

# Leukocyte filtration of the cardiotomy suction. Does it affect systemic leukocyte activation or pulmonary function?

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## Abstract

**Background:** Cardiopulmonary bypass is thought to propagate a global systemic response through contact with the non-physiological surfaces of the extracorporeal circuit, leading to the stimulation of leukocytes, their adherence to endothelial cells and the release of cytotoxic molecules. This, in turn, has been shown to accelerate pulmonary injury. This study tested a new leukocyte-filtration system (RemoweLL) against a conventional system with no leukocyte-depleting capacity to determine the efficacy of the filtration system and its effects on pulmonary function.

**Methods:** Thirty patients underwent coronary artery bypass graft surgery using either the RemoweLL filtration system (15 patients) or a conventional cardiopulmonary bypass circuit (15 patients). Data were collected on the total number of leukocytes, their differentiation and activation, using the leukocyte adhesion integrin CD11b as a surrogate marker. Pulmonary function was assessed using the Alveolar-arterial Oxygenation Index (AaOI) and patients were categorized using the Berlin definition of acute respiratory distress syndrome (ARDS).

**Results:** Both groups showed significant increases in leukocyte numbers during CPB ( $p < 0.001$ ), with no differences noted between the groups. CD11b showed a significant increase in both groups, with peak activation occurring at the end of CPB, but no difference between the groups ( $p = 0.8$ ). There was a trend towards lower AaOI increases in the filtration group, but this did not reach significance ( $p = 0.075$ ) and there was no difference in ARDS definitions ( $p = 0.33$ ).

**Conclusions:** Leukocyte filtration of cardiotomy suction did not influence total leukocyte counts or activation as measured by CD11b upregulation. Furthermore, no evidence could be found to suggest improved pulmonary function.

## Keywords

leukocytes; cardiopulmonary bypass; pulmonary dysfunction; leukodepletion; coronary artery bypass grafts

## Introduction

The inflammatory response to cardiac surgery is multifactorial and can be categorized as either material dependent (i.e., the exposure of blood to non-physiological surfaces and conditions associated with cardiopulmonary bypass (CPB)) or material independent (surgical trauma, release of endotoxin and changes in body temperature). It can also be due to allogeneic blood exposure in response to the haemodilution that occurs during CPB.<sup>1</sup> Of major interest for more than a decade is the role that activated leukocytes play in the inflammatory response. Leukocyte action can be mediated by the complement C3a molecule, enabling local release of elastase, myeloperoxidase and lactoferrin upon adhesion to endothelial cells, promoting endothelial damage and neutrophil extravasation.<sup>2</sup> This is

especially prevalent in the pulmonary system where CPB has been shown to induce increased pulmonary

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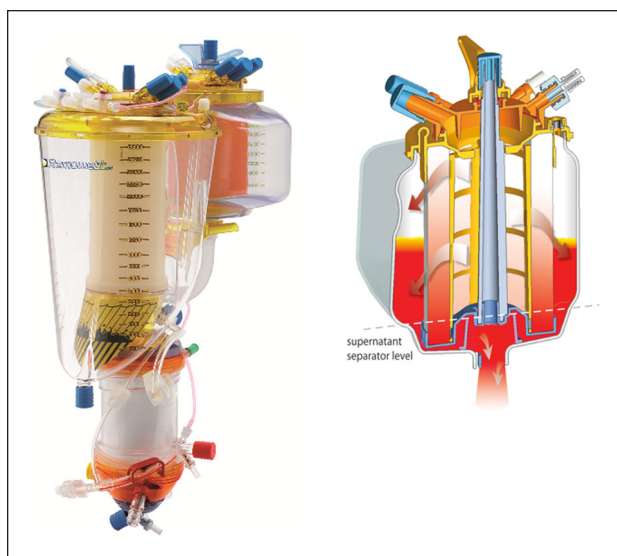
vascular permeability and arterial–alveolar gradients and decreased pulmonary compliance, resulting in acute respiratory distress syndrome (ARDS).<sup>2</sup> This was refined in 2012 by the European Society of Intensive Care Medicine as “an acute, diffuse, inflammatory lung injury, leading to increased pulmonary vascular permeability, increased lung weight and loss of aerated lung tissue...(with) hypoxemia and bilateral radiographic opacities, associated with increased venous admixture, increased physiological dead space and decreased lung compliance.”<sup>3</sup> Furthermore, the group categorized ARDS into three grades: mild, moderate and severe.

