the highest with 12.94 ng/mL (Table 3 and Figure 5). In the very early period of CPB (time point 2), lactoferrin levels became dramatically higher and there were statistically significant differences (\( p < 0.01 \)) among the groups – the average concentration in the TLD group was still the lowest (14.93 ng/mL) with a significant difference from the control group (52.16 ng/mL, \( p < 0.01 \)) and the SLD group (89.65 ng/mL, \( p = 0.01 \)). Both the TLD and CLD groups had significantly lower concentrations of lactoferrin than the SLD group. Plasma lactoferrin concentrations gradually increased over time, and reached a peak at 15 min after release of the aortic crossclamp, which was the early period of myocardial reperfusion. Plasma lactoferrin levels in both the TLD and CLD groups were significantly lower than in the SLD group (\( p < 0.01 \)) at the third time point, but there was no significant difference between the SLD and control groups, or between the TLD and CLD groups. At 1 hour after the end of bypass, there were statistical differences between the CLD and SLD and between the TLD and SLD groups (\( p = 0.02 \)). Eventually, plasma lactoferrin levels in each group declined to

Table 2 Intraoperative data

<table>
<thead>
<tr>
<th>Categories</th>
<th>Control (n = 10)</th>
<th>SLD (n = 10)</th>
<th>CLD (n = 10)</th>
<th>TLD (n = 10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of grafts</td>
<td>3 (1–3)</td>
<td>3 (3–4)</td>
<td>3 (3–4)</td>
<td>3 (2–4)</td>
<td>0.08</td>
</tr>
<tr>
<td>CPB flow rate (L/min)</td>
<td>4.07 ± 0.44</td>
<td>4.09 ± 0.57</td>
<td>3.76 ± 0.4</td>
<td>4.03 ± 0.55</td>
<td>0.42</td>
</tr>
<tr>
<td>Aortic crossclamp time (min)</td>
<td>39.40 ± 16.6</td>
<td>42.60 ± 13.47</td>
<td>37.50 ± 9.64</td>
<td>38.00 ± 14.7</td>
<td>0.84</td>
</tr>
<tr>
<td>Total bypass time (min)</td>
<td>68.20 ± 21.93</td>
<td>77.80 ± 20.51</td>
<td>66.80 ± 12.49</td>
<td>64.80 ± 18.67</td>
<td>0.42</td>
</tr>
</tbody>
</table>

\* Mean arterial pressures during CPB, CPB flow rate, aortic crossclamping time and total bypass time are shown as mean ± SD.

\( \text{Hct}_{\text{obs}} \) = observed haematocrit at a specific time point.

Table 3 Plasma lactoferrin levels (ng/mL) corrected to the postinduction haematocrit: mean and 95% confidence intervals of mean

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>SLD</th>
<th>CLD</th>
<th>TLD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td></td>
<td>12.94</td>
<td>10.26</td>
<td>7.46</td>
<td>2.15</td>
</tr>
<tr>
<td></td>
<td>(5.43–20.45)</td>
<td>(6.62–13.90)</td>
<td>(1.64–3.30)</td>
<td>(0.19–1.117)</td>
</tr>
<tr>
<td></td>
<td>52.16</td>
<td>89.65</td>
<td>38.79</td>
<td>14.93</td>
</tr>
<tr>
<td></td>
<td>(30.53–73.78)</td>
<td>(21.45–157.9)</td>
<td>(9.34–68.24)</td>
<td>(5.07–24.80)</td>
</tr>
<tr>
<td></td>
<td>121.7</td>
<td>176.4</td>
<td>78.63</td>
<td>68.31</td>
</tr>
<tr>
<td>5 min before reperfusion</td>
<td>(79.36–164.1)</td>
<td>(121.8–231.0)</td>
<td>(37.32–120)</td>
<td>(3.68–132.9)</td>
</tr>
<tr>
<td>15 min after reperfusion</td>
<td>151.9</td>
<td>190.7</td>
<td>114.6</td>
<td>141.9</td>
</tr>
<tr>
<td>1 hour after CPB</td>
<td>(93.32–210.4)</td>
<td>(169.2–212.2)</td>
<td>(72.92–156.2)</td>
<td>(84.65–199.2)</td>
</tr>
<tr>
<td>24 hours after CPB</td>
<td>57.90</td>
<td>113.9</td>
<td>35.52</td>
<td>53.00</td>
</tr>
<tr>
<td></td>
<td>(26.93–88.87)</td>
<td>(42.41–185.3)</td>
<td>(9.68–61.35)</td>
<td>(27.31–78.69)</td>
</tr>
</tbody>
</table>

