UNIVERSITY OF SOUTHAMPTON

FACULTY OF HEALTH SCIENCES

Centre for Innovation and Leadership in Health Sciences

QUANTIFICATION OF LIPID AND LEUCOCYTE FILTRATION AND THE EFFECTS ON CEREBRAL AND RENAL INJURY MARKERS AND PULMONARY FUNCTION DURING CARDIOPULMONARY BYPASS

By

Richard William Issitt B.Sc. (Hons) PgDip FCCP AACP

Thesis for the degree of Doctorate in Clinical Practice

March 2017

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ABSTRACT

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Background: Despite the extremely low mortality and morbidity rates of coronary artery bypass graft (CABG) surgery involving cardiopulmonary bypass (CPB), there are a number of pathological injuries believed to be due to the CPB circuit. Lipid Microemboli (LME) are produced when fats are released from bone marrow during a sternotomy, which then mix with blood in the pericardium during surgery. This blood is passed into the CPB circuit and reintroduced into the systemic circulation and is thought to promote an increased inflammatory response and ischaemia-inducing blockages. There are at present no adequate methods of dealing with this problem.

Methods: A randomised controlled trial was undertaken to determine if a new Oxygenator, the RemoweLL, which contains a lipid and leucocyte filter, can effectively remove LME from the patient's circulation compared with current gold standard CPB technologies and examine the effects on markers of cerebral, renal injury; neuron specific enolase, Cystatin C and pulmonary function. The Null hypothesis was that there would be no difference in peak neuron specific enolase concentrations.

Results: The data presented in this Thesis provide the first biochemical evidence for a direct effect of LME upon neuron specific enolase release, which correlates in a linear fashion with increasing numbers of LME. The filtration of LME does not appear to significantly reduce systemic levels of activated leucocytes, which is reflected in similar pulmonary functions of those patients with and without LME filtration. However, there is evidence of a weak association towards renal protection with LME, with peak Cystatin C clearance lower than non-filtered patients. This met statistical trial stopping criteria.

Conclusions: The RemoweLL CPB system removes significant numbers of LME compared to current CPB technology. Significant differences in the release of neuron specific enolase in the immediate postoperative period have been demonstrated. Furthermore, there is weak evidence towards improved clearance of Cystatin C. Further work is now planned to determine if LME filtration translates into longer-term neurocognitive and renal protection. The statistical differences between groups were so great from neuron specific enolase that the Ethics Committee advised early termination of the trial.

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Declaration of Authorship

I, Richard Issitt declare that this thesis and the work presented in it are my own and have been generated by me as the result of my own original research.

Quantification of Lipid and Leucocyte Filtration and the Effects on Cerebral and Renal Injury Markers and Pulmonary Function during Cardiopulmonary Bypass

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
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5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Either none of this work has been published before submission, or parts of this work have been published as: [please list references below]:

Signed: ……

Date: 07/02/17

Publications

The following publications have appeared from this study and are reproduced in full at the end of the Thesis:

- **Issitt, R**., and Sheppard, S. Dealing with pericardial suction blood and residual pump volume: a review of current practices in the UK. *Perfusion* 2011; 26:51-55.
- **Issitt, R**., Sheppard, S.V., Voegeli, D., and Walsh, B., Design of a Prospective Clinical Study on the Quantification of Lipid and Leucocyte Filtration and the Effects on Cerebral and Renal Injury Markers and Pulmonary Function during Cardiopulmonary Bypass, in *Working Papers in the Health Sciences*. 2013.
- **Issitt, R.W**., Harvey, I., Walsh, B., and Voegeli, D. Quantification of lipid filtration and the effects on cerebral injury during cardiopulmonary bypass. *Annals of Thoracic Surgery*. 2017; **104**: 884-890.
- **Issitt, R**., James, T., Walsh, B., and Voegeli, D. Do Lipid Microemboli Induce Acute Kidney Injury during Cardiopulmonary Bypass? *Perfusion* 2017; **32**(6): 466–473.
- **Issitt, R**., Ball, J., Bilhkoo, I., Mani, A., Walsh, B., and Voegeli, D. Leucocyte Filtration of the Cardiotomy Suction. Does it affect Systemic Leucocyte Activation or Pulmonary Function? *Perfusion* 2017; **32**(7) 574–582.

Presentations

The following presentations have been made of work from this Thesis:

- **Issitt, R**. Quantification of lipid and leucocyte filtration and the effects on cerebral and renal injury markers and pulmonary function during cardiopulmonary bypass. The Society of Clinical Perfusion Scientists of Great Britain and Ireland 42nd Annual Congress, October 2016, Manchester, United Kingdom.

Prizes

The following prizes have been awarded for work from this Thesis:

- **2016 Cliff Dawson Memorial Prize** - Best Presentation at the 2016 Congress on Perfusion.
- **2017 College of Clinical Perfusion Scientists Research Award** – Best Research Publication 2017

Definitions and Abbreviations

AaOI	Alveolar-Arterial Oxygenation Index
ADP	Adenosine Diphosphate
AKI	Acute Kidney Injury
ALI	Acute Lung Injury
ANOVA	Analysis of Variance
ARDS	Acute Respiratory Distress Syndrome
ARF	Acute Renal Failure
ATP	Adenosine Triphosphate
BMI	Body Mass Index
BSA	Body Surface Area
CABG	Coronary Artery Bypass Grafting
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
COPD	Chronic Obstructive Pulmonary Disease
CPB	Cardiopulmonary Bypass
CQC	Care Quality Commission
CRF	Case Report Form
CRP	C-Reactive Protein
CS	Cell Salvage
CT	Computerised Tomography
CVA	Cerebrovascular Accident
Cys C	Cystatin C
DCHA	Deep Hypothermic Circulatory Arrest
ECC	Extracorporeal Circuit
ECMO	Extracorporeal Membrane Oxygenation
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EPCR	Endothelial Cell Protein C Receptor
FACS	Fluorescence-Activated Cell Sorting
FFP	Fresh Frozen Plasma
GFR	Glomerular Filtration Rate
HCT	Haematocrit

HDL	High Density Lipoprotein
HLA-DR	Human Leucocyte Antigen – antigen D Related
HRP	Horseradish Peroxidase
ICAM-1	Intercellular Adhesion Molecule 1
IHD	Ischaemic Heart Disease
IQR	Interquartile Range
FFA	Free Fatty Acids
F_iO_2	Fraction of Inspired Oxygen
GFR	Glomerular Filtration Rate
LDL	Low Density Lipoprotein
LIMA	Left Internal Mammary Artery
LME	Lipid Microemboli
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCPB	Miniaturised Cardiopulmonary Bypass
MCV	Mean Corpuscular Volume
MFC	Mean Fluorescence Channel
MPO	Myeloperoxidase
MRI	Magnetic Resonance Imaging
NHS	National Health Service
NO	Nitric Oxide
NSE	Neuron Specific Enolase
OPCAB	Off Pump Coronary Artery Bypass
ORH	Oxford Radcliffe Hospitals
$PaCO_2$	Arterial Partial Pressure of Carbon Dioxide
PAD	Peripheral Artery Disease
PAF	Platelet-Activating Factor
PaO_2	Arterial Partial Pressure of Oxygen
PAP	Pulmonary Artery Pressure
PCI	Percutaneous Coronary Interventions
PE	Phycoerythrin
PSB	Pericardial Suction Blood
PSGL-1	P-selectin glycoprotein ligand 1

PVR	Pulmonary Vascular Resistance
RAP	Retrograde Autologous Prime
RBC	Red Blood Cells
RDW	Red blood cell Distribution Width
RIFLE	Risk, Injury, and Failure; and Loss; and End-stage kidney disease
RPV	Residual Pump Volume
SCAD	Small Capillary Arteriolar Dilatations
SD	Standard Deviation
SIRS	Systemic Inflammatory Response Syndrome
SNP	Sodium Nitroprusside
SSC	Side-Scattered Light
TCD	Transcranial Doppler
TGs	Triglycerides
TIA	Transient Ischaemic Attack
TMB	3,3',5,5'-Tetramethylbenzidine
TNFα	Tumour Necrosis Factor α
X-Clamp	Aortic Cross Clamp
WBC	White Blood Cells

1. Literature Review

1.1. The Role of Cardiopulmonary Bypass

Cardiopulmonary Bypass (CPB) using an Extracorporeal Circuit (ECC) facilitates surgical intervention of the heart and was first used by John Gibbon Jr in 1953. The general principle of CPB is to act as the heart and the lungs and requires the continuous removal and subsequent return of the patient's entire circulating volume allowing the surgeon to operate on a flaccid, bloodless environment. An ECC circuit consists of 4 major components; a venous reservoir, an arterial pump, an oxygenator and an arterial line filter (Figure 1). The deoxygenated blood enters the venous reservoir from a cannula in the right atrial appendage, which drains the patient's venous circulation. The venous reservoir (1) serves two purposes; acting as a capacitance chamber and a filter. As a capacitance chamber, the venous reservoir can cope with acute volume shifts that occur as a result of surgical manipulation of the heart (this allows surgery to areas of the heart otherwise inaccessible). The reservoir contains a central column of porous plastic foam and a polypropylene woven screen providing filtration to at least 40μm. This filter removes most particulate matter and gaseous emboli that might enter the circuit via the venous cannula. From the venous reservoir, blood passes into the arterial pump which functions as the heart, providing cardiac output (2). When the blood leaves the arterial pump it enters the oxygenator (3); this is made of a porous polypropylene membrane arranged into hollow fibres. Integral to the oxygenator is a plastic coated aluminium heat exchanger that enables control of the blood (and therefore the patient's) temperature during the operation. The oxygenator works on a similar concept to the lungs; it has a large surface area but with tiny holes in the hollow fibres creating a blood-gas interface allowing the addition and removal of oxygen and carbon dioxide respectively. The final component is the arterial line filter (4), which removes any further microemboli and is a gaseous bubble trap. However, microemboli formed from fat globules are deformable and therefore pass through unfiltered. At present there are no effective inline filtration methods for Lipid Microemboli (LME; discussed later).

Whilst the CPB circuit is made of non-toxic, non-immunogenic materials such as polycarbonate and polyvinyl chloride, contact of blood with the artificial surfaces of the circuit causes activation of the clotting cascade (discussed in section 1.2). To enable CPB to take place full anticoagulation is required. This is attained using Heparin, a naturally occurring anticoagulant produced by basophils and mast cells, which has the added advantage of being reversible using Protamine once the operation is completed.

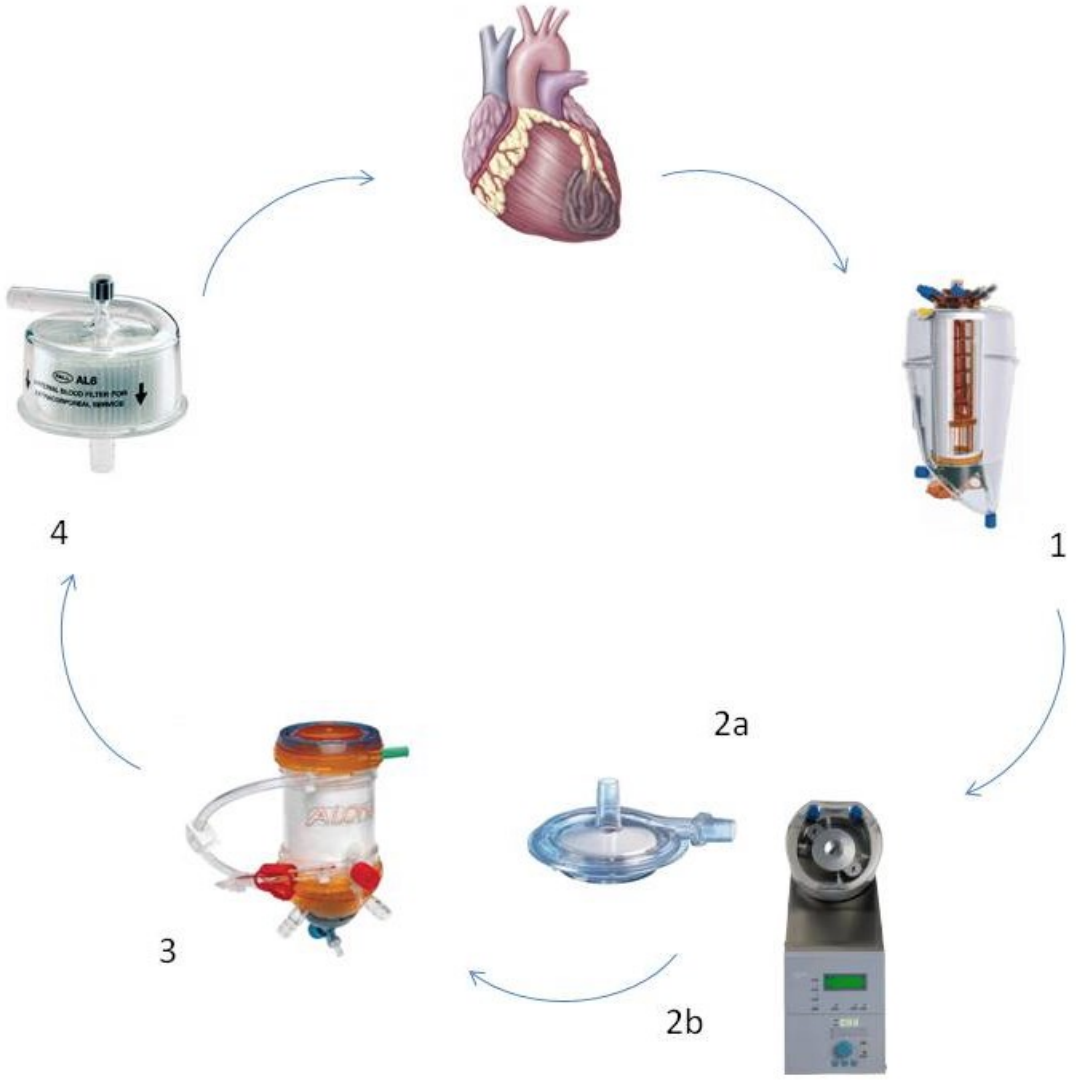


Figure 1. Components of the CPB circuit. 1, Venous Reservoir. 2a, Centrifugal Arterial Pump. 2b Roller Arterial Pump. 3, Oxygenator. 4, Arterial Line Filter. See text for details. The centrifugal pump is a non-occlusive constrained vortex pump whilst a roller pump acts as a partially occlusive peristaltic pump. Image created by Richard Issitt and Medical Illustration, Great Ormond Street Hospital.