The leukocyte integrin CD11b/CD18 promotes leukocyte-endothelial adhesion and has been shown to be a reliable surrogate marker of leukocyte activation<sup>4</sup> and is, in turn, itself activated by the fatty acid oleic acid, the major component of lipid microemboli (LME) in pericardial suction blood (PSB).<sup>5</sup> Leukocyte filtration (leukodepletion) has been proposed as a technique to attenuate the inflammatory response to bypass, with numerous studies investigating differing methodologies to address this although most have been small studies with limitations noted in the methodology, leading to inconsistent evidence for its use.<sup>4</sup> Moreover, due to technical impracticalities, such as the saturation of the filter, there is a trend to attempt targeted leukodepletion; for example, during the administration of blood cardioplegia to prevent ischaemic-reperfusion injury.<sup>4</sup>

Previous work has demonstrated that there are no ideal methods of treating PSB in order to remove activated leukocytes whilst simultaneously retaining significant quantities of plasma clotting factors.<sup>6</sup> We have previously shown that it is possible to remove LME from PSB using a new oxygenation system (RemoweLL, Eurosets s.r.l, Mirandola, Italy).<sup>7</sup> This combines a cardiomy reservoir consisting of a 40- $\mu$ m membrane to provide multilayer filtration for leukocytes and lipids, with a sedimentation chamber that allows the siphoning of a lipid-rich supernatant (Figure 1). The next step was to assess whether this system could attenuate systemic leukocyte activation and how this might affect leukocyte differentiation and pulmonary function.

## Methods

Thirty patients undergoing coronary artery bypass grafting (CABG) with CPB were prospectively randomised to either the RemoweLL (intervention) or conventional extracorporeal circuit (control; Admiral, Eurosets S.r.l. Medolla (MO), Italy), using a previously compiled randomization table system (QuickCalcs Randomise1, GraphPad Software Inc., San Diego, CA, USA). The study was approved by the Institutional Review Board and Research Ethics Committee (10/H0606/30). Exclusion criteria included emergency or



**Figure 1.** RemoweLL Cardiomy Schematic. The RemoweLL® extracorporeal circulation system comprising a leukocyte filter and lipid microemboli siphon.

previous cardiac surgery, morbid obesity, pre-existing renal or pulmonary dysfunction and evidence of existing cognitive impairment. All patients provided written, informed consent. Anaesthesia was induced with midazolam, fentanyl and pancuronium and maintained during CPB using a propofol infusion. The CPB circuit consisted of an oxygenation system with integrated cardiomy reservoir. This contained either a conventional cardiomy filter (Control; Admiral, Eurosets s.r.l.) or a lipid/leukocyte filter (Intervention; RemoweLL, Eurosets S.r.l). The circuit was primed with 2 L lactated Ringer’s solution (Baxter, Thetford, UK) and mannitol 20% w/v (2.5mL/kg; Baxter) that contained 5000 units of unfractionated heparin (Wockhart, Wrexham, UK). Alpha-stat blood gas management was used and analysis of activated clotting time was checked every 20 to 30 minutes, with a minimum activated clotting time of 480 seconds maintained during bypass. Cardiac output was based on a cardiac index of 2.4 L/m<sup>2</sup>/min, with mean arterial pressures maintained between 50-60 mmHg with pharmacological manipulation, using 0.5-1.0 mg boluses of metaraminol as required. All patients were systemically cooled to nasopharyngeal temperatures between 32-34°C and myocardial protection was provided using cold (4°C) blood cardioplegia (Harefield Hospital Formulation, Terumo BCT Ltd., Larne, UK). Distal anastomoses were completed during a single period of aortic clamping whilst proximal anastomoses were performed with a beating heart, using an aortic partial occluding clamp. Termination of CPB occurred once the patient was re-warmed to a nasopharyngeal temperature of 36°C. The PSB was sent to the integrated cardiomy reservoir where it was kept separated from

the systemic circulation until immediately prior to the end of the CPB period whereupon it was reintroduced via the venous reservoir back into the systemic circulation. After the operation, the patients were kept ventilated until standard extubation criteria were met. The ventilation protocol comprised a respiratory rate of 10, a tidal volume of 10 mL/kg of body weight, a fraction of inspired oxygen of 60%, pressure support of 20 cmH<sub>2</sub>O, positive end expiratory pressure of 5 cmH<sub>2</sub>O and an inspiratory to expiratory ratio of 1:2. Hydration was achieved with the intravenous administration of Dextrose 5% solution infused at 1 mL/kg/h. Blood, Gelofusine or human albumin solution was given to maintain adequate filling and systemic perfusion pressures and plasma haemoglobin levels above 8.5 g/dL.

Leukocyte differentiation was undertaken using standard laboratory techniques. Samples were taken pre-CPB, pre-reintroduction of PSB back into the systemic circulation, post-reintroduction of the PSB, immediately preceding the end of CPB and 1 hour and 24 hours post-CPB. Data were collected on erythrocytes, haemoglobin, haematocrit, platelets and leukocytes plus differential (neutrophils, lymphocytes and monocytes).