\* p value: calculated by a comparison using ANOVA among four groups altogether at any time point. For the detail on p value in comparison between any two individual groups please see text.
baseline levels at some time between 1 and 24 hours after CPB.

**Troponin-I**

At the first time point, mean levels of troponin-I in each group were statistically comparable ($p = 0.73$), as shown in Table 4 and Figure 6. Subsequently, from the beginning of CPB to the early period of myocardial reperfusion, there was no significant change in concentrations of cardiac troponin-I in any group. Plasma troponin-I concentrations increased gradually from 1 hour after releasing the aortic crossclamp onwards. There was no statistically significant difference in any comparison between any two groups ($p > 0.05$). Nevertheless, when compared with those of the other groups, the troponin-I levels in the CLD group seemed to be the lowest at 5 min on CPB, and 5 min before and 15 min after aortic declamping. Plasma cardiac troponin-I concentrations in each group levelled off at 24 hours after the termination bypass with no statistically significant differences ($p = 0.2$).

**Postoperative clinical outcomes**

Previous studies have demonstrated a potential blood sparing property associated with the use of leucocyte depletion.$^9$ For this reason, blood loss and blood transfusion were recorded for all patients.

**Blood loss in the first 24 hours.** Although the lowest amounts of blood loss and blood transfusion were in the CLD group (Table 5), there was no statistically significant difference between this group and any of the other groups.

**Non-red blood cell volume replacement and inotropic drug administration.** Volume replacement calculated in this category includes every type of fluid that is free of red blood cells. The inotropic drug used in this study was dopamine. Both volume replacement and inotropic drug use are summarized together in Table 6.

There was no statistically significant difference in the amount of volume replacement among groups, but the CLD group had the lowest average volume of fluid added while the highest was in the TLD group ($p = 0.34$). Inotropic drug dosage was lowest in the CLD group.

**Postoperative morbidity and mortality**

Severe postoperative bleeding occurred in one patient in the TLD group, and this affected the blood and volume replacement in this group as a whole. The bleeding was probably caused by coagulopathy. Apart from this bleeding, this patient's postoperative period was uneventful.

There was one patient in the CLD group who died of a postoperative acute myocardial infarction. One non-insulin-dependent diabetic patient in the SLD group had a sternal wound infection, and she consequently developed sternal dehiscence. Eventually, she made a complete recovery. There was also one patient in this group suffering from a cerebrovascular accident – a cerebral thrombosis. He had a
transient period of right hemiparesis. Accordingly, the mean hospital stay of this group was elevated.

Atrial fibrillation was detected in two patients, one from the control group and the other from the SLD group. It was controlled by medication in both cases. These patients made a complete and otherwise uneventful recovery.

**Discussion**

Perioperative myocardial dysfunction and infarction are still two of the most important predictors of outcome after cardiac surgery. Despite the advances in myocardial preservation techniques, a large number of patients undergoing various open cardiac procedures, in particular CABG surgery, demonstrate some degree of myocardial dysfunction. Several mechanisms have been proposed as causes of perioperative myocardial dysfunction – SIR and ischaemia reperfusion injury are two of these.

