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Preoperative neutrophil response as a predictive marker of clinical outcome following open heart surgery and the impact of leukocyte filtration☆,☆☆,☆☆☆

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Abstract

Objective: Open heart surgery is associated with a massive systemic inflammatory response. Neutrophils, are the main mediator of this response. We hypothesised that the degree of neutrophil activation and inflammatory response to open heart surgery varies individually and correlates with clinical outcome. The aim of this study was to determine if individual clinical outcome can be predicted preoperatively through assessment of in-vitro stimulated neutrophil responses. Following that, the effects of neutrophil depletion through leukocyte filters are examined. **Methods:** Neutrophil responses were assessed preoperatively ($n=40$) through change in neutrophil adhesion molecule [CD11b, CD62L and P Selectin Glycoprotein-1 (PSGL-1)] expression before and after in-vitro stimulation with Phorbol 12-myristate 13-acetate, PMA (1 ng/ml), lipopolysaccharide, LPS (1 μg/ml) and N-Formyl-Met-Leu-Phe, fMLP (1 ng/ml). Stimulated neutrophil responses were then correlated with postoperative clinical outcome. Patients were then randomised to leukocyte filtration ($n=20$) and a control group ($n=20$) and the effect of leukocyte filtration on neutrophil response and clinical outcome were investigated. **Results:** An individual variation in in-vitro stimulated neutrophil responses was demonstrated. Significant correlations were shown between neutrophil responses and maximum serum creatinine change, CKMB-fraction, adrenaline requirement, noradrenaline requirement, duration of adrenaline required and time to extubation. White cell count and percentage neutrophils were lower in the LD group ($P=0.05$). CD11b expression ($P=0.005$) and PSGL-1 expression ($P=0.043$) across leukocyte filters were also increased. However, no significant difference was detected in clinical outcome between the LD and control groups. **Conclusion:** Preoperative neutrophil responses to in-vitro stimuli can predict clinical outcome following open heart surgery. However, leukocyte filtration did not offer significant benefit in clinical outcome in our study.

Keywords: Cardiopulmonary bypass; Neutrophil; Cardiac surgery; Complications; Leukocyte filtration

1. Introduction

The introduction of cardiopulmonary bypass (CPB) in the 1950s by Gibbon has revolutionised cardiac surgery. In the present era, cardiac procedures are safe and reproducible. However, despite technological advancement, the use of CPB is still associated with massive systemic inflammatory response resulting in postoperative morbidity. During CPB, the patient's circulation is passed through an extracorporeal circuit which leads to activation of inflammatory cells. The main mediator of damage being activated neutrophils.

Neutrophils contain a wide array of toxic granular enzymes and have the capacity to generate oxygen free radicals forming a formidable defence against offending micro-organism. However, in cardiac surgery, these destructive mechanisms contribute to tissue damage. During reperfusion, activated neutrophils undergo transendothelial migration to reach the target tissue. This process involves the interaction between adhesion molecules on the surface of neutrophils and endothelial cells, namely, the selectins (L-Selectin, CD62L), integrins (CD11b/CD18) and the immunoglobulins. Eventually, neutrophils migrate between cells to reach their target organ, a process known as 'diapedesis'.

To modify the perioperative inflammatory response caused by neutrophils, various attempts have been made to deplete neutrophils. These attempts include the use of direct neutrophil antibody blockade [1], inhibition of neutrophil adhesion [2, 3] and inhibition of neutrophil chemokine [4]. However, results of these methods have not been translated to clinical application. Currently, the only clinically utilised strategy to deplete neutrophils is by way of the leukocyte-depleting (LD) filters. LD filters are used traditionally in the blood transfusion service to filter whole blood donations. Clinical results to date have been conflicting and hence these filters have not been adopted univers-

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ally. Experimentally, some groups have found that these filters cause neutrophil activation, upregulating neutrophil markers, such as CD11b and increase the release of neutrophil enzymes, such as elastase and melondialdehyde [5, 6]. However, contrary results have also been reported [7].