#### Assessment of leukocyte activation

A 5-mL blood sample was taken for analysis of CD11b pre-CPB 5 minutes before the end of CPB and 1 and 24 hours post-CPB. Samples were kept on ice and were analyzed within 24 hours by fluorescence-activated cell sorting (FACS) after staining with CD11b phycoerythrin (PE). The samples were then mixed and incubated at 4-8°C for 30 minutes. Following lysing, the samples were washed and centrifuged at 3000rpm for 2 minutes and, then, the supernatant was removed. The remaining pellet was re-suspended in cell wash and placed into the FACS machine for analysis. Samples were also stained for CD45 as a control marker and concentrations of monocytes, lymphocytes and total leukocytes were reported. Adjustment of the positions of displayed and preconfigured polygon gates to capture all populations of interest, including neutrophils, monocytes and lymphocytes using the CD45/SSC plot, were performed by Dr. Adnan Mani, Head of the Flow Cytometry Unit, Southampton General Hospital.

#### Assessment of pulmonary function

Alveolar-arterial oxygenation index (AaOI) was calculated from blood gases and ventilation data taken pre-CPB, immediately post-CPB and 1, 2, 4, 8 and 18 hours post-CPB. For the 48-hour sample, the patient breathed room air for 10 minutes to allow for equilibration and, then, samples of arterial blood were taken for oxygen

partial pressure (PaO<sub>2</sub>), % saturation and carbon dioxide partial pressure (PaCO<sub>2</sub>). Samples obtained at 1, 2 and 4 hours reflect intubation whilst 8 and 18 hours reflected oxygen administered by facemask. The AaOI was determined using the following formula:

$$\text{AaOI} = ((760-47)F_{iO_2} - (\text{PaCO}_2 \times 7.6)1.25) - (\text{PaO}_2 \times 7.6) / (\text{PaO}_2 \times 7.6)$$

Where:

F<sub>i</sub>O<sub>2</sub> = the inspired oxygen concentration (%)

PaCO<sub>2</sub> = the partial pressure of arterial CO<sub>2</sub> (kPa)

PaO<sub>2</sub> = the partial pressure of arterial O<sub>2</sub> (kPa).

ARDS classification was calculated using PaO<sub>2</sub>/F<sub>i</sub>O<sub>2</sub> ratios at 1 and 2 hours post-CPB, with the lowest value used for categorization according to the Berlin definition. Each category was given a number indicating severity, 1: PaO<sub>2</sub>/F<sub>i</sub>O<sub>2</sub> >300, none; 2: PaO<sub>2</sub>/F<sub>i</sub>O<sub>2</sub> 200-300, mild; 3: PaO<sub>2</sub>/F<sub>i</sub>O<sub>2</sub> 100-200, moderate; 4: PaO<sub>2</sub>/F<sub>i</sub>O<sub>2</sub> <100, severe.

#### Statistics

Assessment of normal distribution was carried out using the Shapiro-Wilk Test and confirmed using a QQ Plot. As many of the parameters were measured at various time points, two factor ANOVA for repeated measures was undertaken to explore differences between the groups. Normally distributed data were analyzed using a t test for two independent samples whilst non-normally distributed values were LOG transformed and, if shown to be normally distributed, analyzed as above. If still non-normally distributed, data were analyzed using the Mann-Whitney test for two independent samples. ARDS categorization was tested using the Chi-square test. All tests were considered to be two tailed. A p-value ≤0.05 was considered significant. Normally distributed data are presented as mean±standard deviation whilst non-normally distributed data are presented as median (IQR). Both are graphically displayed as box and whisker plots with boxes representing 25th-75th centiles, with median and whiskers as maximum and minimum values.

#### Results

Thirty patients successfully underwent the study assessments (15 per group). The demographics of the patients undergoing the study are given in Table 1. Both groups were well matched in terms of male:female ratio, preoperative statin regimen and number of patients with diabetes mellitus (p>0.05). All patients were on aspirin and clopidogrel anticoagulation therapy preoperatively. In line with hospital protocol, both treatments were stopped 10 days before surgery. There were no

**Table 1.** Demographic data. Data presented as mean±standard deviations or median (IQR).

	Admiral	RemoweLL	p
Male (n)	12.00	11.00	
Diabetes (n)	2.00	3.00	
Statin (n)	8.00	8.00	
Age (years)	69.93 ± 7.54	69.33 ± 7.29	0.83
Height (m)	1.76 ± 0.10	1.71 ± 0.08	0.10
Weight (kg)	87.51 ± 13.37	82.84 ± 14.90	0.37
Body Mass Index	28.15 ± 3.56	28.31 ± 4.15	0.91
Body Surface Area	2.07 ± 0.20	1.98 ± 0.21	0.24
Calculated Flow (L/min)	4.96 ± 0.47	4.74 ± 0.50	0.24
Bypass Time (min)	101.40 ± 22.01	88.47 ± 23.51	0.13
X-clamp Time (min)	62.67 ± 17.67	51.20 ± 17.11	0.08
Procedure (CABG x n)	3.33 ± 0.49	3.13 ± 0.83	0.43
Fluid Balance (mL)	1678.60 ± 842.38	1562.27 ± 867.16	0.71
Time of Cardiotomy Release (min)	74.93 ± 19.27	67 ± 17	0.23
Volume in Cardiotomy Reservoir (mL)	776.67 ± 632.14	780.00 ± 567.20	0.99
Pre-CPB LME Count (n/μL)	400 (200)	400 (400)	0.47
Post-CPB LME Count (n/μL)	1200 (2000)	100 (75)	<0.001*