Recently, activated leucocytes have been shown to play a fundamental role in the development of SIR and ischaemia reperfusion injury. Researchers have been investigating widely to prevent these undesired consequences of leucocyte activation. Pharmacological strategy, for example aprotinin, is an effective therapeutic method for preventing or at least modifying the adverse phenomenon of SIR, but it does not eliminate the basic causative factor. A biocompatible bypass circuit, for instance, heparin-coated extracorporeal circuits, is another strategy

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**Table 4** Plasma troponin-I levels (ng/mL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Postinduction</th>
<th>5 min on CPB</th>
<th>5 min before reperfusion</th>
<th>15 min after reperfusion</th>
<th>1 hour after CPB</th>
<th>24 hours after CPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(n = 10)</td>
<td>2.753</td>
<td>2.717</td>
<td>3.161</td>
<td>2.95</td>
<td>4.00</td>
<td>4.84</td>
</tr>
<tr>
<td>SLD</td>
<td>2.65</td>
<td>(1.47–4.04)</td>
<td>(1.01–4.43)</td>
<td>(1.08–5.24)</td>
<td>(0.51–5.39)</td>
<td>(1.06–6.95)</td>
<td>(1.51–8.16)</td>
</tr>
<tr>
<td>CLD</td>
<td>(n = 10)</td>
<td>2.01</td>
<td>1.49</td>
<td>1.76</td>
<td>1.374</td>
<td>4.13</td>
<td>5.55</td>
</tr>
<tr>
<td>TLD</td>
<td>2.044</td>
<td>(0.00–4.057)</td>
<td>(0.00–3.28)</td>
<td>(0.15–3.37)</td>
<td>(0.00–3.82)</td>
<td>(0.53–7.73)</td>
<td>(1.83–9.27)</td>
</tr>
<tr>
<td>p value*</td>
<td>0.73</td>
<td>0.16</td>
<td>0.12</td>
<td>0.46</td>
<td>0.06</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

*p value: calculated from a comparison using ANOVA among four groups altogether at any time point. For the detail on p value in comparison between any two individual groups please see text.
that has been employed to attenuate the inflammatory reaction.\textsuperscript{18–20} The leucocyte filter that preferentially removes only activated neutrophils is a new, and apparently successful, addition to the anti-inflammatory arsenal.\textsuperscript{21}

Leucocyte depletion has been studied for either attenuation of SIR or reduction in myocardial injury after cardiac operations. We were keen to determine which strategy or combination of strategies offered the optimal level of protection. We studied three strategies in this regard – systemic, cardioplegic and total leucocyte depletion.

Our study focussed on both SIR, as demonstrated by changes in plasma lactoferrin, and myocardial damage, as demonstrated by troponin-I changes. In addition, the normal range of outcome measures and demographic data were compared. The robust selection criteria for patients were justified by the fact that there were absolutely no statistically significant differences between the groups with regards to the demographic and outcome measures. Lactoferrin levels showed a normal CPB profile, rising at the onset of CPB, rising further throughout bypass and then decreasing gradually, returning to baseline levels around 24 hours postoperatively.

Lactoferrin is selected as a marker to represent SIR; leucocytes are the only source of lactoferrin in plasma. The previous study by Stefanou and coworkers demonstrated changes of lactoferrin concentration with time – there was an initial increase followed by a decrease and, finally, levelling off to baseline level at the third postoperative hour.\textsuperscript{9} In this study, we compared lactoferrin concentrations between groups. Although, on the one hand, there was some statistically significant difference between two pairs of groups at some time points, e.g., between the cardioplegic filter group and the systemic group at timepoints 2, 3, 4 and 5, this study shows some advantages of the cardioplegic leucocyte filter over other methods. It demonstrates the lowest lactoferrin concentration in this group. On the other hand, the total cardioplegic filter group also showed inflammation-reducing effects: once again, lactoferrin levels were lower than the systemic group. Nevertheless, the optimal leucocyte depletion strategy has not been identified as a substantial reduction in neutrophil activation in this small-scale study, but cardioplegic filtration appears to be the most effective strategy in the attenuation of SIR. The previous study by Mathis and coworkers also concluded that reperfusion injury might be based mainly on oxidative stress and leucocyte-mediated disturbance of endothelial integrity. Moreover, despite numerous advances in CPB, including more physiological surfaces of the bypass circuit used in all patients of their study, they found that myocardial injury due to ischaemia reperfusion injury after release of the aortic cross-clamp was still unaffected by this modification. They also found that the filter group had more favourable results than the control group, although both groups used heparin-coated systems.\textsuperscript{22} This benefit of cardioplegic filtration may be due to the possible mechanism that myocardial ischaemia reperfusion injury is mainly contributed by oxidative stress and leucocyte-mediated alteration of endothelial functions and cytokine release.