Previous studies in our laboratory have demonstrated that individual neutrophil response correlated with severity of cardiac transplant rejection [8]. Furthermore, various genetic studies have demonstrated that polymorphisms may be responsible for individual's susceptibility to develop inflammatory disease. In this study, we aim to determine if individual neutrophil responses to stimulation assessed preoperatively can act as a predictive marker for postoperative morbidity. Also, we aim to determine the impact of LD filters and if the beneficial effect evident in some individuals is due to their ability to mount an inflammatory response.

2. Materials and methods

2.1. Patient population

This study was approved by the Mater Misericordiae University Hospital Ethics Committee. All patients undergoing coronary artery bypass grafting (CABG) surgery and valvular heart surgery were invited to participate. Written informed consent was obtained prior to participation. For the pilot study developing the preoperative in-vitro stimulation model, 20 patients ($n=20$) were recruited (group 1). The demographic data of these patients is summarised in Table 1. To determine the impact of leukocyte filtration on neutrophil function and clinical outcome, 40 patients were recruited (group 2). These patients were randomly allocated to the LD group ($n=20$) or a control group ($n=20$). Randomisation was achieved with sealed envelopes given to the perfusion department, whose staff then set up the bypass circuit using the appropriate filter. A record of the filter used for each patient was kept by the perfusion staff. This record was revealed only during data analysis. All other investigators were blinded to the patient allocation for the purpose of the study. Exclusion criteria were active infection, emergency operation, preoperative corticosteroid therapy and severe asthma or chronic obstructive lung disease. The demographic data of patients in both groups are summarised in Table 2.

Table 1. Demographic data of patients in pilot study for preoperative artificial stimulation study

Variable	$n=20$
Age (years)	64±10
Gender	
Male	15
Female	5
Type of procedure	
CABG	15
CABG+AVR	3
MVR	1
Redo CABG	1
CPB time (min)	111±30
Aortic cross-clamp time (min)	80±22
Systemic hypothermia (°C)	31.5±1.6
Left ventricular EF	
Good	14
Moderate	5
Poor	1

CABG, coronary artery bypass grafting; AVR, aortic valve replacement; MVR, mitral valve replacement; CPB, cardiopulmonary bypass; EF, ejection fraction.

Table 2. Demographic data of patients randomised into leukocyte depletion (LD) or control group. No significant difference was detected between the two groups

Variable	Control ($n=20$)	LD ($n=20$)	P-value
Age (years)	60±18	64±11	0.512
Gender			
Male	15	14	
Female	5	6	
Type of procedure			
CABG	9	8	
Redo CABG	–	1	
AVR	5	4	
Redo AVR	2	–	
MVR	–	1	
MVRp	2	2	
CABG+AVR	–	2	
CABG+MVRp	–	1	
AVR+MVR	–	1	
AVR+MVRp	1	–	
MVRp+TVRp	1	–	
CPB time (min)	93±28	83±27	0.273
Aortic cross-clamp time (min)	62±29	55±18	0.422
Systemic hypothermia (°C)	31±1	31±1	0.689
Left ventricular EF			
Good	12	14	
Moderate	6	4	
Poor	2	2	

CABG, coronary artery bypass grafting; AVR, aortic valve replacement; MVR, mitral valve replacement; MVRp, mitral valve repair; TVRp, tricuspid valve repair; CPB, cardiopulmonary bypass; EF, ejection fraction.

2.2. Anaesthetic technique, conduction of CPB, myocardial protection and leukocyte depletion

Similar anaesthesia (propofol, fentanyl, pancuronium) and monitoring techniques (electrocardiogram, central venous and arterial catheter, urinary catheter, temperature probes) were used in both groups of patients. No patient received steroids before or during the operation.

All operations were performed through a median sternotomy. Sodium heparin at a dose of 300 U/kg of body weight was administered intravenously before CPB to achieve an activated clotting time >470 seconds. Standard cannulation technique of ascending aortic and right atrium or bicaval cannulation was employed.

A standard CPB circuit was used in all patients. This consisted of a membrane oxygenator (COBE, Cardiovascular, SORIN Biomedica, Gloucester, UK) and a roller pump (Computer Assisted Perfusion System, CAPS, STÖKERT, SORIN Biomedica, Gloucester, UK) primed with 1.0–1.2 l of crystalloid solution. A similar myocardial protection strategy was used in both groups. This consisted of intermittent cold blood cardioplegia solution administered via the aortic root. Mild hypothermia (32 °C) was induced with the heat exchanger of the extracorporeal circuit and topical slush used concomitantly. In the LD group, a LD arterial blood filter for extracorporeal circulation (LeukoGuard-6, Pall Biomedical Product Corp, East Hillis, NY, USA) was placed

instead of the conventional arterial filter prior to commencement of CPB.