X-clamp; aortic cross-clamp. CABG; coronary artery bypass grafts; LME: lipid microemboli. Time of cardiotomy release is the amount of time the pericardial suction blood (PSB) was left separated from the systemic circulation. There were no significant differences between the groups of patients with regards to morbidity, preoperative drug regimens and perioperative details. \* A p-value ≤0.05 was considered significant.

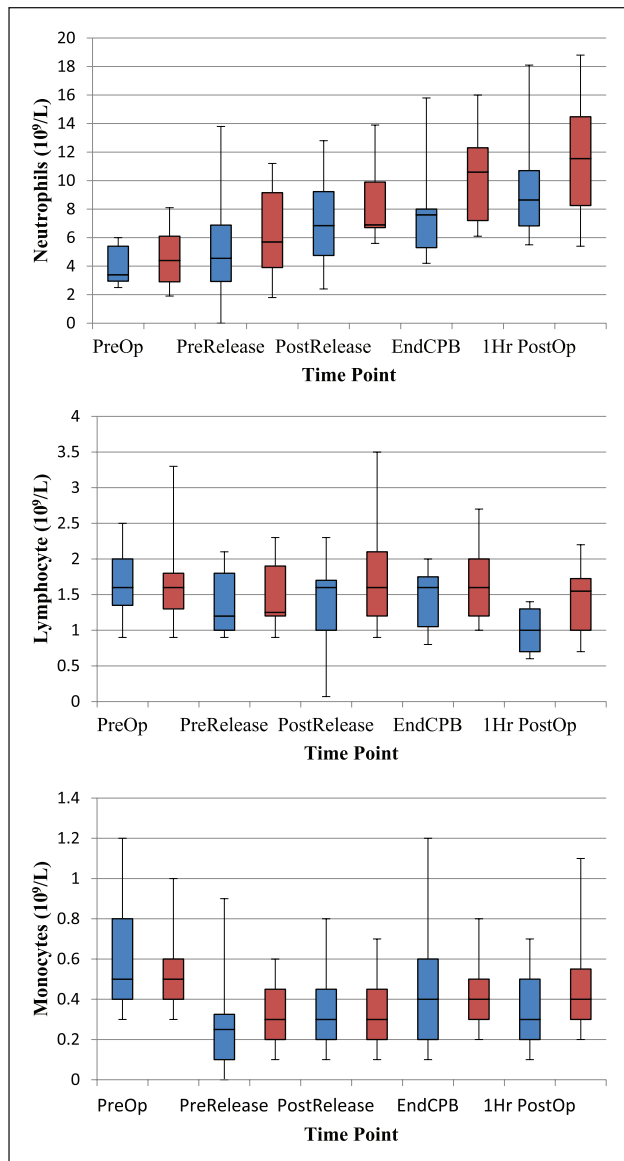
significant differences in terms of perioperative details, including CPB time, number of grafts and fluid balance. There were no significant differences in transfusion rates or haemoglobin levels between the two groups at any time point ( $p>0.05$ ).

The number of total leukocytes rose significantly throughout the CPB period ( $p<0.001$  for both groups). Whilst there were no significant differences between the two groups, there was a tendency for greater leukocyte numbers in the RemoweLL group at 1 hour post CPB (Admiral 9.7 (4.5)  $\times 10^9/L$  vs. RemoweLL 13.4 (7.2)  $\times 10^9/L$ ;  $p=0.06$ ; Figure 2). Differential leukocyte analysis showed a similar pattern for neutrophils. There were no significant differences in neutrophil counts between the groups at any sample point and both groups showed significant increases in neutrophil numbers following CPB ( $p<0.001$ ). Lymphocytes were similar in both groups during the procedure, but a significant difference was noted between the two groups in the immediate post-op period (Admiral 1 (0.6)  $\times 10^9/L$  vs. RemoweLL 1.55 (0.73)  $\times 10^9/L$ ;  $p=0.018$ ). Pre- and post-analysis showed a significant decrease in the Admiral group, but similar levels in the RemoweLL group (Admiral 1.6 (0.65)  $\times 10^9/L$  vs. 1 (0.6)  $\times 10^9/L$ ;  $p<0.001$ ; RemoweLL 1.6 (0.5)  $\times 10^9/L$  vs. 1.55 (0.73)  $\times 10^9/L$ ;  $p=0.46$ ). The number of monocytes dropped significantly in both groups upon commencing bypass (Admiral 0.5 (0.4)  $\times 10^9/L$  vs. 0.25 (0.23)  $\times 10^9/L$ ;  $p=0.004$ ; RemoweLL 0.5 (0.2)  $\times 10^9/L$  vs. 0.3 (0.25)  $\times 10^9/L$ ;  $p=0.005$ ). Post-CPB, the numbers of monocytes had recovered in the RemoweLL group (0.5 (0.2)  $\times 10^9/L$  vs. 0.4 (0.25)  $\times 10^9/L$ ;  $p=0.15$ ), but remained