**Table 5** Blood loss and blood transfusion in the first 24 hours

<table>
<thead>
<tr>
<th>Categories</th>
<th>Control (n = 10)</th>
<th>SLD (n = 10)</th>
<th>CLD (n = 10)</th>
<th>TLD (n = 10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drainage in first 24 hours (mL)</td>
<td>936 (729.7–1142)</td>
<td>980.5 (651.9–1309)</td>
<td>802.0 (634.5–969.5)</td>
<td>1179 (880.4–1477)</td>
<td>0.1539</td>
</tr>
<tr>
<td>Blood transfusion (units)</td>
<td>1.0 (0.5–2.0)</td>
<td>1.5 (0–3.5)</td>
<td>0.5 (0–1.5)</td>
<td>2.0 (0–4.5)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

**Table 6** Volume (mL) of colloid and crystalloid fluid replacement and inotropic drug dosage (\(\mu g/\text{kg/min}\)): mean and 95% confidence intervals of mean

<table>
<thead>
<tr>
<th>Categories</th>
<th>Control (n = 10)</th>
<th>SLD (n = 10)</th>
<th>CLD (n = 10)</th>
<th>TLD (n = 10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume replacement (mL)</td>
<td>6516.10 (5469.9–7563.3)</td>
<td>6470.00 (5714.3–7226.7)</td>
<td>6268.10 (5763.8–6772.4)</td>
<td>7191.40 (6211.1–8171.7)</td>
<td>0.34</td>
</tr>
<tr>
<td>Inotropic drug use ((\mu g/\text{kg/min}))</td>
<td>1.68 (0.12–3.24)</td>
<td>1.35 (0.17–2.52)</td>
<td>0.88 (0–2.05)</td>
<td>0.98 (0–2.14)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

**Myocardial ischaemia reperfusion injury**

Cardiac troponin-I is used as a highly myocardium-specific marker in the diagnosis of myocardial damage\textsuperscript{23,24} because it is not detected in other types of muscle.\textsuperscript{25–29} Also, from previous studies, plasma troponin-I is detectable between 3 and 12 hours after the onset of myocardial infarction, and usually reaches a peak at 24 hours. Montgomery et al. have also demonstrated the prognostic ability of cardiac troponin-I in paediatric cardiac patients.\textsuperscript{30} As a result of reperfusion of the myocardium, plasma troponin-I levels elevated after releasing...
the aortic crossclamp. This phenomenon can be explained on the basis of ischaemia reperfusion injury because the elevated concentration of troponin-I cannot be detected until the unclamping of the aorta. The cardioplegic filtration was the most beneficial in lower troponin-I levels, although the difference was not statistically significant on comparison with other groups. This also supports that myocardial ischaemic reperfusion is probably a major source of inflammatory response.

**Postoperative care, morbidity and mortality**
This study was unable to show any statistically significant difference in blood loss or amount of blood transfused in the first 24 hours after CPB ($p = 0.15$ and $0.26$, respectively). One possible reason is that the patients were not in a high-risk group. Although this study did not demonstrate any substantial statistical significance, it provided the same outcome of blood-sparing effects of the leucocyte filter as previous studies. The CLD group had beneficial effects on blood loss with the lowest average amount. Surprisingly, the TLD group had the highest blood loss, but this was probably due to one patient in this group who had severe postoperative bleeding.

The average amount of volume replacement was comparable in each group. The CLD group had the lowest amount of volume replacement while the TLD group had the highest. However, once again, the differences were not statistically significant.

Interestingly, the highest average dose of inotropic drug was found in the control group; this possibly reflected the better myocardial functional preservation associated with the filter. Similarly, ICU and hospital stay in the filter group did not significantly differ from the control (Table 7). Again, it was inconclusive in this small study, but a tendency for shorter hospital stay was observed in all filter groups.