2.3. Timing of blood sampling

Blood was collected from patients preoperatively within 12 hours of their operation through venopuncture. Preoperative samples were obtained to assess baseline expression of neutrophil markers and stimulated with artificial stimulant as outlined in the next section to assess for functional responses. Intraoperative samples consisted of both venous and arterial samples taken before and after the filter at 5 minutes after cross-clamp release. These samples were obtained to assess the degree of surgically induced neutrophil activation and the differential effect of the filtration strategy. Blood samples were collected in lithium heparin tube (Coagulation 9 NC/10 ml, SARSTEDT Monovette, SARSTEDT AG & Co., Nümbrecht, Germany).

2.4. Neutrophil activation markers

Neutrophil activation was assessed in this study through surface expression of neutrophil adhesion molecules CD62L, CD11b, P Selectin Glycoprotein-1 (PSGL-1). Adhesion molecule expression was assessed using the Coulter EPICS® XL-MCL (Beckman Coulter, High Wycombe, UK) flow cytometer by direct immunofluorescence staining. Briefly, 50 μl of whole blood from different conditions was incubated with 5 μl of the relevant antibody for 20 minutes at room temperature in the dark. After incubation, antibody stained whole blood was treated with BD FACS lysing solution (BD Bioscience, Oxford, UK) for 10 minutes at room temperature to lyse the red blood cells. The resulting pellet was then washed with Dulbeccos Modified Eagle Medium (Gibco, Invitrogen life technologies, Paisley, UK) and stored on ice until assessment of neutrophil surface adhesion molecule expression using flow cytometry. Data was expressed as mean channel fluorescence. Antibodies to CD62L (PE-CD62L) and CD11b (PE-CD11b) were purchased from BD Bioscience (BD Bioscience, Oxford, UK) and the antibody to PSGL-1 (PE-conjugated anti-mouse CD162) was purchased from Fitzgerald Intl. (Fitzgerald Industries International, Concord, MA, USA).

2.5. In-vitro neutrophil stimulation

To assess individual neutrophil activation, preoperative whole blood samples (50 μl) were incubated with lipopolysaccharide, LPS (1 μg/ml), Phorbol 12-myristate 13-acetate, PMA (1 ng/ml) and N-Formyl-Met-Leu-Phe, fMLP (1 ng/ml) for specific lengths of time (1 hour for LPS, fMLP and 20 minutes for PMA) at 37 °C for optimal stimulation. LPS, PMA and fMLP were purchased from Sigma (Sigma-Aldrich Ireland Ltd., Dublin, Ireland). Following stimulation, neutrophil adhesion molecule expression was assessed using flow cytometry as outlined in the next section and described by Molloy et al. [9] The degree of individual neutrophil response was assessed through the difference in adhesion molecule expression pre- and poststimulation.

Table 3. Clinical outcome parameters measured in group 1 patients ($n=20$). The median and range of the data for each clinical parameter are given

Clinical outcome parameter	$n=20$
Time to extubation (hour, median and range)	15 (11–192)
Respiratory index (PaO_2/FiO_2) before extubation (mmHg, median and range)	43.1 (20.6–64.0)
Total mediastinal chest drainage (ml, median and range)	820 (320–4520)
Cumulative adrenaline requirement (μg/kg/hour, median and range)	31 (7.6–99)
Duration of adrenaline usage (hour, median and range)	12 (5–25)
Cumulative noradrenaline requirement (μg/kg/hour, median and range)	67.5 (0–494)
Duration of noradrenaline usage (hour, median and range)	20.5 (0–83)
Highest lactate level (median and range)	4.6 (0.8–8.6)
Maximal change in serum creatinine level (median and range)	19 (−40 to 93)
ICU stay (hour, median and range)	47 (22–240)

ICU, intensive care unit.

2.6. Intraoperative sample

As mentioned previously, intraoperative samples were obtained at 5 minutes after cross-clamp release. Both pre- and postfilter samples were obtained and assessed for adhesion molecule expression as in the preoperative sample but without artificial stimulation.