significantly suppressed in the Admiral group (0.5 (0.4)  $\times 10^9/L$  vs. 0.3 (0.3)  $\times 10^9/L$ ;  $p=0.02$ ).

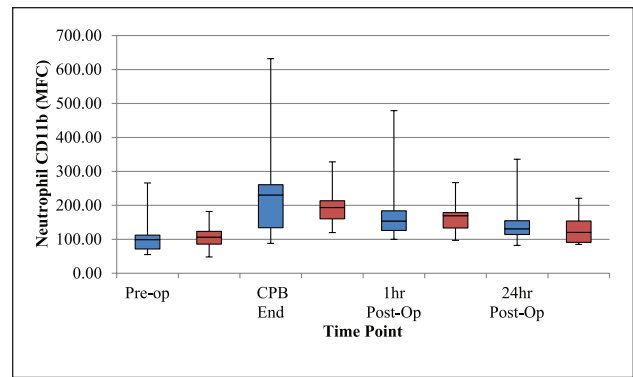
Leukocyte activation, as measured by CD11b, showed a significant increase in both groups, with peak activation occurring at the end of CPB, although there was no difference between the groups in terms of peak fluorescence (Admiral 230.5 (126.5) mean fluorescence channel (MFC) vs. RemoweLL 193.5 (53) MFC;  $p=0.8$ ; Figure 3). A significant decrease in activation was observed in the RemoweLL group between 1 and 24 hours post-CPB (169.5 (45.3) MFC vs. 120.5 (63) MFC;  $p=0.04$ ) whilst the Admiral group saw an insignificant decrease (154 (58) MFC vs. 131 (40.5) MFC;  $p=0.43$ ). Twenty-four hours post-op, the levels of activation had returned to baseline in the RemoweLL group (106.5 (37.5) MFC vs. 120.5 (63) MFC;  $p=0.24$ ), but remained elevated in the Admiral group (99 (40.75) MFC vs. 131 (40.5) MFC;  $p=0.016$ ).

Baseline data for AaOI were similar in both groups although the RemoweLL group had slightly lower values (Admiral 12.4 (3.2) vs. RemoweLL 10.4 (5.9);  $p=0.1$ ; Figure 4). ANOVA results showed a trend for lower AaOI in the RemoweLL group although this did not reach significance ( $p=0.075$ ). Further examination using pairwise analysis with a t test for two independent samples showed significantly lower AaOI post-CPB in the RemoweLL group (Admiral 17.67 (14.15) vs. RemoweLL 13.25 (4);  $p=0.012$ ). There were no differences between the two groups at any other time points. Pre-CPB vs. 18 hr post-CPB showed significant differences in both groups (Admiral 12.4 (3.12) vs. 5.81 (6.6);

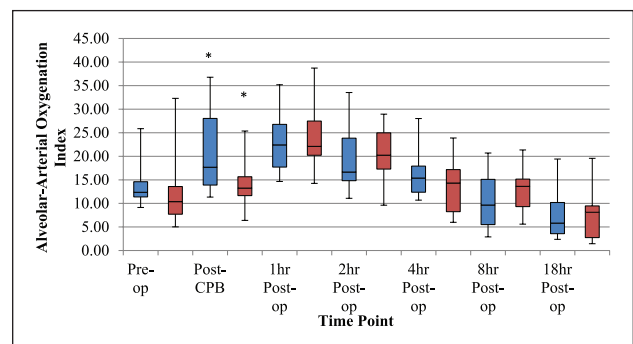


**Figure 2.** Cell differential during cardiopulmonary bypass. Samples taken pre-CPB, pre-PSB release into the systemic circulation, post release of PSB into the systemic circulation, 5 minutes before the end of CPB and 1 hour post-CPB. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots, with boxes representing 25th-75th centiles, with median and whiskers as maximum and minimum values.

$p < 0.001$  and RemoweLL 10.4 (5.9) vs. 8.12 (6.7);  $p = 0.033$ ). Repeated measures ANOVA showed significant interactions in both  $pO_2$  and  $pCO_2$  ( $p < 0.001$ ; Table 2), but this was not reflected in the lowest  $PaO_2/FiO_2$  ratios and ARDS definitions, which showed no differences between the two groups ( $p = 0.33$ ; Table 3). Although data were not collected on the length of ventilation, as the study was not powered to detect such differences, all patients were extubated within 24 hours.