There are various components that can be added to the CPB circuit depending on the diagnosis and treatment of the patient. A separate cardiotomy suction reservoir (such as the one seen in Figure 2) can be added to manage low-pressure suction of blood from the open chest cavity, termed Pericardial Suction Blood (PSB). This blood contains the major source of microemboli and activated inflammatory markers (discussed in more detail later). When major blood loss is expected, in emergency procedures for example, a Cell Saver can be added. This machine collects blood that would otherwise be discarded, spins and washes it with saline and then returns concentrated packed red blood cells for reinfusion back into the patient (Figure 3).

To enable the surgeon to open the heart and operate on the inner structures (such as valves, heart walls etc.), a clamp must be placed across the aorta to prevent air from entering the systemic circulation. This "Aortic Cross Clamp" also prevents blood from entering the heart keeping the operating field clear but has the undesirable effect of preventing blood entering the coronary arteries that supply oxygen to the myocardial tissue and muscle. Myocardial protection must therefore be applied in order to prevent the heart from becoming ischaemic; this is accomplished using a a high potassium solution: Cardioplegia (final potassium concentration 20mmol). Diastolic arrest caused by Cardioplegia greatly reduces the heart's metabolic requirement allowing a period during which the heart is isolated from the systemic circulation to take place without damaging the heart. A large initial dose is given (normally 1L) which is mixed in a 4:1 ratio with cold blood, with subsequent doses given, if required, at an 8:1 ratio. The temperature of the blood further reduces the metabolic demand of the heart.

1.1.1.**Summary**

Cardiopulmonary bypass takes over the functions of the heart and lungs allowing surgical intervention on both the inside and outside of the heart. In order to prevent the heart from becoming ischaemic, a cross clamp is applied and cold blood cardioplegia is delivered to the coronary arteries causing a controlled diastolic arrest. The ECC is designed to combat particulate and gaseous microemboli, which it does with considerable success. However, there has never been a component of the ECC designed for deformable fat globules, or LME.

Figure 2. Cardiotomy Suction Reservoir. Similar in make up to the venous reservoir, a cardiotomy reservoir is used as a separate suction system. Blood from the operating field, Pericardial Suction Blood, is returned by roller pump to this reservoir where, depending on surgical preference, it can be discarded, re-introduced into the systemic circulation or processed using Cell Salvage. Image supplied by LivaNova and reproduced with Permission.

Figure 3. Autologous Cell Saver. Cell savers perform a similar role to the cardiotomy suction; blood from the surgical field is sucked into the reservoir where it is sent to a "Latham bowl". The spinning of the bowl creates centrifugal force pushing the heavier red blood cells to the bottom and the lighter plasma to the top. Once full of red blood cells, saline is pumped into the bowl to wash lysed red cells into a waste bag. When the blood is "clean" (as assessed by the level of plasma free haemoglobin) the red blood cells are sent to an autologous blood bag where it can then be re-infused directly into the ECC or the patient. Image supplied by LivaNova and reproduced with Permission.

1.2. **The Pathological Effects of Cardiopulmonary Bypass**

1.2.1.**The Inflammatory Response to Cardiopulmonary Bypass**

Since the beginning of cardiac surgery, facilitated through CPB, there has been an interest in the inflammatory response, assumed to be precipitated by the ECC (Cremer 1996). A review by Wan *et al.,*(Wan 1997) suggests that the inflammatory response is multifactorial and can be either material dependent (in the exposure of blood to non-physiological surfaces and conditions), or material independent (surgical trauma, release of endotoxin and changes in body temperature). It can also be due to allogeneic blood exposure that occurs due to the haemodilution necessary for CPB (Fransen, Maessen et al. 1999). All of these types of inducing events lead to a complex inflammatory reaction involving complement activation, release of cytokines and leucocyte activation. These in turn can cause the production of various substances including oxygen-free radicals, platelet-activating factor (PAF) and nitric oxide. This inflammatory cascade is believed to contribute to the development of postoperative complications such as respiratory failure, renal dysfunction and ultimately, multiple organ failure. The term "systemic inflammatory response syndrome" (SIRS) was introduced to describe the wide variety of insults that occur following CPB.

The following provides a brief description of some key pathophysiological mechanisms.

1.2.2.**Mechanisms Involved**

The primary mechanism involved in the inflammatory response is the complement cascade. This system consists of approximately 20 proteins and forms a major part of the body's defence mechanism. Activation involves the classical and alternative pathways, although in practice there is considerable overlap and reinforcement between the two types. The alternative pathway is activated by blood coming into contact with the non-physiological surfaces of the ECC leading to formation of C3a and C5a (Utley 1990), whilst protamine reversal of heparin's anticoagulant effect following CPB activates C4a in the classical pathway. Activation of C3a causes the aggregation of platelets, while activation of C5a stimulates neutrophil adherence to endothelial cells (Utley 1990). Elevated C3a levels following CPB are associated with the length of CPB, although it is interesting to note that the same is not seen in patients undergoing Extracorporeal Membrane Oxygenation (ECMO; a form of extracorporeal support) suggesting that the length of CPB time is not as important as other activators of C3a (Westfall 1991), and further still, there are data that show the patients that receive off bypass cardiac surgery also see an inflammatory response, suggesting that the effects of surgery itself are the root cause of any response.

Critically, this debate may never be answered, as a Randomised Controlled Trial is impossible; CPB is required to keep the patients alive during the majority of cardiothoracic procedures. It is also difficult to comment on the clinical effects of complement activation, as there are conflicting views within the literature. Kirklin and colleagues (Kirklin 1983) suggested that elevated C3a levels following CPB could predict the occurrence of complications, whilst Bando *et al*., (Bando 1990) found no correlation between complement activation and acute lung injury. These differences may possibly be due to the complexity of the inflammatory reaction and the nature of the complement system being just one of the many interrelated factors involved. It is widely accepted that a systemic inflammatory response is present in all patients following CPB (Sablotzki 2001) although the incidence and severity is variable; most patients experience very few clinical symptoms (Kirklin 1987) and only a minority will develop severe haemodynamic changes or organ failure (Cremer 1996).

Of major interest in the last decade is the role that activated neutrophils and leucocytes play in the inflammatory response and coagulation cascades. Neutrophil action can be mediated by the complement C3a molecule enabling local release of damaging substances upon adhesion to endothelial cells. The most common activation marker for neutrophils is the specific adhesion molecule CD11b/CD18 which has been observed by several groups both experimentally and clinically (Guillinov 1993, Dreyer 1995). Dreyer and colleagues (Dreyer 1995) have previously reported that activated neutrophils may be responsible for pulmonary injury whilst Byrne *et al*., (Byrne 1992) have shown that blocking of neutrophil adhesion molecules leads to an amelioration of myocardial injury. In their review, Wan *et al*., (Wan 1997) suggest that preventing neutrophil adhesion should provide a practical benefit, although these should be weighed against the increased risk of infection. More recent evidence however, has offered a slightly different picture on thrombus formation and interaction with the coagulation pathways. In particular it is thought that microparticles containing the leucocyte protein P-selectin glycoprotein ligand 1 (PSGL-1) are essential for the developing thrombus. As the thrombus forms initially and platelets are recruited, tissue factor concentrates through the PSGL-1-P-selectin interactions propagating full thrombus formation. This suggests that the leucocyte adhesion molecule was originally thought to be involved in leucocyte trafficking is actually a dominant molecule in thrombus development (Esmon 2005). This is particularly relevant when contemplating the effects of cardiac surgery and the interactions between coagulation and inflammation. CD11b/CD18 is thought to have dual function in neutrophils and monocytes facilitating the tight cell-cell adhesion required for an inflammatory reaction, but also to endothelial cell protein C receptor, a key factor in the naturally occurring anticoagulant protein C pathway (Esmon 2005). Any upset in balance to this action might result in a vicious cycle of inflammation and coagulation; the failure of the natural anticoagulant process would increase the inflammatory process. Activated

leucocytes also lead to the release of large amounts of oxygen-free radicals including the superoxide anion, hydrogen peroxide and the hydroxyl radical. It believed that these molecules attack the lung parenchyma allowing fluid extravasation. These effects are more pronounced during CPB as the colloid osmotic pressure of the blood is lowered due to haemodilution. Natural anticoagulants are currently being considered as treatments for acute inflammatory diseases/episodes which explain why the use of heparin to facilitate CPB tips the balance in favour of keeping control of inflammation during the operative period and cardiac patients are very rarely pro-thrombotic (Esmon 2005). As the inflammatory reaction is initiated by the incision made by the surgeon, and not the CPB circuit *per se*, it is the opinion of the author that little use can be gained by examining this process further within the scope of the study undertaken here, and should be used purely to provide context. Until a non-inflammatory provoking incision can be invented, attempting to moderate inflammation during CPB is perhaps futile.

1.2.3.**Summary**

Whilst pathological effects associated with CPB have been demonstrated in all of the vital organs, the major effects can be seen in the kidneys, lungs and brain. This thesis will focus on the effects of CPB on these specific organs. Neutrophils act as a major inflammatory mediator and measurement of CD11b has been proven to correlate to neutrophil activation, making it an excellent marker of the inflammatory response.

1.3. **Cerebral Injury Associated with Cardiopulmonary Bypass**

Major neurological injury has an incidence of 1-5%, although select populations may have a stroke rate as high as 8-9% (Redmond 1996, Wolman 1999). Equally, studies have observed postoperative cognitive and intellectual dysfunction in almost 50% of patients when examined by neuropsychological tests (Murkin 2000). There have been several reports of increased markers of neurologic dysfunction correlating to the duration of CPB (Westaby, Saatvedt et al. 2000), the number of grafts (Anderson, Hansson et al. 2000), and the number of emboli detected by Transcranial Doppler (TCD)(Grocott, Amory et al. 1998). Furthermore, early postoperative neurological dysfunction correlates with progression of cognitive decline and impaired quality of life during later years (Newman, Grocott et al. 2001, Newman, Kirchner et al. 2001). Neurological outcomes following CPB have been divided into type I; fatal or non-fatal stroke, stupor, or coma at discharge and type II; deterioration in intellectual function, memory deficit or seizures (Roach, Kanchuger et al. 1996). Whilst those patients undergoing open-heart procedures are believed to be most at risk of embolization from vegetations, thrombi and gaseous emboli (Wolman, Nussmeier et al. 1999), the repetitive occlusion of the atherosclerotic aorta during CABG surgery also results in increased levels of particulate emboli (Abu-Omar, Balacumaraswami et al.

2004). Combined procedures (open-chamber + CABG), however, show two to three times higher rate of stroke than single procedures (Hogue, Murphy et al. 1999, Hogue, Barzilai et al. 2001).

Whilst CPB has been identified as a major contributor to neurological injury, there are many other factors that may contribute to the overall effect. Firstly, there is a body of evidence to suggest that the transient effects of anaesthetic drugs such as narcotics and benzodiazepines may heavily influence the immediate perioperative period. Secondly, late decline in neurological function may be due to recurrent injury or depression (Newman, Mathew et al. 2006). The cost of treating stoke associated with cardiac surgery has been well documented. In the USA, the frequency of stroke after CABG is the leading cause of iatrogenic stroke and accounts for approximately 25% of the resources expended annually on stroke treatments (Mangano 1990, Stamou, Hill et al. 2001). It has also been shown to increase intensive care unit stay from 3 to 9 days, hospital length of stay from 7 to 11 days and reduce quality of life measures at 5 years follow up (Tuman, McCarthy et al. 1992, Hlatky, Bacon et al. 1997, Stamou, Hill et al. 2001). Neurological injury from CPB has also been attributed to the non-pulsatile nature of blood flow and low blood pressure (Gilman 1965). Caplan *et al*., observed a reduced small emboli washout from areas of infarction in patients with low blood pressure, suggesting that hypoperfusion may contribute to areas of neurological injury (Caplan and Hennerici 1998) although further studies failed to find any difference in neurological outcome in patients treated with low or high perfusion pressure strategies (Gold, Charlson et al. 1995). What is not often accounted for however is any pre-existing cerebrovascular disease; in a study by Goto and colleagues including 421 patients scheduled for CABG, they found that 30% had small brain infarctions and 20% had multiple infarctions preoperatively (Goto, Baba et al. 2001). This suggests that many patients undergoing CABG surgery have latent, undiagnosed neurological complications, which may determine the level of postoperative cognitive decline seen. Of much interest in the literature are the effects that LME have on neurological function. Whilst this will be the major focus of §1.6.1, it is worth noting at this point that research has shown the presence of thousands of LME present in the brains of patients who have died following CPB, but there has never been a definitive causal relationship shown between LME and neurological dysfunction (Moody, Brown et al. 1995).