2.7. Clinical outcome parameters

Outcome variables measured in this study include duration of intensive care unit (ICU) stay, chest tube drainage and a range of organ function indices (Table 3). Duration of ICU stay (hours) was measured from time of ICU admission to time of discharge. Postoperative chest tube drainage was recorded at hourly interval and cumulative drainage was calculated 24 hours after patient's admission to ICU. Cardiac function was assessed using cardiac enzyme assay (CKMB-fraction), duration of inotropic support (hours) and cumulative inotropic requirement. Cardiac enzymes (CKMB-fraction) were assayed at 24 hours postadmission to ICU. Respiratory function was assessed using the duration of postoperative ventilation (hour) and respiratory index (PaO_2/FiO_2). Renal function was assessed by recording the ratio of maximum postoperative creatinine level to the preoperative value. Clinical parameters were obtained through review of charts and laboratory parameters were obtained through the Hospital Information System (HIS) in place in the Mater Misericordiae University Hospital.

2.8. Statistical analysis

Statistical analysis was performed using Minitab Release 13 statistical software package (Minitab Ltd., Coventry, UK). Correlations were performed using Pearson correlation test and comparisons between groups were performed using Student t-test. A $P \leq 0.05$ was considered to be significant.

3. Results

3.1. Preoperative in-vitro stimulated neutrophil response

Neutrophils isolated from whole blood, of patients undergoing routine open heart surgery (group 1, $n=20$) demon-

strated significant upregulation of CD11b expression following stimulation with PMA ($P<0.001$) and LPS ($P<0.0001$). fMLP stimulation had no significant effect ($P=0.119$).

Whole blood stimulation using PMA and LPS also led to a significant reduction in neutrophil surface CD62L expression (PMA, $P=0.009$, LPS, $P=0.001$). fMLP stimulation again demonstrated no significant change in CD62L expression ($P=0.296$).

Preoperative stimulation of neutrophils using PMA resulted in a significant upregulation of PSGL-1 expression ($P=0.015$). No significant upregulation of PSGL-1 expression was detected following stimulation with LPS ($P=0.145$) and fMLP ($P=0.564$).

3.2. Determination of individual variation in neutrophil response

We have previously demonstrated an individual variation in neutrophil responses upon artificial stimulation [8]. Similar responses were found in this study population, $n=20$ (data not shown).

3.3. In-vitro stimulated neutrophil response simulate surgical trauma

Open heart surgery causes significant neutrophil activation as a consequence of surgical trauma and the use of CPB. The aim of the preoperative in-vitro neutrophil stimulation model was to mimic this surgically induced neutrophil activation. We have previously performed this [8] and found a number of significant correlations between preoperative in-vitro stimulated neutrophil responses with surgically stimulated neutrophil responses validating our in-vitro whole blood preoperative stimulation model for subsequent correlations with patient outcome (data not shown).

3.4. Preoperative artificial stimulated neutrophil response predicts clinical outcome

With confirmation of the individual variation in neutrophil response, we correlated preoperative artificial stimulated neutrophil response with clinical outcome. In part I of the study, correlation was performed on patients in group 1 ($n=20$) as a pilot study. In part II of the study, patients in group 2 who were randomised into the control group ($n=20$), were also included in the correlation test. In Table 4, significant correlation between preoperative in-vitro neutrophil responses and clinical outcome parameters were shown. Significant correlations were shown between neutrophil responses with maximum serum creatinine change (CD11b-LPS, $P=0.023$; PSGL-1-PMA, $P=0.026$), CKMB-fraction on postoperative day 1 (CD11b-PMA, $P=0.041$; PSGL-1-PMA, $P=0.012$), cumulative requirement of adrenaline (CD62L-PMA, $P=0.001$, CD62L-fMLP, $P=0.015$) and noradrenaline (CD62L-PMA, $P=0.001$, CD62L-fMLP, $P=0.003$), duration of adrenaline required (PSGL-1-PMA, $P=0.017$) and time to extubation (PSGL-1-PMA, $P=0.012$). A positive correlation was found between preoperative in-vitro stimulated responses (Fig. 1) and clinical outcome variables signifying that a higher preoperatively stimulated neutrophil response is predictive of an unfavourable clinical outcome.