**Figure 3.** Neutrophil activation profile. MFC; Mean fluorescence channel. Activation of neutrophil adhesion molecule CD11b used as an indicator of neutrophil activation as measured by FACS analysis. Samples taken pre-CPB, 5 minutes before the end of CPB and 1 And 24 hours post-CPB. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75th centiles, with median and whiskers as maximum and minimum values.



**Figure 4.** Alveolar-arterial Oxygenation Index. Samples taken pre-CPB, immediately post-CPB and then 1, 2, 4, 8 and 18 hours post-CPB. For the 48 hour sample, the patient breathed room air for 10 minutes to allow for equilibration and then samples of arterial blood were taken. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75th centiles, with median and whiskers as maximum and minimum values. \* $p \leq 0.05$ .

## Discussion

The initial filtration step of the RemoweLL filter is designed to remove activated leukocytes from the cardiotomy suction via two layers of 40- $\mu$ m, non-woven polyester, the second treated with a polymeric coating. It is believed that leukocytes play a major role in the formation of ‘post-pump’ syndrome which can affect lungs, kidneys and the brain.<sup>8</sup> The aim of the current study was to determine the effects the RemoweLL system had on the numbers and activation status of leukocytes, as measured by CD11b, and post-operative

**Table 2.** Ventilation Settings.

	Time	Admiral	RemoweLL	p
F <sub>I</sub> O <sub>2</sub>	Pre-op	0.57 (0.02)	0.57 (0.02)	0.69
	Post-CPB	0.58 (0.03)	0.57 (0.04)	0.06
	1 Hr Post-op	0.5 (0.07)	0.5 (0.08)	0.79
	2 Hr Post-op	0.5 (0.1)	0.45 (0.08)	0.65
	4 Hr Post-op	0.4 (0)	0.4 (0.08)	0.09
	8 Hr Post-op	0.35 (0.1)	0.35 (0.05)	0.55
	18 Hr Post-op	0.3 (0.09)	0.28 (0.09)	0.98
	p <sub>a</sub> O <sub>2</sub> (kPa)	Pre-op	33.5 (3.2)	36.3 (4.6)
Post-CPB		30.2 (11.8)	33.4 (2.3)	0.13
1 Hr Post-op		19.3 (5.9)	18.6 (2.4)	0.82
2 Hr Post-op		16.4 (3.6)	16.2 (3.2)	0.37
4 Hr Post-op		15.9 (3.9)	14.8 (2.7)	0.12
8 Hr Post-op		14.9 (3.2)	12 (2)	<0.001
18 Hr Post-op		11.2 (2.2)	11.6 (1.3)	0.87
p <sub>a</sub> CO <sub>2</sub> (kPa)		Pre-op	5.32 ± 0.42	5.42 ± 0.56
	Post-CPB	5.68 ± 0.64	5.38 ± 0.29	0.18
	1 Hr Post-op	5.39 ± 0.49	4.89 ± 0.63	0.05
	2 Hr Post-op	5.28 ± 0.55	4.66 ± 0.37	0.002
	4 Hr Post-op	5.22 ± 0.54	4.94 ± 0.53	0.21
	8 Hr Post-p	5.12 ± 0.39	5.59 ± 0.43	0.02
	18 Hr Post-op	5.03 ± 0.33	5.34 ± 0.22	0.01

F<sub>I</sub>O<sub>2</sub>: fraction of inspired oxygen; p<sub>a</sub>O<sub>2</sub>: arterial partial pressure of oxygen; p<sub>a</sub>CO<sub>2</sub>: arterial partial pressure of carbon dioxide. Samples taken pre-CPB, immediately post-CPB and then 1, 2, 4, 8 and 18 hours post-CPB. A p-value ≤0.05 was considered significant.

**Table 3.** ARDS Classification.

ARDS Classification	Admiral	RemoweLL	p
None	2	0	0.33
Mild	10	11	
Moderate	3	4	
Severe	0	0	

Classifications based upon Berlin definitions; None, PaO<sub>2</sub>/FiO<sub>2</sub> >300; Mild, PaO<sub>2</sub>/FiO<sub>2</sub> = 200 – 300; Moderate, PaO<sub>2</sub>/FiO<sub>2</sub> = 100 – 200; Severe, PaO<sub>2</sub>/FiO<sub>2</sub> <100. A p-value ≤0.05 was considered significant.

pulmonary function as judged by the ventilatory requirements of patients undergoing CABG surgery.