The ability to be able to diagnose neurological dysfunction using biochemical markers would potentially allow earlier therapeutic intervention than possible using standard neuropsychological assessment, as well as highlight subclinical levels of change in neurological status (Whitaker, Green et al. 2007). Much of the research thus far has focussed on the astroglial cell neuroprotein S100β that has been linked to both neurological and neuropsychological outcome following cardiac surgery (Ali, Harmer et al. 2000, Anderson, Hansson et al. 2001, Vaage and Anderson 2003). However, this has been shown to be unspecific as S100β is also contained within the heart, aorta and mediastinal tissues which are disrupted

during cardiac surgery causing potential contamination from non-cerebral sources (Zimmer, Cornwall et al. 1995, Jonsson, Johnsson et al. 1999, Fazio, Bhudia et al. 2004). Studies by Westaby and Whitaker have also shown that when the cardiotomy suction is avoided, the S100β released from cerebral sources did not correlate to neurological or neuropsychological outcome (Westaby, Saatvedt et al. 2000, Whitaker, Green et al. 2007). Another neurological marker, Neuron Specific Enolase (NSE), is an enzyme that catalyses the conversion of 2-phospho-D-glycerate to phosphoenolpyruvate in the glycolytic pathway and is found in neurons and neuroendocrine cells, with α and γ subunits being specific to neurons (Karkela, Bock et al. 1993). Animal studies have demonstrated a link between increased NSE and cortical injury (Woertgen, Rothoerl et al. 2000, Woertgen, Rothoerl et al. 2002), whilst it has also been shown to increase following cardiac arrest (Tiainen, Roine et al. 2003, Sulaj, Saniova et al. 2009). NSE demonstrates an increased response to cardiopulmonary bypass (Herrmann, Ebert et al. 2000), and serum levels of NSE exhibit a significant association with postoperative neurocognitive outcomes (Ramlawi, Rudolph et al. 2006).

1.3.1.**Summary**

Neurological dysfunction following CPB is a major source of morbidity and mortality in the cardiac surgical population. There are multiple factors involved but a number of studies are focussing on the influence of LME in causing ischaemia-inducing blockages (§ 1.6.1). Much research has been conducted trying to define a biochemical marker of neurological injury, with NSE providing the best correlation to neurological outcomes following CPB.

1.4. **Pulmonary Dysfunction Associated with Cardiopulmonary Bypass**

Pulmonary dysfunction is one of the most frequent complications associated with CPB (Taggart, el-Fiky et al. 1993), ranging from mild postoperative dyspnoea to acute respiratory distress syndrome (ARDS) which, although only affecting 2% of patients, has a 50% mortality rate (Clark 2006). The mechanisms behind CPB-induced injury are believed to be related to the inflammatory response but are influenced by general anaesthesia, surgical injury and perioperative pain whilst smokers are at a higher risk due to a greater preoperative prevalence of Chronic Obstructive Pulmonary Disease (COPD)(Taggart 2000). Pulmonary dysfunction prolongs mechanical ventilation, intensive care and hospitalisation stay and increases treatment costs (Andrejaitiene, Sirvinskas et al. 2004).

Contact with the ECC initiates the activation of leucocytes as well as a host of proinflammatory mediators (discussed above), including the CD18 and CD11b surface adhesion molecules on leucocytes, which promote adhesion to specific ligands on pulmonary endothelium. These ligands (such as ICAM-1)

are themselves upregulated during CPB and facilitate the transmigrating of leucocytes into the lung parenchyma under the influence of IL-8. There, the activated leucocytes release proteolytic enzymes (such as elastase and collagenase) and oxygen free radicals, which enter the systemic circulation and mediate lung parenchymal damage through cellular and tissue injury (Clark 2006). The resulting injury destroys the ultrastructure of the lung increasing the permeability of alveolar-endothelium.

During CPB the blood flow to the lungs is highly diminished and the majority of blood flow is via the bronchial arteries, which has been shown to be significantly diminished during CPB, suggesting a role for metabolic-induced lung ischemia (Schlensak and Beyersdorf 2005). There appears to be a mechanism unrelated to the surface-induced effects of CPB, as intermittent lung perfusion via the pulmonary artery improves postoperative lung function, possibly by reducing the metabolic and pathological features associated with ischaemia (Sievers, Elliott et al. 2007). This suggests that there are other mechanisms involved, not mediated by inflammatory markers. The main consequences of CPB on the lungs are reduced lung compliance, increased alveolar-arterial oxygen pressure difference, intrapulmonary shunt fraction and pulmonary vascular resistance. There is also an increase in permeability and interstitial oedema affected lung surfactant and oxygen transfer (Griese, Wilnhammer et al. 1999). Lung injury can be clearly seen histologically as alveolar oedema with leucocyte extravasation, whilst the tissue appears necrotic and swollen. Weiss and colleagues proposed that atelectasis was the major cause of an increase in intrapulmonary shunting following CPB with poorly oxygenated blood from the atelectatic areas lowering the arterial partial oxygen pressure (Weiss, Merin et al. 2000).

Blood that is shed into the mediastinal/pericardial space (known as Pericardial Suction Blood, PSB §2) contains, among other things, high levels of LME that are thought to affect the pulmonary vasculature and impair gas exchange although only a weak association could be shown, with no significant differences in those patients that had PSB treated with cell salvage and those that did not (Boodhwani, Nathan et al. 2008). Interestingly, there is further debate in the literature as to the effects of PSB on Pulmonary Vascular Resistance (PVR) with studies associating both increases and decreases in PVR following reinfusion of PSB (Westerberg, Gabel et al. 2006, Boodhwani, Nathan et al. 2008). However, it has yet to be proved whether any difference in pulmonary function (which at present has not been observed) is due to LME or other vasoactive mediators present in the PSB (§1.6.1).

Postoperative lung dysfunction commonly exhibits as a predictable and consistent deterioration in gas exchange, (such as the widening of the alveolar-arterial oxygen gradient). In addition, pulmonary compliance is reduced, in particular affecting the lower lobe of the left lung. Calculation of the Alveolar-Arterial Oxygenation Index (AaOI) is an established method for the assessment of peri-operative changes in lung function and is invariably elevated after cardiac surgery (Alexiou, Tang et al. 2004). Furthermore,

postoperative lung dysfunction can be classified using the Berlin definitions of ARDS; None PaO_2/FiO_2 >300, Mild PaO_2/FiO_2 = 200 – 300, Moderate PaO_2/FiO_2 = 100 – 200, and Severe PaO_2/FiO_2 < 100 (Cartotto, Li et al. 2016).

1.4.1. **Summary**

Lung function following CPB is heavily influenced by the volume of fluid used within the bypass circuitry and the actions of activated leucocytes. It has yet to be shown whether LME influence pulmonary function. This can be inferred easily using the AaOI and classified using the Berlin definitions of ARDS.

1.5. **Renal Injury Associated with Cardiopulmonary Bypass**

Approximately 1-5% of all patients undergoing CABG surgery suffer from Acute Kidney Injury (AKI) postoperatively, which remains a major cause of morbidity and mortality (Abu-Omar, Mussa et al. 2005). The severity of AKI can range from subclinical injury to established renal failure requiring dialysis and is often exacerbated by other co-morbidities such as diabetes mellitus (Zanardo, Michielon et al. 1994). The pathophysiology of renal function is incompletely understood but is deemed multifactorial and can be related to perioperative renal hypoperfusion and the presence of endogenous and exogenous nephrotoxins (such as free radicals, anaesthetic agents etc.), which result in glomerular and tubular injury. It is interesting to note that a study in patients undergoing CABG surgery performed without CPB (off pump CABG; OPCAB), showed that there is a smaller increase in markers of renal injury compared to those with CPB, suggesting that CPB is a major factor (Abu-Omar, Mussa et al. 2005). However, the study had a small sample size and the patients were not randomised. Also, the removal of outlying results reduced the observed associations detected and therefore the power of the findings. Equally, there is much debate over the benefits of OPCAB with other studies indicating that there is no significant difference in postoperative renal failure between CPB and OPCAB (Gamoso, Phillips-Bute et al. 2000, Tang, Knott et al. 2002, Puskas, Williams et al. 2003). As a highly vascularised organ, the kidney is at risk from emboli, particularly LME which have been shown in great numbers in the renal vasculature of patients undergoing CPB, and might act through either a direct mechanical mechanism, or through the cytotoxicity of the lipids and free fatty acids that make up LME (§ 1.6.1). Renal function is also dependent upon higher perfusion pressures and is therefore susceptible to periods of hypoperfusion during the surgical period (Rosner and Okusa 2006).

Measurement of renal injury has traditionally been undertaken using creatinine clearance and Glomerular Filtration Rate (GFR). The Acute Dialysis Quality Initiative set up in 2002 defined acute renal injury according to the RIFLE criteria (Risk, Injury, and Failure; and Loss; and End-stage kidney disease)

this was further refined in 2004 by the Acute Kidney Injury Network to define AKI as an abrupt increase in absolute serum creatinine ≥3mg/dL (26.4µmol/L) or percentage increase greater than 50% (1.5-fold from baseline). However, creatinine clearance, whilst specific, is not very sensitive; serum creatinine concentrations do not significantly increase until the GFR has reduced to less than 50% of its baseline (Perrone, Madias et al. 1992) and is dependent upon several other factors such as muscle mass. The assay is also susceptible to interference from various drugs and endogenous substances. Equally, GFR requires meticulous collection of urine over a fixed period of time which is laborious and impractical in many clinical settings. A cysteine protease inhibitor, Cystatin C, which is produced by all nucleated cells, is exclusively eliminated from the body by glomerular filtration and its serum concentration is used to estimate GFR from spot serum samples rather than using serial urine collections. Additionally, the assay required is less susceptible to methodological interference, which is inherent in the method of creatinine estimation and there is less inter-individual variation than serum creatinine allowing for an earlier detection of AKI (Page, Bukki et al. 2000). Typically markers of renal dysfunction peak at 1-2 days postoperatively (Abu-Omar, Mussa et al. 2005).

1.5.1. **Summary**

Due to the highly vascularised nature of the kidneys, they are highly susceptible to ischaemic damage and hypoperfusion during the CPB period. Many methods are available to evaluate glomerular filtration although most lack sensitivity or require meticulous measurement of urine output. However, Cystatin C is exclusively excreted by the kidneys and is highly correlated to glomerular filtration. Acute kidney injury is defined as an increase in absolute serum creatinine ≥3mg/dL (26.4µmol/L) or 1.5-fold from baseline.

1.6. **Lipid Microemboli**

Although the terms “fat” and “lipid” are used interchangeably, it is worth noting that fats are a subsection of lipids, made up of fatty acids and glycerol whilst lipids can be distinguished into 3 forms; Simple, Compound and Derived. Fats are grouped within Simple lipids and make up the majority of the body’s lipid store, forming neutral triglycerides. The major components of fatty acids are palmitoleic acid, oleic acid, linoleic acid and arachidonic acid (de Vries, Gu et al. 2002). Compound lipids are mostly found in the cellular membranes as phospholipids and glycolipids and in the myelinated parts of the central nervous system. Derived lipids, such as steroids, are a combination of Compound and Simple lipids produced by a hydrolysis reaction. The main fats that concern cardiac surgery are subcutaneous fat, fat around the organs (especially the heart), and fat in the bone marrow and in the blood. The main

fatty acid composition of subcutaneous fat is Oleic Acid (41-44%), Palmitic Acid (19-21%) and Linoleic Acid (10-12%). Although all are important, due to the advances in surgical technique and CPB circuitry (such as depth filters), subcutaneous fats and organ surrounding fats, do not play a significant role in CPB related morbidity, as these do not enter the systemic circulation. For this reason the rest of this Thesis will concentrate on fats in the blood and from the bone marrow.