Table 4. Preoperative in-vitro stimulated neutrophil response predicts clinical outcome

Clinical outcome parameter	$n=40$
Maximal change in serum creatinine level	
CD11b-LPS response	$P=0.023$
PSGL-PMA response	$P=0.026$
CKMB fraction on postoperative day 1	
CD11b-PMA response	$P=0.041$
PSGL-1 PMA response	$P=0.012$
Duration of adrenaline support required	
PSGL-1 PMA	$P=0.017$
Cumulative dosage of adrenaline required	
CD62L-PMA	$P=0.001$
CD62L-fMLP	$P=0.015$
Cumulative dosage of noradrenaline	
CD62L-PMA	$P=0.001$
CD62L-fMLP	$P=0.003$
Time to extubation	
PSGL-1 PMA	$P=0.012$

Whole blood (50 μl) was obtained from patients preoperatively and stimulated with lipopolysaccharide, LPS (1 μg/ml), Phorbol 12-myristate 13-acetate, PMA (1 ng/ml) and N-Formyl-Met-Leu-Phe, fMLP (1 ng/ml) for specific length of time (1 hour for LPS, fMLP and 20 minutes for PMA). Neutrophil response (change in adhesion molecule expression) is then assessed and correlated with clinical outcome. The table above shows the correlation between preoperative in-vitro stimulated neutrophil response and clinical outcome. Only significant correlations are shown. PSGL-1, P Selectin Glycoprotein-1.

3.5. Effect of leukocyte filtration on leukocyte numbers, leukocyte function and clinical outcome

Leukocyte filters are an innovative device recently introduced into cardiac surgery. They are the only clinically available strategy to manipulate neutrophils. In the LD group, significant decreases in white cell count and percentage of neutrophils in the white cell population was

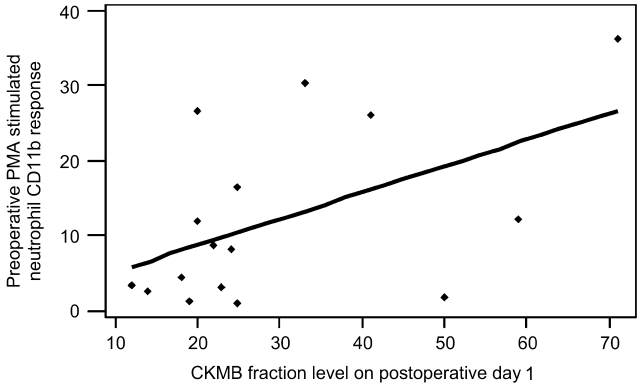

Fig. 1. Preoperative in-vitro stimulated CD11b response predicts CKMB fraction level on postoperative day 1. Whole blood (50 μl) was obtained from recruited patients preoperatively and stimulated with PMA (1 ng/ml) for 20 minutes. Neutrophil surface CD11b expression pre- and poststimulation was then assessed by flow cytometry. Neutrophil response was determined as the difference of CD11b expression pre-(basal level) and poststimulation. PMA stimulated neutrophil response was then correlated with CKMB fraction level obtained on postoperative day 1. Correlation was performed using Pearson's correlation test. Preoperative PMA stimulated neutrophil CD11b response showed a significant positive correlation ($R=0.514$, $P=0.041$) with CKMB fraction level on postoperative day 1. PMA, phorbol 12-myristate 13-acetate.

Table 5. Differences of white cell count, % neutrophil and neutrophil surface CD11b, CD62L and PSGL-1 expression across both leukocyte filter (LD) group and conventional arterial filter (control) group

	Prefilter	Postfilter	*P*-value
White cell count ($\times 10^9$)			
LD group	11.1±4.0	10.1±3.8	0.05
Control	11.7±6.5	11.4±6.4	0.75
% Neutrophil ($\times 10^9$)			
LD group	8.2±3.5	7.4±3.0	0.05
Control	8.8±5.9	8.7±5.6	1.0
CD11b			
LD group	14.8±5.4	15.9±4.8	0.543
Control	11.1±4.4	12.5±4.8	0.384
CD62L			
LD group	7.6±3.0	7.4±3.1	0.88
Control	7.9±2.9	8.3±3.5	0.725
PSGL-1			
LD group	3.1±0.8	3.2±0.8	0.826
Control	3.9±1.0	3.7±1.1	0.698

Intraoperative blood samples (50 μl) are obtained at 5 minutes after the release of aortic cross-clamp. Samples are obtained pre- and postfilter from all patients ($n=40$). Samples obtained are then assessed for total white cell count, % neutrophil, and neutrophil surface adhesion molecule expression. The differences across the filter in these parameters were compared between the groups. LD, leukocyte depletion; PSGL-1, P Selectin Glycoprotein-1.