### Leukocyte depletion and activation

Our results did not demonstrate any leukocyte reduction compared to the control group. In fact, there was a tendency for the RemoweLL group to have higher levels of leukocytes one-hour post-CPB (p=0.06). Leukocyte differential data showed that the number of neutrophils rose significantly in both groups compared to baseline (p<0.001). This is in contrast to previous studies which used leukocyte-depleting filters and have shown a decrease in neutrophil concentration. In particular, Dell'Amore and colleagues showed a decrease in neutrophil concentration from 76.2x10<sup>3</sup>/mL to 69.7x10<sup>3</sup>/mL using the same RemoweLL system.<sup>9</sup> However, the

25th–75th percentiles were 56–78x10<sup>3</sup>/mL in the control group and 55–77x10<sup>3</sup>/mL in the RemoweLL group, so it is debatable as to whether this was an effect of small sample size (10 patients per group). Furthermore, it would seem likely that the samples were taken directly from the cardiotomy reservoir and not from the systemic circulation, as was the case in the current study. Santa Ursula Tolosa et al. previously reported a 'neutrophilic leukocytosis' after cardiac surgery which persists for 2–3 days, which represents the acute response to stress, the mobilization of marginalized neutrophils and the release of new neutrophils from the bone marrow and pulmonary circulation.<sup>10</sup> A common observation following cardiac surgery is the acute reduction in circulating lymphocytes, as observed in the current study. Rinder et al. have suggested that the loss of lymphocytes might be due to numerous factors, including haemodilution, redistribution of cells to the tissues and loss due to bleeding and adhesion to CPB circuitry.<sup>11</sup> Further work by the group proposed that cellular activation causes adhesion activation of lymphocytes and endothelial cells, promoting exit from the circulation.<sup>12,13</sup> This study also noticed a significant decrease in monocytes from induction to during CPB. Rinder et al. have previously shown that monocytes have increased expression of activation-dependent adhesion receptors immediately post-CPB.<sup>12</sup> In contrast to this study, they report observing a rise in monocytes to almost double baseline

levels. However, this was on the 1<sup>st</sup> post-op day, later than measured in this study and they did not examine changes in the perioperative period. Interestingly, in this study, after the initial reduction in monocyte numbers, there was a trend towards a return to baseline levels in both groups although, in the Admiral group, this remained significantly lower than baseline ( $p=0.017$ ). Evidence suggests that monocytes, like lymphocytes, are removed from the circulation to tissues as another marker, CD16, which is expressed on mature monocytes, decreases despite overall increases in monocyte numbers, suggesting immature monocyte recruitment from the bone marrow.<sup>14</sup> Therefore, this study could not demonstrate significant differences in total or leukocyte differential counts between the two groups or the published literature using the RemoweLL cardiomy reservoir.

In order to assess the extent of leukocyte activation that occurred during the procedure, CD11b (a surface adhesion molecule that regulates the adhesion of activated leukocytes) was measured using FACS analysis. The CD11b integrin is a key factor in allowing the transendothelial migration of leukocytes through the modulation of the endothelial cytoskeleton. When inappropriate activation occurs, this is a primary cause of tissue oedema.<sup>15</sup> Previous studies have demonstrated reduced expression of CD11b in patients undergoing CABG surgery, with continuous arterial line leukocyte depletion.<sup>16,17</sup> In contrast to inline leukocyte filtration, this study showed no statistical difference in peak CD11b expression between the Admiral and RemoweLL groups ( $p=0.8$ ). Of particular interest to this study is the observation of Mastrangelo et al. who demonstrated a direct interaction between the CD11b integrin and oleic acid, the major fatty acid component of bone marrow-derived fat emboli<sup>5</sup> that is also removed by the RemoweLL cardiomy reservoir.<sup>7</sup> This interaction, they reported, caused the leukocytes to aggregate and adhere with CD11b/CD18 to endothelial membranes. Moreover, previous work by the group showed that free fatty acids caused and amplified the mobilization of myeloperoxidase-containing granules within the leukocyte.<sup>18</sup> As the 2<sup>nd</sup> time point (end CPB) would have been shortly after the release of cardiomy PSB in both groups, it is of little surprise that this is where peak activation occurs as this is the point at which oleic acid, in the form of LME, would enter the circulation. However, there appears to have been little, if any, research on the length of action of oleic acid on leukocytes. Mastrangelo et al. saw increasing expression of CD11b with time following the addition of oleic acid to leukocytes, but the experiments only measured expression for 10 minutes post-treatment and was undertaken in an *in vitro* environment, so translation to the present study is difficult. Furthermore, leukocyte activation does not occur solely

from oleic acid interactions; exposure to artificial surfaces, anaesthesia, even the surgery itself can cause neutrophil activation.<sup>19</sup> Therefore, this study could not demonstrate any significant differences in leukocyte activation status between the two groups using the RemoweLL cardiomy reservoir, a phenomenon that has been described with in-line leukodepletion.