1.6.1. **Lipid Microemboli and Organ Injury**

Many of the neutral lipids are broken down by the enzyme lipase A forming glycerol and fatty acids. Although the quantities of these fatty acids are small, they are metabolically active and are able to uncouple oxidative phosphorylation and inhibit the ADP-ATP exchange across mitochondrial membranes leading to cell destruction (Wojczak 1976). To facilitate cardiac surgery, a median sternotomy must be performed. Unlike the long bones (i.e. the femur) which contains yellow bone marrow that is 85% fat, the sternum contains red bone marrow that is 5% fat (Goodsitt, Hoover et al. 1994). By splitting the sternum, bone marrow is exposed allowing fats to leach into the chest cavity. Shed blood from surgical manipulation and incision of the heart collects in the chest cavity with the fats from the sternum and are suctioned into the CPB circuit forming emboli varying in size from 10-60µm (Kaza, Cope et al. 2003, Brondén, Dencker et al. 2008). A recent study by Brondén and colleagues showed that the emboli distribute through the organs, especially the kidneys, spleen and brain (Brondén, Dencker et al. 2006). This was confirmed by the work of Brooker *et al*., (Brooker, Brown et al. 1998) who studied the brains of dogs that had undergone CPB. They found Small Capillary Arterial Dilatations (SCADs), in afferent microvessels consisting of oily material indicating embolic episodes, only in the animals that had had shed blood returned to the CPB circuit. These fatty intravascular collections were approximately 10-70µm in diameter, larger than the vessel in which they were found. SCADs were first detected after Moody *et al*., introduced a staining method based upon alkaline phosphatase which allowed studies of cerebral microvasculature to take place (Moody, Bell et al. 1990). In the brain specimens of patients who had died shortly after CPB, Moody found evidence of thousands of microemboli which tended to distend the vessels to their fullest extent, and were distributed proportional throughout the brain, often found at vascular bifurcations, where due to the size of the SCAD further progression through the cerebral vasculature was impossible. Eyjolfsson *et al*., 2008 found hundreds of thousands of lipid emboli in the range of 10-60µm and observed that when lipids are added to a hydrophilic liquid such as blood, they do not dissolve but remain in suspension (Eyjolfsson *et al*., 2008). For lipids to dissolve in water or blood, emulsifiers and energy must be added. It is believed that once a SCAD becomes established, more lipid emboli get caught within it leading to the vessel dilatation. More recent work by Brown (Brown, Moody

et al. 2000) has shown that not only is CPB associated with an increased embolic load, but that the embolic load was proportional to the length of time on CPB; for every 1 hour increase in CPB time, the embolic load increases by 90.5%. Interestingly, the authors also noticed that with increasing survival time after CPB, the emboli load declined. Work by Appelblad and Engström has shown that the addition of liquid fat to blood impairs the capillary flow function which might suggest that the reason for the observation of Moody *et al*., is that lipid emboli travel slowly though the brain and therefore require many days to exit the cerebral microvasculature (Appelblad and Engström 2002). Whilst there is little data to show a direct correlation between neurological injury and the number of emboli detected, this may be due to the inability of many emboli detectors to distinguish between less harmful gaseous emboli and more dangerous particulate emboli (Newman, Mathew et al. 2006). It appears more likely that the location of injury will determine the severity of postoperative neurological dysfunction more than the size of the injury. For example, Newman *et al*., propose a far more devastating stoke caused by a small injury in the internal capsule compared to a large frontal or cerebellar infarct which would only be detectable upon detailed functional investigation (Newman, Mathew et al. 2006). Furthermore, there are no definitive studies identifying a causal effect of LME and organ dysfunction.

It has been observed in orthopaedic or trauma surgery involving breakage of the long bones, that when fat mixes with blood a "fat embolism syndrome" can occur. Whilst cardiac surgery is not associated with this particular syndrome (the fat content is significantly different), there is described a "pump syndrome" which has been observed for many decades and associated with the use of CPB (Torres, Frank et al. 1959). This referred to a spectrum of neurological dysfunctions ranging from disorientation and delirium to seizures, strokes and coma. Brown and colleagues (Brown, Moody et al. 1999) suggested that this "pump syndrome" may be a milder form of "fat embolism syndrome", a fact that is supported by the associated rise in blood lipid content on the first postoperative day following CPB-mediated cardiac surgery (Arrants, Gadsden et al. 1973). It is also supported by the observation that SCADs persist for many days following CPB, similar to Warrens' finding that there is a lack of breakdown of fat emboli from traumatic fat embolism (Warren 1946). Animal studies have also provided a link between Oleic Acid (the main component of lipid microemboli) and an experimental model of acute lung injury (Grotjohan, van der Heijde et al. 1993). Oleic acid induces pulmonary hypertension, decrease in oxygenation, increase in extravascular lung water and lung weight, and a decrease in compliance (Young, Rayhrer et al. 2000). It is believed that the oleic acid exerts a direct toxic effect on endothelial walls (Jefferson and Necheles 1948, Hofman and Ehrhart 1984), possibly by the inhibition of the calcium pump and ($Na^+ + K^+$)-ATPase leading to dysfunction of the cell membrane (Pine, Vincenzi et al. 1983, Lamers, Stinis et al. 1984).

1.6.2. **Summary**

Lipid Microemboli are formed in PSB which, when returned to the CPB circuit, pass through filter materials and are returned to the arterial cannula. From here LME have been observed to enter all major organs and have been associated with SCADs in the brains of patients who have died following CPB. However, there has never been a causal relationship demonstrating a correlation between LME and organ dysfunction, or whether removal of LME results in improved organ function post CPB.

1.7. **Techniques to Attenuate the Pathological Response to Cardiopulmonary Bypass**

1.7.1. **Leucocyte Depletion**

Leucocyte filtration has been used with varying efficacy in a number of studies (Mihaljevic, Tonz et al. 1995, Gu, deVries et al. 1996, Wan 1997, Tang, Alexiou et al. 2002). Alexiou and colleagues showed that continuous leucodepletion of arterial blood during CPB reduced the postoperative total and activated leucocyte count with correlated attenuation of pulmonary inflammation and improvement of AaOI (Alexiou, Tang et al. 2004), They did not, however, observe improved outcomes in patients undergoing elective CABG surgery (although it should be noted that the study was not powered for such outcomes) but did observe a reduction in inotropic support and a lower requirement for duration of ventilation with leucodepletion. A limitation noted in the study was the apparent saturation (i.e. a plateau in leucocyte removal) that occurred to the leucocyte-depleting filter, especially shortly after cross-clamp removal (at which point leucocytes, sequestrated in the pulmonary vasculature, re-enter the systemic circulation) where the filter is exposed to an increased leucocyte load. Some have suggested that leucocyte-depleting filters are unable to consistently reduce the systemic white cell count (Baksaas, Videm et al. 1998), possibly due to the rapid replacement of leucocytes released from bone marrow ‘stores’ (Alexiou, Tang et al. 2004). It has been noted however, that the leucocyte-depleting filters may selectively remove activated leucocytes (Ohto, Yamamoto et al. 2000). Furthermore clinical markers of leucocyte activation such as the CD11b and CD18 surface adhesion molecules (which regulate the adhesion of activated leucocytes) have shown reduced expression in patients undergoing CABG surgery with continuous arterial line leucocyte depletion (Hurst, Johnson et al. 1997, Chen, Tsai et al. 2002). Interestingly, there is broad agreement that leucodepletion has no effect on postoperative total and activated leucocyte count (Hurst, Johnson et al. 1997, Chen, Tsai et al. 2002, Alexiou, Tang et al. 2004).

It has previously been shown that in low-risk patients with no respiratory or other organ dysfunction, there is an ability to compensate for the detrimental effects of CPB (Alexiou, Tang et al. 2004), but in patients requiring CPB for longer than 90 minutes or Deep Hypothermic Circulatory Arrest (DHCA), leucodepletion reduced mechanical ventilation and improved lung function (Sheppard 1999,

Alexiou, Tang et al. 2004). This suggests that it is the higher risk patient group that may benefit the most from leucodepletion. This was further supported by a recent systematic review (Warren 2007) that concluded that whilst there were many randomised controlled trials investigating leucocyte filtration, most were small and had limitations; although some benefit was seen in a subsection of patients with certain co-morbidities, such as preoperative renal impairment.

1.7.2. **Lipid Filtration**

Inline lipid filtration would be the easiest adopted method to remove fat but has been technically difficult to achieve and there are conflicting opinions about its efficacy. A major problem associated with filtration is the deformability of fats, allowing them to pass through filters and into the systemic circulation of the patient (de Vries, Gu et al. 2004). Fats will adhere to surfaces and separate by density in a temperature dependent way, with authors reporting superior filtration at low temperatures (10°C) (Engström 2003). This however, is unrealistic in the clinical setting where the current standard of treatment is to perform mild hypothermia (32-34°C) management in patients undergoing CABG surgery. Much of the research examining lipid filtration is fundamentally flawed with several studies utilising soya oil as a reference fat which is substantially different from the liquid fat seen in human pericardial fat (Chen, Ratnayake et al. 1995, Booke, Fobker et al. 1997) or using excessive fat in order to gain higher measurement resolution which lead to the saturation and decrease in efficacy of the filter (Booke, Fobker et al. 1997). Another study showed filtration at 10°C to be associated with filter occlusion and haemolysis which might also reflect the fragility of erythrocytes in cold blood (Engström 2003). Therefore, at present there is no suitable inline filtration method for efficacious LME removal in the clinical setting.

1.7.3. **Cell Salvage**

Cell salvage allows the processing of shed and stagnant blood in the mediastinal cavity before reinfusion of the processed red blood cells, the quality of which has been demonstrated by numerous studies (Stokke, Burchardi et al. 1986, Griffith, Billman et al. 1989, Sieunarine, Lawrence-Brown et al. 1992, Tawes and Duvall 1996, Reents, Babin-Ebell et al. 1999).

In order to determine the efficacy of cell salvage in reducing the circulating levels of inflammatory markers, Amand *et al*., compared 5 clinically available cell salvage units (Amand, Pincemail et al. 2002). They observed a decreased in *IL-2, IL-6, IL-8, TNFα,* Elastase and Myeloperoxidase (MPO) levels but surprisingly an increase of up to 220% in *IL-1β*, a lymphocyte activating factor which plays in important role in the inflammatory response. There has been a suggestion that this may be due to an induced stress response affecting leucocyte activation (Haeffner-Cavaillon, Roussellier et al. 1989). It remains to

be determined whether the transfusion of blood containing increased levels of *IL-1β* is detrimental to the patient but this indicates that the act of cell salvage can activate leucocytes through a stress response (Dewitz, McIntire et al. 1979).

The Cardiotomy Trial was the largest randomised, double blind study ever undertaken looking at the effects of cell salvage on neurological outcome following processing of shed mediastinal blood (Rubens, Boodhwani et al. 2007). They observed an incidence of postoperative cognitive dysfunction of 45.3% in patients who had cell salvage compared to 39% in those without cell salvage with no benefit in early or late quality of life parameters. It was also noted that there was an increased incidence of Red Blood Cells (RBC) and non-RBC transfusions (p<0.001), increased output from chest tubes (p=0.04) and a tendency for lower platelet counts (p=0.05). Both groups exhibited significantly raised coagulation parameters (p<0.01). The reason for such differences is attributed to the quantity of cardiotomy blood processed by the cell saver (636±577mL). Therefore it is clear that cell salvage is not the correct method of PSB processing and a different process is required.

1.7.4. **Off Pump Coronary Artery Bypass (OPCAB)**

Off pump coronary artery bypass (OPCAB) was invented to avoid the deleterious effects of CPB and is thought to be superior in patients with atherosclerotic aortas due to the minimization of aortic manipulation (Abu-Omar, Balacumaraswami et al. 2004). However, whilst the data show less cognitive decline and stroke associated with OPCAB, the studies are heavily flawed by non-randomisation bias and have shown no difference from CABG at 1 or 5 year follow up (Hlatky, Bacon et al. 1997, Van Dijk D et al. 2002, Cheng, Bainbridge et al. 2005). There are trends in low-risk trials but these suffer from being underpowered (Mark D 2002). Furthermore, many other factors involved in initiating the inflammatory response and contributing to embolization during CPB are still present during OPCAB, such as sternotomy, heparin administration, and manipulation (even if less than CABG) of the ascending aorta (Newman, Mathew et al. 2006) and there are concerns that OPCAB is associated with incomplete revascularisation (Puskas, Williams et al. 2004). It is believed that OPCAB may provide additional protection to the lungs, as the inflammatory cascade is initiated predominantly by the artificial surfaces associated with the extracorporeal circuit, and OPCAB has been shown to reduce neutrophil elastase along with lipid hydroperoxides, nitrotyrosines and is associated with lower levels of oxidative stress (Matata, Sosnowski et al. 2000). Furthermore, as the lungs continue to ventilate, there is less opportunity for metabolic-induced lung ischemia (discussed in section 1.4). However, no significant differences in alveolar-arterial oxygen pressure, intrapulmonary shunt fraction or ventilation times have been demonstrated (Cox, Ascione et al. 2000, Taggart 2000).

1.8. **Summary**

The pathological effects of CPB have been extensively studied and are widely understood to provoke a whole body response in which lipids and leucocytes play a pivotal role. However, there are no data providing clear, causal connections between LME and organ dysfunction and whilst various methods to attenuate this whole-body response have been attempted, these have been met with varying levels of success. When coupled with the fact that there are limited technologies available in order to treat LME, it becomes a major issue to elicit a suitable methodology to protect against LME-induced pathology.

This Thesis will now explore the current practices and methodologies used within the UK for dealing with LME-rich PSB in order to gain insight into how UK cardiac units are adapting their routine care in light of the incoherent evidence base. It will then examine the efficacy of a new lipid/leucocyte filter on reducing LME within the patient's systemic circulation. The effects of the lipid/leucocyte filter on biochemical markers of cerebral and renal injury/function and pulmonary function will also be determined.

2. The Study Context: the National Audit

2.1. Introduction

Numerous studies have proposed that Pericardial Suction Blood (PSB), re-introduced into the systemic circulation via the cardiotomy suction, are responsible for much of the organ dysfunction following CPB (Spanier, Tector et al. 2000, Svenmarker, Engström et al. 2004, Westerberg, Gabel et al. 2006). Several methods have been suggested to alleviate this problem including cell salvage and the discarding of pericardial suction blood. These methods however, are far from ideal as both remove large quantities of clotting factors and haemoglobin (Rubens, Boodhwani et al. 2007). Therefore, the ideal method of attenuating the pathological effects of LME is yet to be determined. For this reason, a national audit was devised to ascertain current practices and methodologies employed throughout the UK. In particular this audit wanted to know how PSB was dealt with, and what happened to this volume at the end of CPB (termed Residual Pump Volume; RPV).