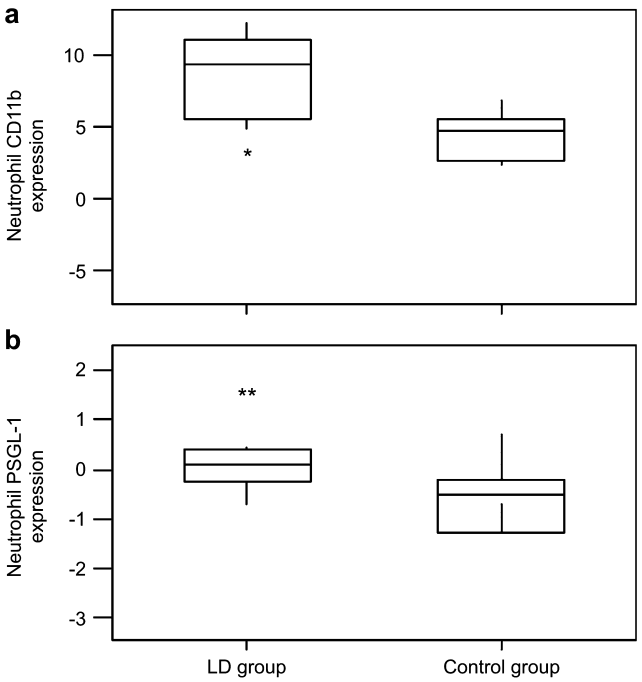

Fig. 2. Intraoperative neutrophil adhesion molecule expression. Whole blood (50 μl) is obtained from a site beyond the filters intraoperatively at 5 minutes after the release of aortic cross-clamp. Neutrophil adhesion molecule expression is assessed through flow cytometry and compared using Student *t*-test (Minitab Inc., USA). Significant upregulation of (a) CD11b expression (*, $P=0.005$) and (b) PSGL-1 expression (**, $P=0.043$) is demonstrated. LD, leukocyte depletion; PSGL-1, P Selectin Glycoprotein-1.

achieved ($P=0.05$) as compared to the control group. Across the filter, leukocyte filtration does not significantly change the expression of CD11b, CD62L and PSGL-1 (Table 5). However, significant increase in CD11b expression ($P=0.005$) and PSGL-1 expression ($P=0.043$) was demonstrated in the LD group as compared to the control group (Fig. 2).

Next, we compared the clinical outcome of the LD and the control group (Table 6). No significant difference in the clinical outcome was detected between the groups.

4. Discussion

Open heart surgery, in the current era, is a routine surgical procedure. Surgical repair of the heart with increasing complexity are being undertaken and increasingly, older patients are being operated on with great success. Much of this success is due to the improved understanding and management of the resulting systemic inflammatory response, the main cause of complications.

The systemic inflammatory response, associated with all forms of surgery, is particularly severe in open heart surgery partially due to the use of the extracorporeal circuit required for the conduction of CPB and reperfusion of the heart after a period of ischaemia. During the conduction of CPB, the patient's circulation and its components are exposed to a foreign surface. This leads to complement activation, the release of various inflammatory cytokines (interleukin-6 and -8, transforming growth factor), and neutrophil activation. However, to perform complex open heart surgery, the patient's heart is excluded from the systemic circulation during the operation. This subjects the heart to a period of ischaemia. To protect the heart, a potassium enriched solution is used and patients are subjected to hypothermia. During this period of ischaemia, endothelial cells of the coronary circulation express adhesion molecules and release chemokines which will in turn attract inflammatory cells during reperfusion. Inflammatory cells attracted to the heart will then exert an inflammatory response leading to myocardial damage. Neutrophils, in particular, are attributed as the main mediators of this reperfusion injury.