### Pulmonary function

Pulmonary dysfunction following surgery is not unique to cardiothoracic surgery involving CPB, with multiple factors indicated, such as general anaesthesia.<sup>20</sup> However, due to the nature of the extracorporeal circuit, its non-endothelial material, the opening of the pleural space for harvesting of the internal mammary artery and the deflation of the lungs during cardiac surgery, the incidence of pulmonary dysfunction associated with CPB-facilitated cardiac surgery is more common and severe.<sup>21</sup> Whilst the manifestation of pulmonary dysfunction can be quantified in terms of a widening of the alveolar-arterial oxygen gradient, the underlying causes are many and varied, as are the physiological characteristics. An increase in AaOI was observed in all patients following CPB. This has also been demonstrated in previous studies and is thought to arise from several factors: the disruption of pulmonary endothelial membranes, haemodilution with the subsequent sudden decrease in colloid osmotic pressure and the use mechanical ventilation.<sup>22</sup> If an increase in alveolar-endothelial permeability did occur, the fluid balance of both groups was similar ( $1678.60 \pm 842.38$  ml vs.  $1562.27 \pm 867.16$  ml;  $p=0.71$ ) and so this study would not be powered to detect any differences in this situation. One of the key components believed to facilitate changes in pulmonary function is the inflammatory response. Previous studies have shown contact with the extracorporeal circuit initiates the activation of leukocytes, facilitating the transmigration of leukocytes into the lung parenchyma, which mediates lung parenchymal damage through cellular and tissue injury.<sup>21</sup> The resulting injury destroys the ultrastructure of the lung, increasing the permeability of alveolar-endothelium. Therefore, theoretically, it could be assumed that filtration of activated leukocytes should provide significant protection from lung injury. This study focussed on the calculation of the AaOI, which is an established method for the assessment of peri-operative changes in lung function and is invariably elevated after cardiac surgery. Furthermore, this study aimed to use the Berlin definitions of ARDS to examine any differences in arterial oxygenation to determine if either group exhibited a more severe rating than the other. Repeated measures ANOVA showed no significant difference between the two groups in terms of AaOI ( $p=0.075$ ). Stepwise analysis of log-transformed

data using unpaired t tests showed a significant difference in the immediate post-op period ( $p=0.01$ ), but any functional difference had resolved by 1 hour following surgery ( $p=0.9$ ) and remained insignificant for the remaining period of observation. Furthermore, analysis revealed that the fraction of inspired oxygen showed a trend for lower values immediately post-CPB, which may explain the differences in the post-op AaOI ( $p=0.06$ ). Chi-square testing of the ARDS classification showed no significant differences between the two groups ( $p=0.33$ ). This study, therefore, could not demonstrate any evidence of improved pulmonary function using the RemoweLL cardiotomy reservoir.

### Limitations

There are several important limitations to this study that should be noted. The numbers of patients in this study is relatively small, therefore, this study may not have the power to detect subtle differences. As the samples for measurement were taken from the sampling port (which is connected to the arterial limb of the CPB circuit) rather than the cardiotomy reservoir, one could speculate that the level of leukocyte activation would be similar in both study groups as this measures activation at the systemic level. Whether leukocyte activation in the cardiotomy suction with the RemoweLL compared to the Admiral group is different cannot be confirmed. Furthermore, this study was not designed to examine differences in length of ventilation (as far greater numbers of patients would be required), so cannot comment on the duration of ventilation with or without leukocyte filtration. Finally, there are issues with using the Berlin definitions of ARDS; in particular, they do not include the underlying aetiology and lack a true measure of lung injury. For example, the use of vasopressors at the time of ARDS diagnosis (which is associated with a much higher mortality) is independent of the  $\text{PaO}_2:\text{FiO}_2$  ratio. It also does not allow the early identification of patients who may be amenable to therapies before ARDS becomes established and, as none of the patients exhibited signs of severe ventilation requirements, the usefulness of this parameter in the current study could also be questioned.

### Conclusion

Contact with the extracorporeal circuit initiates the activation of leukocytes as well as a host of proinflammatory mediators, including the CD18 and CD11b surface adhesion molecules on leukocytes which promote adhesion to specific ligands on the pulmonary endothelium, with the resulting injury destroying the ultrastructure of the lung and increasing the permeability of the alveolar endothelium. This study aimed to determine whether

leukocyte filtration of PSB, which is known to contain high concentrations of activated leukocytes, using the RemoweLL cardiotomy reservoir, could attenuate these adverse effects. An increase in total numbers and activation status of leukocytes was confirmed in both groups with no statistical differences between the two groups. Furthermore, analysis of pulmonary function revealed no significant differences between the two groups during the post-operative period. Therefore, this study was unable to demonstrate the clinical efficacy of the RemoweLL oxygenation system in providing targeted leukodepletion.

### Declaration of Conflicting Interests

The manufacturer of the medical device under trial funded the biochemical analysis used in this study. The authors had full control of the design of the study, methods used, outcome measurements, analysis of data and production of the written report.

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