2.2. Methods

A national audit of adult perfusion departments across the United Kingdom was undertaken to analyse current practices and was published in Perfusion in 2011 (Issitt and Sheppard 2011). Those perfusion units contacted were those listed on the Care Quality Commission (CQC) Heart Surgery website undertaking a minimum of 600 cardiac cases per annum. The audit asked the following questions:

1. What methods of blood conservation do you use?
2. Does your unit routinely use a cell salvage device?
3. If yes, then when is it used?
4. How do you deal with pericardial suction blood (PSB)?
5. Do you use a lipid/leucocyte filter when re-infusing blood?
6. How do you deal with residual pump volume (RPV)?

2.3. Results

Thirty-four (34) of the 38 perfusion departments across the UK completed the audit (89.5%), their blood conservation techniques are given in Figure 4. Of those, 71% did use routine cell salvage, 20% used cell salvage sometimes if the case was a re-operation, it was OPCAB and surgery involving more than one procedure (i.e. CABG + valve replacement), and 9% did not (Figure 5).

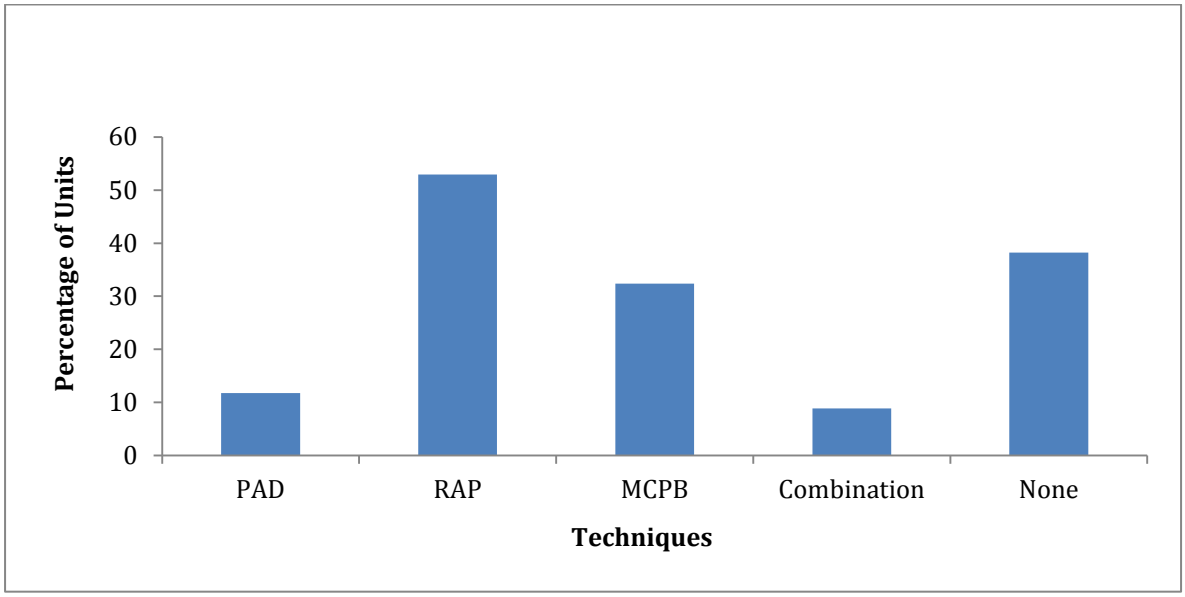


Figure 4. Blood conservation techniques used in UK Perfusion units. PAD; Preoperative Autologous Donation, RAP; Retrograde Autologous Priming, MCPB; Miniaturised Cardiopulmonary Bypass

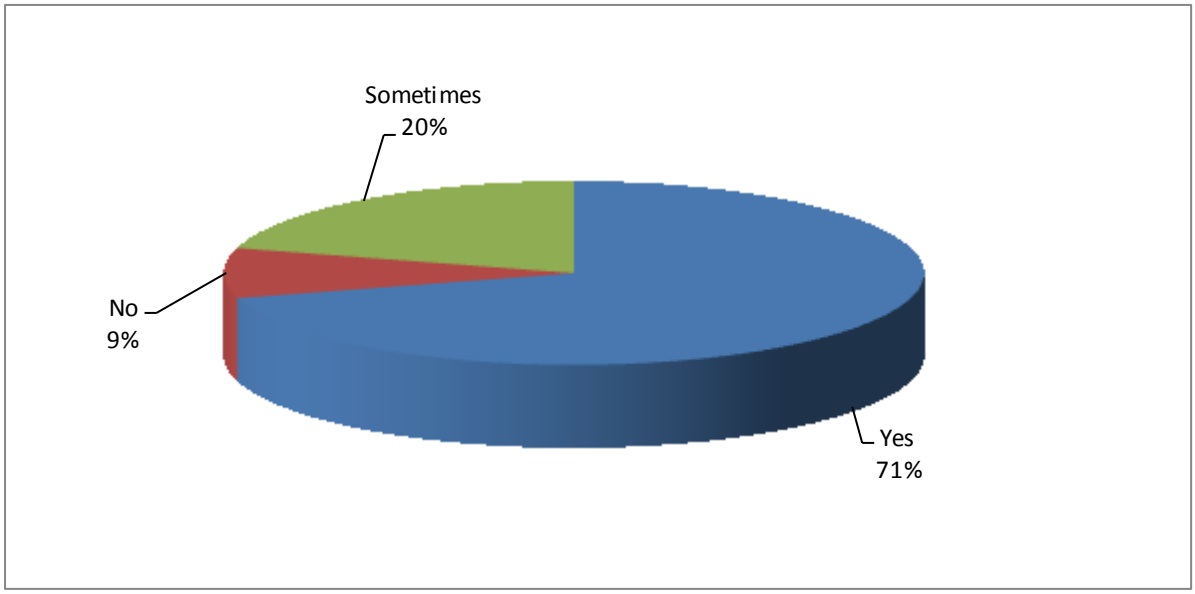


Figure 5. Percentage of perfusion units using routine cell salvage for cardiac cases.

Half of the units returned PSB directly into the systemic circulation whilst 29% collected PSB in a separate cardiotomy reservoir before discarding or re-infusing into the systemic circulation depending on the surgeon and anaesthetist's preference. Twelve per cent (12%) of the units cell save PSB, 6% sent PSB to a separate cardiotomy reservoir where it remained until processing at the end of CPB and 3% separated PSB into a cardiotomy reservoir and re-infused it to the systemic circulation at the end of CPB (Figure 6).

One unit routinely used a leucocyte filter when re-infusing shed blood back to the patient whilst 3 others used filtration only when the patient was suspected of having a carcinoma. The majority (88%) do not routinely use lipid/leucocyte filters when re-infusing blood (Figure 7).

Residual Pump Volume (RPV) was processed via cell saver in 19 units, re-infused via the aortic cannula in 3 units whilst 10 units used both methods. One centre would either cell save, re-infuse or haemoconcentrate RPV depending on the surgeon and anaesthetist involved in the operation. One centre would routinely haemoconcentrate the RPV into a collection bag and hand this to the anaesthetist for re-infusion via the central venous line (Figure 8).

2.1. **Discussion**

Whilst the efficacy of CS processing PSB or RPV has been questioned by some studies, in an emergency situation (such as ventricular rupture upon sternotomy), the benefits of collecting large quantities of blood in a CS reservoir, as opposed to completely discarding it, cannot be understated. However, the argument changes once heparinisation occurs. As the Cardiotomy Trial demonstrates, CS-processed PSB results in a higher Fresh Frozen Plasma (FFP) transfusion rate, with no benefits in terms of neurocognitive outcome or reduction in bleeding postoperatively. Therefore, in terms of preventing transfusion-related morbidity and postoperative blood loss, one may feel justified in returning the PSB directly into the systemic circulation. The audit shows that half of cardiac units have adopted this approach. In so doing, however, the perfusionist is again allowing LME-rich blood to reach sensitive organs such as the kidney and brain. Every perfusionist spoken to whose unit re-introduced PSB back into the systemic circulation was apologetic in their response, which demonstrates that the issue is well understood by all those in the profession and that the need for directly re-infusing PSB has come about by a lack of superior alternatives. Filtration of PSB would appear to be the ideal solution to this problem, although, currently, this technology is not available. The results show that only one cardiac unit in the UK routinely uses a filter when re-infusing blood into a patient. Due to the high deformability of fat particles, standard arterial line and blood transfusion filters are unable to prevent even large fat globules from

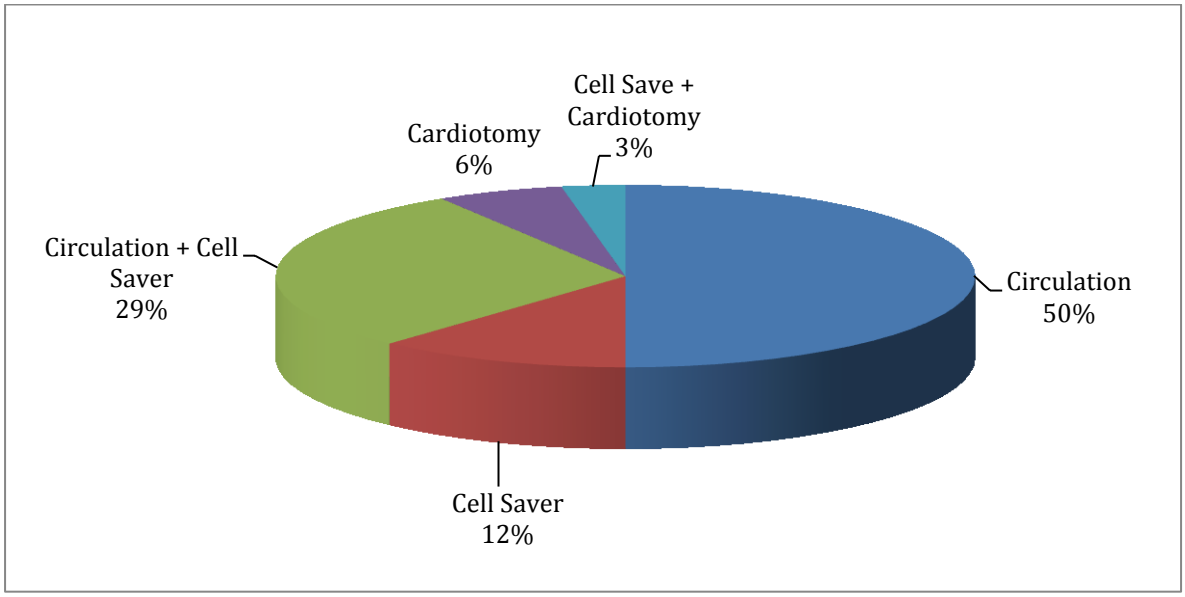


Figure 6. Processing pericardial suction blood. Circulation: PSB returned directly into systemic circulation. Cardiotomy: PSB collected in a separate cardiotomy reservoir before discarding or re-infusing into the systemic circulation. Cell Save: PSB collected and processed with cell salvage. Number given as percentages of perfusion units.

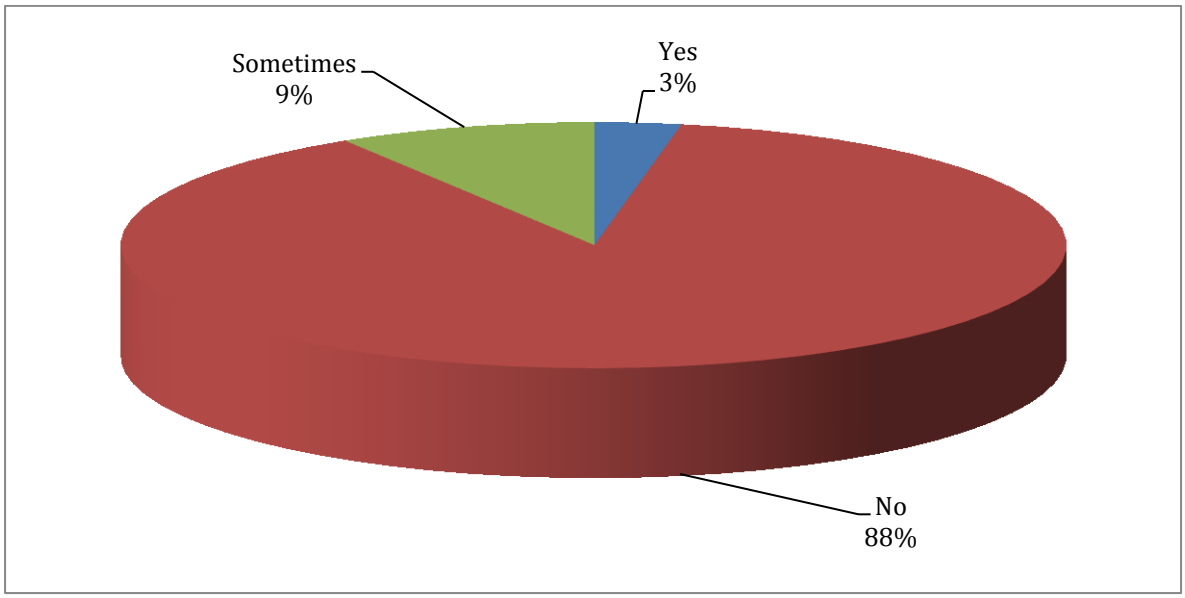


Figure 7. Percentage of perfusion units using fat or leucocyte filters.

passing to the patient (de Vries, Gu et al. 2002). de Vries and colleagues have shown that a filter based upon a leucocyte-depleting filter design can remove 40% of LME from PSB (de Vries, Gu et al. 2004). However, when one considers that the number of lipid particles in PSB may exceed 650,000/mL (Eyjolfsson, Scicluna et al. 2008), a higher removal rate than 40% is required in practice.
At present there appear to be no devices that will offer suitable lipid emboli removal.