Neutrophils are the most abundant inflammatory cells in the body and are the main cell type responsible for host defence. They contain a wide array of granular enzymes, such as myeloperoxidase (MPO) and elastase. When released, these enzymes cause severe toxic effect. This is an extremely useful defence mechanism against foreign invading pathogen. However, released in the wrong setting, these enzymes can also cause damage to the host, which is the case in reperfusion injury. In order for neutrophils to reach the site of interest, they have to transmigrate through endothelial cells. Neutrophil transmigration is a process facilitated by the interactions between surface adhesion molecules on the neutrophils (e.g. CD62L/L-Selectin, CD11b/Mac-1, PSGL-1) and the endothelial cells (e.g. ICAM-1).

The degree of the systemic inflammatory response and reperfusion injury is related to factors, such as the duration of CPB and ischaemia of the heart. It is often observed that despite maintaining short durations of CPB and ischaemic time, some patients still develop systemic inflammatory response especially in an experimental setting through measuring biomarkers of inflammation. This may be contributed to by the variation in individual neutrophil responses and activity as a result of genetic polymorphism.

Table 6. Clinical parameters of leukocyte depletion and control group

	Control ($n=20$)	Leukocyte depletion ($n=20$)
Time to extubation (hour, median and range)	15.5 (10–129)	14 (5–304)
Respiratory index (PaO_2/FiO_2) before extubation (mmHg, median and range)	53.9 (32.2–68.5)	42.6 (19.9–65.9)
Total mediastinal chest drainage (ml, median and range)	915 (340–7320)	920 (230–1610)
Cumulative adrenaline usage (μg/kg/hour, median and range)	19 (0–463)	20.5 (0–500)
Duration of adrenaline usage (hour, median and range)	6.5 (0–117)	11.5 (0–288)
Cumulative noradrenaline usage (μg/kg/hour, median and range)	36 (0–651)	85.5 (0–500)
Duration of noradrenaline usage (hour, median and range)	10 (0–165)	26 (0–288)
Highest lactate level (median and range)	4.7 (2.3–13)	4.5 (0.8–10.6)
Maximal change in serum creatinine level (median and range)	13 (−30 to 295)	14.5 (−28 to 100)
ICU stay (hour, median and range)	25 (18–1272)	24 (23–312)

ICU, intensive care unit.

Polymorphism of genes related to neutrophil functional activity may lead to differential levels of enzyme activity, such as neutrophil elastase and MPO, hence leading to differential levels of neutrophil functional activity which could influence disease development and progression [10–15]. Neutrophil elastase, another significant neutrophil granular enzyme, has also been studied and linked with certain diseases. Most significantly is its association with the development of lung cancer. A genotype indicative of A1ATD and/or an excess of neutrophil elastase are significantly associated with lung cancer risk [15, 16]. Some polymorphisms are associated with the survival of the neutrophil. The G(-248)A polymorphism in the promoter region of the bax gene is associated with prolonged peripheral blood neutrophil survival in leukaemic patients. However, these patients are more susceptible to the development of osteomyelitis [11]. Previous study in our laboratory have demonstrated a correlation between individual neutrophil responses and rejection severity detected on the first post-transplant biopsy following heart transplantation [17]. We therefore hypothesise that the individual neutrophil response to surgical activation varies and contributes to the difference in clinical outcome. To study this, we developed an in-vitro stimulation model using artificial stimulant LPS, PMA and fMLP to artificially stimulate neutrophils. Patient's neutrophils were stimulated in a whole blood setting and the degree of neutrophil activation examined through upregulation in the expression of neutrophil adhesion molecules. Using this model, we successfully simulated neutrophil activation. CD11b expression which is involved in the 'adhesion' phase of neutrophil transmigration, was significantly upregulated after exposure to PMA and LPS. Significant shedding of CD62L, which is involved in the 'rolling' phase of neutrophil transmigration, was seen after PMA and LPS stimulation. PSGL-1 expression was significantly upregulated after exposure to PMA. However, to be useful, artificially stimulated neutrophil responses should mimic surgical neutrophil activation. We have previously demonstrated that artificially stimulated neutrophil response can mimic surgical response [8]. Having confirmed this, we undertook to correlate the preoperative artificially stimulated neutrophil response with clinical variables. In 40 patients undergoing open heart surgery, we found that a number of artificially stimulated neutrophil responses correlated positively with clinical variables (Table 4, Fig. 1) signifying that a higher artificially stimulated neutrophil response was associated with an unfavourable outcome.