Our clinical data were in agreement with other studies regarding the favourable outcome of leucocyte filters, especially cardioplegic (Table 8 and Figure 7)\(^1,8,9,13,15,31,32\) These benefits may be more apparent in high-risk patients, particularly those with severe myocardial impairment.\(^13\)

**Comparison between leucocyte depletion techniques**
It seemed that the systemic leucocyte filter had no obvious beneficial effects, either in moderating the observed degree of neutrophil activation or in myocardial protection. In addition, the results were reasonably consistent throughout the course of the study. This was at odds with other studies.\(^8\)

This study, in contrast, has established consistent benefits with regard to lactoferrin and troponin-I levels when using cardioplegic filters. However, this trial failed to reach a statistically significant difference between the cardioplegic filter and the control group. The cardioplegic leucocyte depletion group had lower levels of both markers than the control group, but there was no substantial difference ($p > 0.05$ for both troponin-I and lactoferrin at most time points). In the study by Wan et al., there was substantial evidence that the main sources of SIR during CPB are the heart and lungs.\(^6\) The cardioplegic leucocyte depletion technique is targeted directly at preventing activated neutrophils in blood cardioplegia from harming the heart, and, subsequently, prevents the inflammatory response and myocardial injury.

Nevertheless, by multiple linear regression analysis, there is no synergistic effect between both types of leucocyte filter, cardioplegic and systemic, on modifying SIR and myocardial preservation. We have found only additive effects, as shown in the results. It is our conclusion that using the cardioplegic leucocyte depletion technique is probably sufficient protection for the myocardium, and that

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**Table 7** ICU stay and postoperative hospital stay: median and interquartile range

<table>
<thead>
<tr>
<th>Categories</th>
<th>Control (n = 10)</th>
<th>SLD (n = 10)</th>
<th>CLD (n = 10)</th>
<th>TLD (n = 10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU stay (hours)</td>
<td>24 (20–24)</td>
<td>24 (20–24)</td>
<td>24 (20–24)</td>
<td>22 (18–24)</td>
<td>0.67</td>
</tr>
<tr>
<td>Postoperative hospital stay (days)</td>
<td>6 (6–10)</td>
<td>7 (6–7.5)</td>
<td>7 (6.5–7.5)</td>
<td>7.5 (6–8.5)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

**Table 8** Postoperative clinical outcomes

<table>
<thead>
<tr>
<th>Categories</th>
<th>Control (n = 10)</th>
<th>SLD (n = 10)</th>
<th>CLD (n = 10)</th>
<th>TLD (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reopen for postoperative bleeding</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cardiac arrhythmia</td>
<td>AF (1)</td>
<td>AF (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Miscellaneous complications</td>
<td>0</td>
<td>CVA (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wound (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>0</td>
<td>Postoperative MI (1)</td>
<td>0</td>
</tr>
</tbody>
</table>
expanding leucocyte depletion to the systemic circulation does not offer any clear additional benefit.

Although any statistical significance between any of the three leucocyte depletion groups and the control group has not yet been established, this pilot study provides fundamental data and trends of clinical applications of leucocyte filters in cardiac surgery. Despite the small numbers in this study, it was powerful enough to show the tendencies for leucocyte depletion to be associated with less SIR and greater myocardial protection. Secondly, we have studied only low-risk patients, who have no substantial clinical challenges. We, therefore, look forward to a larger-scale study that will have more power and can indicate high-risk patients who may benefit more from this technique.

Conclusion

This small-scale study has confirmed the clinical benefit of leucocyte-depleting technology and, further, it has shown differences between therapeutic leucocyte-depleting strategies. In particular, this study has confirmed that cardioplegic leucocyte depletion offers sufficient myocardial protection, and that adding systemic leucocyte depletion offers no additional advantage. This is an interesting finding and has implications, particularly with regard to cost effectiveness.

This study has also indicated the direction for further studies; in particular:

1) To conduct a larger scale, randomized, controlled trial to investigate whether the leucocyte filter has beneficial effects on low-risk patients.
2) To identify the precise inclusion criteria for the application of filter technology.
3) To determine a specific group of patients to whom leucocyte depletion technology is suitable.
4) To verify the most beneficial combination of filter strategy and other interventions, e.g., pharmacological and other mechanical methods, in attenuating SIR.

References