One of the aims of this audit was to explore what the current practices were with regards to residual pump volume, that volume of blood that is left in the venous reservoir and connecting tubing, which contains LME from the PSB. Over 30% of units interviewed indicated that their practice was dependent upon surgical and anaesthetic preference. Some stated that many surgeons felt that there was no difference between RPV and the patient's systemic circulation whilst others indicated that anaesthetists considered RPV as "dirty" blood and required RPV to be washed with CS so that "cleaner" and a lower volume of fluid was returned for re-infusion through the central venous line. This indicates conflicting theories on which clinical decision-making is based, thus, preventing coherent guidelines for perfusion practice being formulated. Whilst there are very few studies comparing outcome measures in cardiac patients having either CS-processed RPV or direct RPV re-infusion, the available literature does not indicate significant benefit of the technique. A potential treatment method would be a form of modified ultrafiltration, as undertaken with many paediatric procedures; by removing excess fluid from the patient's circulating volume and concentrating the RPV, the increase in haematocrit may reduce allogeneic blood transfusion whilst ensuring that clotting factors and platelets are not removed in a washing process. However, without large studies examining potential benefits and adverse effects, this approach should be seen as purely theoretical at present. Based upon the knowledge gained during this review, it is the opinion of the author that the following represents the best current protocol for dealing with PSB and RPV:

- Pericardial suction blood should be removed to the cell saver only when limited blood loss is anticipated; moderate to high blood loss should be managed through separate cardiotomy suction, ideally with a lipid filter before re-infusion into the systemic circulation where possible.

- Residual pump volume should be infused directly into the aorta before de-cannulation and/or via a blood bag and lipid filter into the central venous line after de- cannulation. Any small remaining quantities of RPV can then be chased through into the cell saver for processing if required.

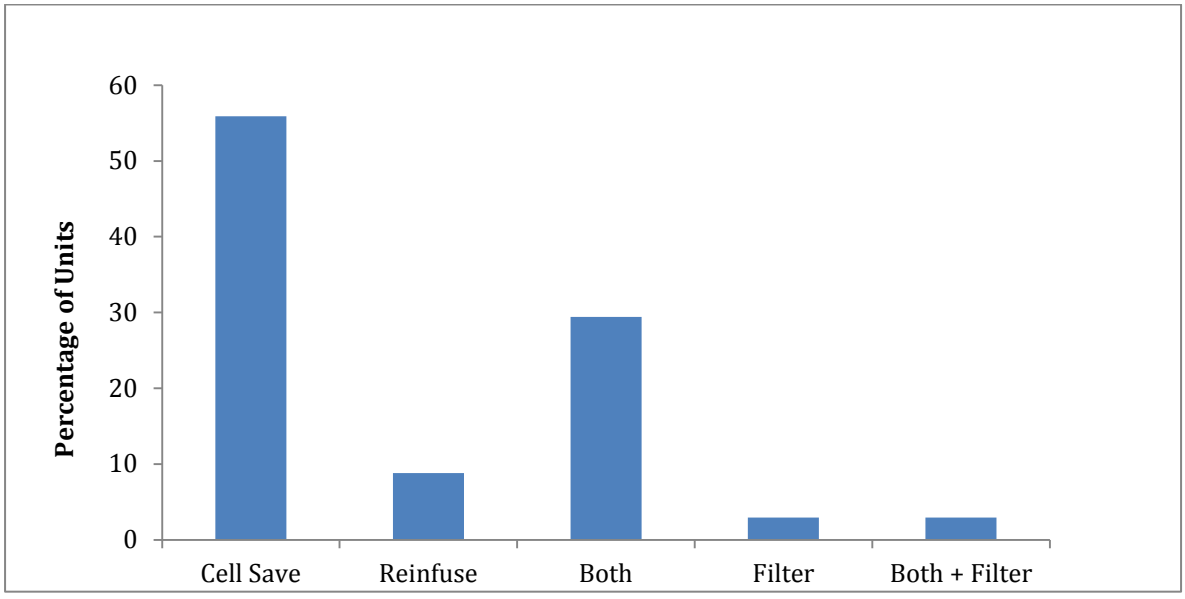


Figure 8. Dealing with residual pump volume. Cell Save: Circuit contents chased into cell saver and processed. Re-infuse: Circuit contents directly re-infused via aortic cannula or chased into a collection bag and directly re-infused via the central venous line. Both: Cell save and re-infuse protocols used. Filter: Contents of the circuit haemoconcentrated into a collection bag and re-infused via the central venous line.

This national audit demonstrates that the issue of PSB processing is confused, with no ideal technology or technique in current use. The practice of re-infusion of cell salvaged RPV should be carefully considered, as theoretical benefits may not be seen in practice. Perfusion protocols and practices are also heavily influenced by surgical and anaesthetic preferences and based on very limited and poor quality evidence.

The key message of this audit is that across the UK there are numerous centres carrying out varying levels of methodologies in order to alleviate their patients of LME. All of the methodologies are flawed; none can demonstrate superiority over the other without sacrificing some element of safety or compromising patient's haemostatic integrity. In short, another methodology is necessary that can demonstrate efficacious LME removal in the clinical setting without:

a) Discarding all PSB, or
b) Discarding all clotting factors and platelets, and
c) Allowing the reinfusion of PSB in an emergency situation

Chapter 3 will now set out the methodology of a randomised study looking at a lipid filter that has the potential to meet these requirements.

3. RemoweLL Study

3.1. **Study Aims and Design**

The aim of this study was to determine if a new oxygenation system with integrated lipid filter (RemoweLL) could remove LME from the PSB and examine what effects, if any this would have on biochemical markers of organ function. The study was a single centre, single blind, randomised, controlled parallel group investigation in patients undergoing CABG with CPB assigned to either the RemoweLL (intervention) or to its sister product, Admiral (control) extracorporeal circuit (Figure 9). The two circuits are identical in all but the filter material in the cardiotomy reservoir (Issitt, Cumberland et al. 2008). The study was performed between March 2013 and December 2015 in Southampton General Hospital, in accordance with the current version of the Declaration of Helsinki. The Central Oxford Research Ethics Committee (COREC) approved the final study protocol and subsequent amendments. The study was conducted under reference 10/H0606/30. The details of the study methodology were published in Working Papers in Health Sciences in 2013 (Issitt 2013). All patients provided written, informed consent.

I was the principle investigator. As such I was responsible for taking informed consent, checking the eligibility criteria and creating the necessary case report form. Furthermore I undertook the randomisation procedure and provided the operating Perfusionist with the correctly assigned circuit. During the procedure I oversaw the conduct, collected the samples and ensured that, where necessary, the samples were kept in the appropriate conditions (i.e. on ice), before personally delivering the samples to the Biochemistry laboratory at Southampton General Hospital. Biochemical samples were analysed by Southampton General Hospital except Cystatin C (which the Southampton laboratory was unable to process), which was measured by Dr Tim James, Head Biomedical Scientist, John Radcliffe Hospital, Oxford following plasma separation at Southampton. Neuron Specific Enolase was measured at King's College Hospital, London (as Southampton was unable to process this). Due to the multiple sites of analysis, I was unable to undertake direct measurement and analysis of Cystatin C and Neuron Specific Enolase. Dr Adnam Mani, Cellular Immunologist, University Hospital Southampton, oversaw the immunological analysis of CD11b. Where possible I participated in the preparation and analysis of this marker, however due to the sampling time points, this was not always possible and so Dr Mani undertook them alone when necessary.

Patients were assessed for eligibility for inclusion at screening. Baseline assessments (Table 1) were laboratory tests (haematology, biochemistry and urine analysis), lipid profile, CD11b assay, C - reactive protein, Cystatin C assay, blood gases and NSE assay. Lipid and inflammation assessment took place

throughout CPB whilst cerebral, pulmonary and renal injury/function investigations took place following CPB. Laboratory tests included:

Haematology

- Red Blood Cells, Haemoglobin, Haematocrit, Platelets and leucocyte count plus differential (Neutrophils, lymphocytes, Monocytes).

Biochemistry

- Calcium (Ionised), Chloride, Creatinine, Urea, Lactate, Magnesium, Potassium, Sodium, Cholesterol, Lipoproteins (LDL, HDL), Triglycerides, C-Reactive Protein.

All haematology and biochemistry results for clinically significant out-of-range findings were recorded on the patient's CRF.

3.1. **Lipid Analysis**

A 5mL blood sample was collected into a lithium heparin tube for analysis of lipid profile (HDL and LDL, total cholesterol, triglycerides) pre-CPB, 5 and 30 minutes on CPB, 5 minutes before cross clamp removal, 5 minutes before end of CPB and 1 and 24 hours post-CPB. Lipid profile was measured using standard laboratory techniques. Lipid emboli detection was carried out using light microscopy. A collection chamber was inserted proximal to the cardiotomy reservoir. At the start of CPB, blood was collected from the chamber as the initial baseline LME count. Following reintroduction of the PSB into the systemic circulation, a sample was taken from the arterial sampling line to give a post-filtration sample. 100µL of the sample was diluted 1/10 with saline (1000µL) and agitated for 2-3 minutes to equilibrate. 10µL was placed onto a Thoma Chamber and lipids counted under light microscopy an eyepiece of 10X magnificantion with a 40X/0.65 objective lens (total magnification 400X). The lipids could be seen as spherical non-nucleated globules. The number of the lipids per µL was obtained by counting the average number of lipids in 4 small squares (Y) and inserted into the formula X=Yx16x100 where 16 equals the number of small squares (total volume 0.1µL) and 100 equals the dilution factor.

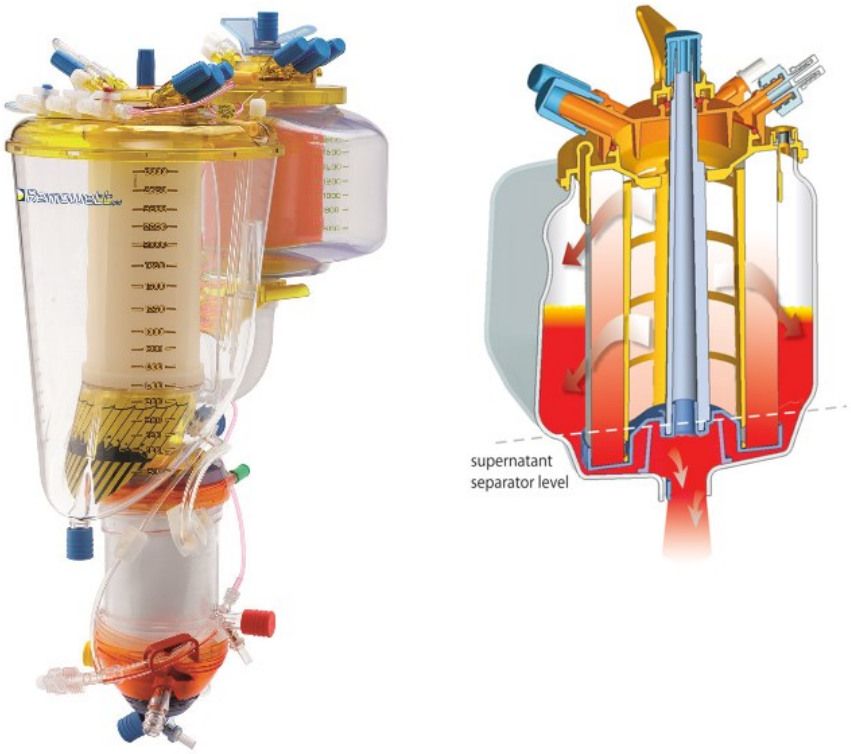


Figure 9. RemoweLL Cardiotomy Schematic. The RemoweLL® ECC system comprising a leucocyte filter and lipid microemboli siphon. See Section 5.4 for details. Image provided and reproduced with permission from Eurosets S.r.l.

Study Assessments											
	Time Point										
	Screen	1	2	3	4	5	6	7	8	9	10	11
Consent	X											
Eligibility	X											
Morbidity		X	X	X	X	X	X	X	X	X	X	X
Mortality		X	X	X	X	X	X	X	X	X	X	X
Full Blood Count		X					X		X			
Blood Gases		X	X	X	X	X	X	X	X	X	X	X
Lipid Profile		X	X	X	X	X	X		X			
CD11b Assay		X				X	X		X			
Electrolytes + C-Reactive Protein		X	X	X	X	X	X		X			
Urea + Creatinine		X								X	X	X
Urine Microalbumin + Osmolarity		X								X	X	X
Cystatin C		X								X	X	X
Neuron Specific Enolase		X				X		X	X			

Table 1. Study Assessments. Breakdown of the assessments taken at each time point. Screen; patient's pre-operative screening assessment, 1; pre-CPB, 2; 5 minutes onto-CPB, 3; 30 minutes on CPB, 4; 5 minutes prior to aortic cross clamp removal, 5; 5 minutes prior to end-CPB, 6; 1 hour post-CPB, 7: 6 hours post-CPB, 8; 24 hours post-CPB, 9; 1st post-operative morning, 10; 2nd post-operative morning, 11; 3rd post-operative morning.