Neutrophil mediated damage in systemic inflammatory response is widely known. Various attempts have been made by researcher to exclude neutrophils in the experimental setting. However, most of these attempts have been hampered by the fact that over depletion of neutrophil, although reducing neutrophil mediated damage, exposed patients to invading pathogens. LD through the use of a leukocyte filter is the only clinically approved device for the reduction of neutrophils in the circulation. Fitted in the CPB circuit, leukocyte filtration has the ability to remove leuokocytes including neutrophils from the circulation causing a transient depletion. These will then be replaced by the bone marrow. Leukocyte filters exert their effects by reducing neutrophil numbers at the time of reperfusion, theoretically reducing the damage inflicted by neutrophils. However, clinical benefits of these filters have not been consistently shown [5–7, 18–24].

In our study, we demonstrate that leukocyte filters do decrease the numbers of leukocyte and neutrophils. However, this does not confer any clinical benefit to the patients. This may be due to the fact that leukocyte filtration leads to neutrophil activation. Neutrophil activation by leukocyte filtration, shown in our study through a higher CD11b and PSGL-1 adhesion molecule expression, is consistent with other studies [5, 6]. Interestingly, there is no significant increase in adhesion molecule expression across the filters in both groups of patients (Table 5). This may be due to the fact that the leukocyte filters are placed from the beginning of the procedure as opposed to some studies where the leukocyte filters are placed only upon reperfusion. In this way, although utilisation of leukocyte filtration may result in a more significant neutrophil activation in general, no significant neutrophil activation is achieved across the filter in one passage. Moreover, the reduction in neutrophil numbers achieved in this study is small and hence may not be clinically significant.

The limitation of this study lies in the fact that we were not able to apply the leukocyte filtration strategy to the patients who will potentially benefit from it. Instead, leukocyte filters were used in all patients in the LD group and these patients have a variation in their ability to mount a neutrophil response. Ideally, the individual's ability to mount a neutrophil response should be identified preoper-

atively and patients separated into high, normal and low responders. These groups of patients should then be randomised into two groups receiving the leukocyte filters (LD) and conventional arterial filters (control). Their clinical outcome could then be followed, measured and compared. We did undertake preliminary analysis of our data divided up in such a way but no significant difference was found between the LD and control group in all three groups of patients (high, normal and low responders). However, the number of patients in each group was small and hence findings derived from this analysis are not conclusive and not included in this paper. Further study is currently underway.

The findings in this study shed further insight into the significance of neutrophils in contributing to adverse outcome in open heart surgery. More importantly, we identified a potential biomarker through artificially stimulating neutrophils, which is able to predict patient responses to surgical stimulation which in turn predicts their clinical course. A patient presenting for open heart surgery will normally be subjected to a battery of routine investigations to determine their risk profile prior to the operation. Risk in open heart surgery is commonly determined preoperatively using a scoring system known as the EuroSCORE. Artificially stimulated neutrophil response could serve as an adjunct that will further delineate the patient's risk profile, accurately predicting postoperative outcome. Hence, measures can then be taken in patients who are identified to be at a higher risk with the aim of improving outcome. Although modern extracorporeal circuit, anaesthesia management and cardioplegia technique has improved the outcome of cardiac surgery, there remains a small proportion of patients that will benefit if systemic inflammatory response and reperfusion injury can be minimised.

Leukocyte filtration is one measure that can be utilised. However, in our study, no significant benefit is offered using this therapy. Alternatively, previous studies in our laboratory have shown that statins could also be used to blunt the migratory potential of patient neutrophils thus improving patients outcomes [25].

5. Conclusion

Preoperative artificially stimulated neutrophil responses can mimic surgical stimulation of neutrophils and predict clinical outcome in open heart surgery. Leukocyte filters have the ability to reduce leukocyte numbers but does not confer significant clinical benefit possibly due to neutrophil activation.

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