3.2. **Inflammatory Response**

A 5mL blood sample was taken into a lithium heparin tube for analysis of C-Reactive Protein pre-CPB, 5 and 30 minutes on CPB, 5 minutes before cross clamp removal, 5 minutes before end of CPB and 1 and 24 hours post-CPB. Leucocyte differential was undertaken using standard laboratory techniques pre-CPB, pre-release of PSB into the systemic circulation, post-release of PSB, end-CPB and 1 hour post-CPB.

3.3. **Leucocyte Activation**

A 5mL blood sample was taken into an EDTA tube for analysis of CD11b pre-CPB, 5 minutes before end of CPB, 1 and 24 hours post-CPB. Samples were kept on ice and were analysed within 24 hours by Fluorescence-Activated Cell Sorting (FACS), after staining with CD11b Phycoerythrin (PE). FACS analysis is a specialized type of flow cytometry that provides a method for sorting a heterogeneous mixture of biological cells, one cell at a time, based upon the specific light scattering and fluorescent characteristics of the label (in this case PE) attached to a particular cell type. The samples were mixed and incubated at 4-8°C for 30 minutes. Following staining, the samples were washed and centrifuged at 3000rpm for 2 minutes and then the supernatant 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 levels of leucocytes, monocytes and lymphocytes were reported. FACS data was reported in two-dimensional dot plots with adjustment of the positions of displayed and preconfigured Polygon gates to capture all population of interest including Neutrophils, monocytes and lymphocytes using the CD45/SSC plot and was done by Dr Adnan Mani, Head of the Flow Cytometry Unit, Southampton General Hospital.

3.4. **Cerebral Injury**

A 5mL blood sample was taken into a serum tube for analysis of NSE pre-CPB, 5 minutes before the end of CPB, 6 and 24 hours post-CPB. Commercially available Enzyme-Linked Immunosorbent Assays (ELISA) were performed at King's College Hospital, London. Briefly, 25µL of sample was added to each well of prepared antibody solution (Horseradish Peroxidase (HRP) Anti-NSE and Biotin Anti-NSE; 100µL) and incubated at room temperature for 1 hour. Following washing the sample in added to a 3,3',5,5'-

Tetramethylbenzidine (TMB) HRP-substrate and incubated for a further 30 minutes before absorbance was read at 620nm.

3.5. **Pulmonary Injury**

Alveolar oxygenation index (AaOI) was calculated from blood gases and ventilation settings taken pre-CPB, immediately post CPB and then 1, 2, 6, 12, 24 and 48 hours post-CPB. For the 48 hours 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_2) and % saturation and carbon dioxide partial pressure ($PaCO_2$). Samples obtained at 1 and 2 hours reflect intubation whilst 6, 12 and 24 hours reflected oxygen administered by facemask. The Alveolar-Arterial Oxygenation Index (AaOI) was determined using the following formula:

$AaOI = ((760\text{-}47)F_iO_2 – (PaCO_2 \times 7.6)1.25)) – (PaO_2 \times 7.6)/(PaO_2 \times 7.6)$

Where:

F_iO_2 = the inspired oxygen concentration (%)

$PaCO_2$ = the partial pressure of arterial CO_2 (kPa)

PaO_2 = the partial pressure of arterial O_2 (kPa).

ARDS classification was calculated using PaO_2/F_iO_2 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_2/F_iO_2 >300; none, 2 – PaO_2/F_iO_2 200-300; mild, 3 – PaO_2/F_iO_2 100-200; moderate and 4 – PaO_2/F_iO_2 <100; severe.

3.6. **Renal Injury**

A 5mL blood sample was taken into a serum tube or a lithium heparin tube for analysis of Cystatin C and electrolytes (including urea and creatinine) respectively pre-CPB, and on the 1st, 2nd and 3rd postoperative mornings. A 10mL urine sample was taken into a plain universal tube for analysis of urine microalbumin and osmolarity. Glomerular Filtration Rate (GFR) was calculated using the CKD-EPI Creatinine Equation (2009). Cystatin C assays were performed at the John Radcliffe Hospital, Oxford. Acute kidney injury as defined as an increase in absolute serum creatinine ≥3mg/dL (26.4µmol/L) or 1.5-fold increase from baseline. Serum and urine electrolytes were collected at all intraoperative time points (pre-CPB, 5 and 30 minutes on CPB, 5 minutes before cross clamp removal, 5 minutes before end of CPB and 1 and 24 hours post-CPB).

3.7. **Anaesthetic and Operative Details**

Both intervention and control groups received the same anaesthetic regime. The patients were pre-medicated with 10 mg of Morphine and 2 mg of Lorazepam. Anaesthesia was induced with Midazolam, Fentanyl and Pancuronium and maintained using intermittent positive pressure ventilation with oxygen-enriched air and isofluorane. During CPB, a Propofol infusion was used to maintain anaesthesia.

The CPB circuit consisted of either the Admiral (control) oxygenation system with integral 40μm cardiotomy filtered reservoir or RemoweLL (intervention) oxygenation system with integral cardiotomy lipid/leucocyte filter (Eurosets s.r.l, Mirandola, Italy; Figure 9). The circuit was primed with 2L lactated Ringer's solution that contained 5000 units of heparin. Prior to the establishment of CPB, 3 mg/kg body weight of heparin were administered and supplemented as required to maintain an activated clotting time of 480s. Continuous, non-pulsatile blood-flow was delivered to the patient using a multi-flow roller pump (HL20, Maquet, Germany) at an indexed flow rate of 2.4L/m^2/min. Alpha stat pH management was used to control acid-base balance. Mean arterial pressure was maintained between 50-60mmHg with pharmacological manipulation if necessary. After aortic clamping, electromechanical diastolic arrest was induced with the delivery of cold (4°C) blood cardioplegic solution. Distal anastomoses were completed during a single period of aortic clamping. Proximal anastomoses were performed with a beating heart using an aortic partial occluding clamp. CPB was terminated after the patient was re-warmed to a nasopharyngeal temperature of 37°C.

After the operation, the patients were kept ventilated until standard extubation criteria were met. The ventilation protocol comprised of a respiratory rate of 10, tidal volume of 10 ml/kg of body weight, fraction of inspired oxygen of 60%, pressure support of 20 cmH_2O, positive end expiratory pressure of 5 cmH_2O and inspiratory to expiratory ratio of 1:2. Hydration was achieved with the intravenous administration of Dextrose 5% solution infused at 1 $mlkg^{-1}h^{-1}$. 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.

3.8. **Patient Numbers and Power Calculations**

3.8.1. **Power Calculations**

In vitro studies have shown that the RemoweLL oxygenator removes 40–50% of leucocytes and 55–70% of lipid microparticles. Recent *in vivo* data (unpublished) show that the numbers of LME in the RemoweLL system compared to a standard circuit is 1095±579 (mean±SD) vs. 2970±1405.29 (mean±SD) particles/mL giving an average percentage removal of 63±8.4% (mean±SD). The number of patients in

each study group (25) was determined by an *a priori* power calculation using G*Power Version 3.1.0 (Universität Kiel, Germany) to achieve a power (1-β) of 0.95 with α=0.001 for an effect size index of 1.745 that may be expected in clinical practice based on the *in vivo* data. These power calculations allow for a 15% drop out rate or loss to follow up. Previous studies have shown that the lower the serum concentrations of NSE, the better the outcome of patients after CPB (Ali, Harmer et al. 2000). For this reason a tentative *a priori* power calculation has been undertaken based on Bonacchi's work (Bonacchi, Prifti et al. 2006) where the average postoperative peak serum NSE concentration was 17.7±6.5µg/L (mean±SD, n=42). An assumption was made that for a significant, clinically relevant, difference in peak circulating NSE, a minimum reduction of 33% (i.e. a one-third reduction) should be seen in the study group compared to control group assuming equal standard deviations in both groups. Based upon these assumptions an effect size of 0.923 can be calculated. Therefore, a sample size of 50 (twenty-five subjects per group) was sufficient, with power (1-β) of 80% and α=0.05, to show a 33% reduction in NSE allowing for a drop out or loss to follow up rate of 15%. However, no data are available to indicate the direct relationship that LME and leucocyte filtration would have on biochemical markers of organ injury; for this reason an Interim Assessment using a statistical stopping rule was undertaken after 20 patients which would allow for adjustment to the participant numbers as appropriate (i.e. this would give pilot data to carry out meaningful power calculations). The Haybittle-Peto boundary was used as the statistical stopping rule to provide interim data at the appropriate statistical level so that a decision can be made on the ethicality of the study, should the early results be promising; i.e. early data is so promising that it is no longer fair to keep patients in one group without giving them the opportunity to change treatment. The boundary states that if an interim analysis shows a probability of equal to or less than 0.001 that the null hypothesis can be rejected and the trial should be stopped early. The final analysis is still evaluated at the normal level of significance (in this case 0.05). The main advantage of using the Haybittle-Peto boundary over other statistical stopping rules is that the final analysis is carried out at the 0.05 level which is easier to understand. The main argument against its use is that it is considered too conservative increasing the change of a type two error. However, this test was chosen to prevent the more important type one error, accepting a false positive and under powering the study. If there was a bigger effect size on the secondary objective than predicted, this would only influence the numbers if the primary objective met the Haybittle-Peto boundary for 2 points (i.e. p=0.001) for the primary objective. The interim analysis is discussed further in Section 4.1.

The Null hypothesis was that there would be no difference between the peak Neuron Specific Enolase concentrations between the two groups.

The Alternative hypothesis was that there would be a difference in peak Neuron Specific Enolase concentration due to LME filtration.

3.8.2. **Inclusion and Exclusion Criteria**

Subjects meeting the following inclusion criteria were eligible for the study:

- Participant is willing and able to give informed consent for participation in the study – any documented history of cognitive impairment will exclude the patient as this may have an effect on biochemical markers of cerebral injury*
- Male or Female, aged 18 years or above
- Patients undergoing elective CABG surgery
- Angiographically proven coronary artery stenosis

The participant may not enter the study if ANY of the following apply:

- Age less than 18 or more than 90 years old
- Emergency CABG surgery
- Previous CABG surgery
- Gross haemodynamic instability: hypertension (systolic blood pressure >160mmHg), hypotension (systolic blood pressure <90mmHg), or bradycardia (heart rate <60 beats/min)
- Morbid Obesity (BMI >35)
- Pre-operative heparin regime.
- Abnormal preoperative white cell count (<4 or >10x10^9 cells/L).
- Renal failure (serum creatinine >150μmol/L)
- Pulmonary dysfunction (e.g. COPD)

*Evidence of existing cognitive impairment was adjudged at the pre-surgical assessment and after consultation with the patient's General Practitioner.

3.8.3. **Randomisation**

The patients were randomised to either the control (Admiral circuit) or intervention (RemoweLL circuit) group on the morning of surgery using a previously compiled randomisation table system (QuickCalcs Randomise1, GraphPad Software Inc., USA), which was held in the Perfusion office at the Southampton General Hospital. The appropriate extracorporeal circuit was then selected. No one else involved in the study were aware of which circuit had been chosen.

3.9. **Data Management and Statistical Analysis**

Data were entered into an EXCEL spread sheet by myself and independently verified by Mr Jon Ball, Chief Perfusionist, Southampton General Hospital. Data was anonymised at this point.

Primary and secondary endpoints were analysed using the SPSS statistical package by me and independently reviewed by Dr Victoria Banks, Statistician, Great Ormond Street Hospital. 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 groups. Normally distributed data were tested using T Test for Two Independent Samples whilst non-normally distributed values were LOG transformed and if shown to be normally distributed tested as above. If still non-normally distributed, data were tested using Mann-Whitney Test for Two Independent Samples. ARDS categorisation was tested using the Chi-square test. All tests were considered to be Two Tailed. Correlation and Regression analysis using Pearson's Coefficient for LME counts and levels of NSE were used to examine any relationships between the 2 parameters. A p value ≤ 0.05 was considered significant. Data are presented as median (IQR) whilst graphically displayed as box and whisker plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values unless stated from continuous variables reported in other papers, in which case data are given as mean±standard deviation with the n = number of subjects.

4. Completeness of Data

4.1. **Interim Statistical Analysis**

The data required for the primary outcome measure (the removal of lipids) were based upon *in-vitro* studies that had not used blood from patients and had pseudo-lipids, which did not replicate the actions of lipids in blood. Therefore a total study population of 50 patients was chosen as a conservative number based upon this data (see 3.8.1). Furthermore, there has never been a direct link made between the secondary outcome measure (NSE) and lipid emboli. For this reason, a caveat was placed into the protocol that a post hoc power calculation would be done. Going on statistician's advice this was to be done at 20 patient stage, which would be sufficient to provide "pilot" data which would then allow for adjustment to the participant numbers as appropriate. The rationale behind this was that actually the effect would be smaller than predicted and therefore this would prevent erroneous conclusions from an underpowered sample size. As all eventualities must be covered, the alternative scenario was that, if there was a bigger effect size on the secondary objective, this would only influence the numbers if the primary objective met the Haybittle-Peto boundary for 2 points (i.e. p=0.001) for the primary objective. This boundary was chosen, as it is more conservative than the Pocock boundary and therefore should prevent the early termination of the trial unnecessarily.

For the primary outcome measure (differences in lipid microemboli), it was calculated that 2 groups of 25 were needed for a power (1-B) of 0.95 with alpha=0.001 for an effect size index of 1.745 based upon *in vitro* data. However, median (IQR) of 1188(442) and 127.2(104.85) have been observed which gives an effect size of 3.5 and a p=0.0002 using a Mann-Whitney Test for two independent samples.

For the secondary outcome measure 2 groups of 25 was also enough for a power of 80% and alpha =0.05 to predict a theoretical 33% drop with an effect size of 0.923. It was believed that this number would be clinically relevant based on previously published data. At the 20 patients (10 per group) stage, the mean±SD in peak NSE levels are 21.13±4.32 and 16.29±4.72 which gives an effect size of 1.2 and a p=0.035 using a T-test with equal variances.

Subject Accountability		
	Admiral	RemoweLL
Screened	30	32
Ineligible	7	5
Enrolled in Study	19	21
Incomplete data	0	0
Patient Withdrew	0	1
Patient Cancelled	4	5
Died	0	0
Completed Study	**15**	**15**

Table 2. Subject accountability. The number of patients undergoing the study assessments. Twelve patients were ruled ineligible (see Table 3), 9 were cancelled and 1 withdrew their consent pre-operatively. In total 30 patients completed the study assessments.

	Admiral	RemoweLL
Ineligibility		
COPD	0	2
Renal Insufficiency	3	1
Morbid Obesity	3	1
Emergency	1	1
Total	**7**	**5**
Cancellation		
No staff	1	2
Lab Unavailable	3	3
Total	**4**	**5**

Table 3. Consort Statement. The number of patients that were unable to undergo the study assessments. Twelve patients matched exclusion criteria whilst 3 patients were cancelled due to lack of Perfusionist. A further 6 were cancelled due to more urgent patients taking the operating sessions. These patients were reallocated to operations on Fridays and have to be withdrawn from the study as CD11b analysis was unable to be undertaken on a Saturday (24 hour post-CPB sample).

Using this data to recalculate sample sizes, 2 groups of 15 are required for the same assumptions as above (power 80, alpha 0.05). Therefore, the Haybittle-Peto Boundary was attained for the 1st point.

4.2. **Completed Statistical Analysis**

Following completion of the 30th patient (15 in each group), the primary objective, LME removal, was tested for normal distribution using the Shapiro-Wilk tests and subsequently two-factor ANOVA for repeated measures. This showed a significant difference between the 2 groups, p=0.000016. Further examination of the post-op differences using the Mann-Whitney Test for Two Independent Samples confirmed this; p=0.0000024. This is well below the threshold for the second (final) analysis using the Haybittle-Peto boundary as a stopping rule (p<0.05).

The secondary objective, differences in NSE, were also tested for normal distribution and underwent two-factor ANOVA for repeated measures which showed significant differences between the 2 groups; p=0.0017. Analysis of the peak concentration between the 2 groups was undertaken using the TTest for Two Independent Samples which showed a significant difference in peak concentrations; p=0.01.

Therefore, the secondary objective confirms the conclusion that the Haybittle-Peto boundary stopping rules have been met and that the null hypothesis can be rejected. Given the implications of neurological outcome following CPB and the relevance and correlation described in section 1.3 of NSE to patient outcomes, the decision to stop this trial early for ethical reasons was made.

4.3. **Subject Accountability**

All patients enrolled in the study had their pre-operative details recorded on their CRF. All patients underwent all study assessments, representing 100% data collection. In total 62 patients undergoing first-time elective CABG surgery were screened for participation in the study (Table 2). Twelve patients were ruled ineligible due to matching exclusion criteria. A breakdown of these is provided in the CONSORT statement (Table 3). In total 40 patients were enrolled into the study. Of these 9 were cancelled and 1 withdrew their consent pre-operatively. Due to the availability of laboratory staff, the FACs analysis for CD11b at time point 8 (24 hours post op) was unable to be carried out on Saturdays, and must be measured within 24 hours of the sample being taken, so if patients were moved to Friday operations, they had to be withdrawn from the study.

	Admiral		RemoweLL		
	Mean	SD	Mean	SD	*p*
Male (n) Female	12.00 3.00		11.00 4.00		
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

Table 4. Demographic data. Data presented as mean with standard deviations. X-Clamp; aortic cross clamp. CABG; coronary artery bypass grafts. Time of cardiotomy release is the amount of time the PSB was left separated from the systemic circulation. There were no significant differences between both groups of patients with regards to morbidity, preoperative drug regimens and perioperative details.

Therefore in total, 30 patients successfully underwent the study assessments (15 per group). The demographics of the patients undergoing the study are given in Table 4. Both groups were equally matched in terms of male:female ratio, preoperative statin regime and number of patients with diabetes mellitus. 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 differences in terms of perioperative details including CPB time, number of grafts and fluid balance.

5. Lipid Filtration

5.1. **Introduction**

Lipid Microemboli are formed in PSB, which when returned to the CPB circuit, pass through filter materials and are returned to the arterial cannula. From here, LME have been observed to enter all major organs and have been associated with SCADs in the brains of patients who have died following CPB. However, there has never been a proven causal relationship showing a definitive correlation between LME and organ dysfunction, or whether removal of LME results in improved organ function post CPB. Several methods of LME filtration have been proposed but as yet there is not a suitable efficacious method for use within the clinical setting. This study tested a new lipid filtration system (RemoweLL) against a normal ECC system with a 40μM cardiotomy filter (Admiral) to determine the efficacy of the filtration system.

5.2. **Methods**

Patients and analytical methods are described in Section 3.1

Descriptive statistics showed a positive skewness to the number of LME, which was confirmed as non-normal data distribution by Shapiro-Wilk test. Log transformation was unsuccessful, therefore Mann-Whitney test for two independent samples was used to compare pre and post filtration samples. Cholesterol, HDL and LDL all conformed to normal distributions and were tested using two factor ANOVA with Repeated Measures test. Cholesterol/HDL ratio and triglycerides were non-normally distributed but log transformation successfully normalised distribution, therefore two factor ANOVA with Repeated Measures test of log transformed data was used.

5.3. **Results**

Both groups processed similar volumes of PSB [Admiral 776.67±632.14mL (mean±SD, n=15) vs. RemoweLL 780.00±567.20mL; (mean±SD, n=15) p=0.99] whilst the sedimentation time (the time PSB left in the cardiotomy reservoir) was similar in both groups [Admiral 74.93±19.27mins(mean±SD, n=15) vs. RemoweLL 67±17mins (mean±SD, n=15) ; p=0.23]. Baseline LME counts (n/μL) were similar in both groups [400(200) vs. 400(400); p=0.47] but there was a significant reduction in LME count with the RemoweLL lipid filter [100 (75); p<0.001] compared with a significant rise in the Admiral circuit [1,200(200); p<0.001] (Table 5, Figure 10). Post op differences between the Admiral and RemoweLL circuits were significant [1,200 (200) vs. 100(75) respectively; p<0.001]. LME were observed under light microscopy and number determined as outlined above, an example of which is shown in Figure 11.

	Time	Admiral	RemoweLL	*p*
LME Count (n/µL)	Pre CPB	400 (200)	400 (400)	0.47
	Post CPB	1200 (200)	100 (75)	<0.001

Table 5. LME Count. LME; Lipid Microemboli counted using light microscopy as detailed in Section 3.1. Data are presented as median (IQR). A *p* value ≤0.05 was considered significant.

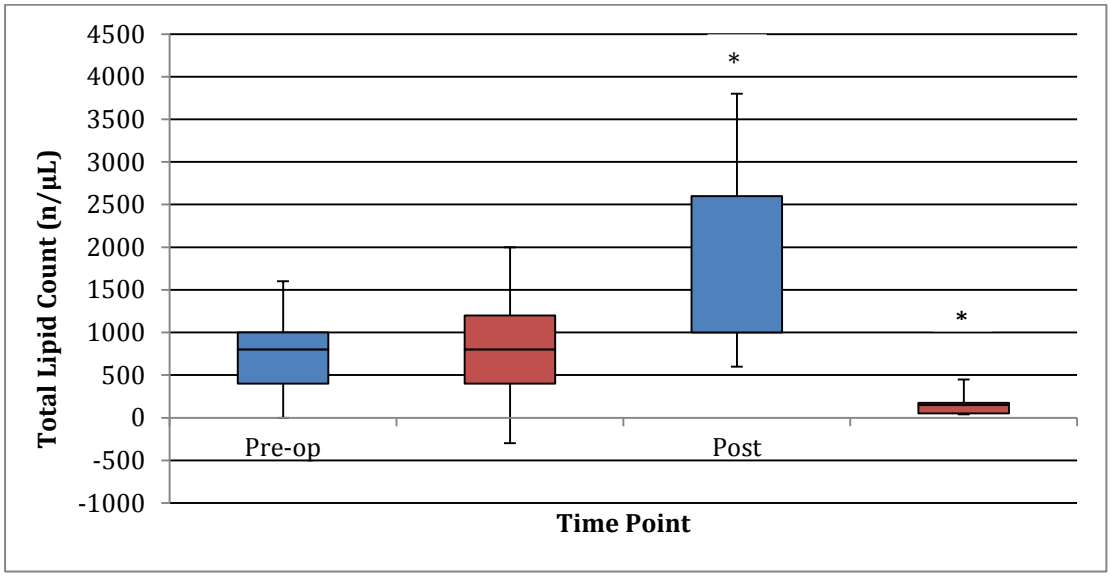


Figure 10. Lipid microemboli count pre and post filtration. Pre sample taken following the administration of heparin and initiation of pericardial suckers. Post sample taken from the arterial sampling manifold following release of PSB into the systemic circulation. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values. *$p \leq 0.05$.

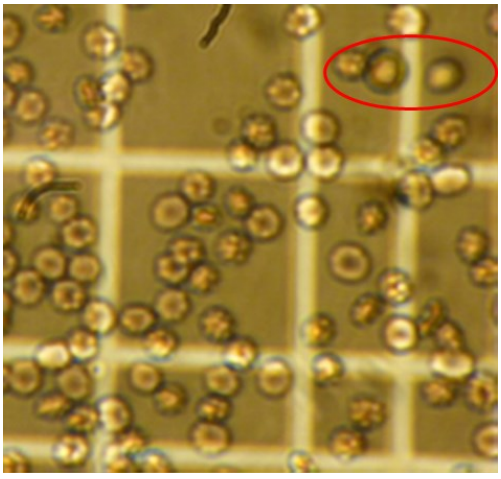


Figure 11. Lipid microemboli under 40/0.65 Optics. Lipid microemboli observed as non-nucleated spherical cells taken under light microscopy with 40/0.65 optics.

Examination of circulating fats showed similar levels of cholesterol, HDL, LDL and triglycerides throughout the CPB period; ANOVA revealed no significant interactions between any parameters and study groups (Table 6; Appendix A). There was a significant reduction in all parameters from baseline to the first sample on CPB, where they stayed stable throughout CPB and into the first 24 hours post-op. There was a small reduction in LDL in the RemoweLL group at 24 hours post-op, whereas the Admiral saw a small increase at the same time point [1.17±0.63 mmol/L vs. 0.91±0.51 mmol/L (mean±SD, n=15), RemoweLL; 0.89±0.34 mmol/L vs. 0.93±0.76 mmol/L (mean±SD, n=15), Admiral]. However, examination using a T Test for two independent samples revealed no significant differences between time points, or groups [p=0.15 and p=0.94 respectively].

There was weak evidence of higher levels of triglycerides in the RemoweLL group but this failed to reach statistical significance [p=0.08]. There was, however, a significant increase in triglyceride levels in both groups in the immediate (1 hour post CPB) period [Admiral p=0.03; RemoweLL p=0.02] but this had then reduced back towards baseline 24 hours later [Admiral 0.8 (0.4) mmol/L; RemoweLL 0.8 (0.5) mmol/L; Table 6].

5.4. **Discussion**

This study set out to establish the efficacy of a new LME filtration system that is situated in the cardiotomy reservoir of a cardiopulmonary bypass circuit. This has been shown to be the major source of LME in patients undergoing CPB (Brooker, Brown et al. 1998). The RemoweLL cardiotomy reservoir consists of 2 filtering mechanisms (Figure 9). The first uses a 40µm membrane to provide multilayer filtration for leucocytes and LME. The blood then passes into the sedimentation chamber where it is kept separate from the circulating volume to obtain a lipid supernatant. The supernatant, rich in lipid particles, is blocked by the siphon (the second filtration method) at the base of the cardiotomy reservoir, which is then discarded following re-infusion of the lipid-filtered PSB. We saw similar volumes of PSB in the cardiotomy suction to those previously reported (Appelblad and Engström 2002, Engström 2004), but noticed discrepancies in numbers of LME between this and other studies. Eyjolfsson *et al*., 2008 reported pre-bypass concentrations of LME in 24 CABG patients as 361±699/µL (mean±SD, n=24) which was similar to the results seen here [400 (200)/µL and 400 (400)/µL, Admiral and RemoweLL respectively].

	Time	Admiral	RemoweLL	*p*
Cholesterol (mmol/L)	Pre Op	2.73±0.57	3.17±091	0.12
	5 mins CPB	1.77±0.38	1.98±0.64	0.27
	30 mins CPB	1.79±0.41	2.05±0.68	0.22
	X Clamp Release	1.81±0.42	2.01±0.64	0.34
	End CPB	1.87±0.44	2.05±0.74	0.43
	1 Hr Post	1.96±0.43	2.27±0.81	0.20
	24 Hr Post	1.77±0.56	2.07±0.78	0.23
HDL (mmol/L)	Pre Op	0.91±0.25	0.94±0.2	0.79
	5 mins CPB	0.58±0.16	0.59±0.15	0.85
	30 mins CPB	0.61±0.17	0.63±0.19	0.73
	X Clamp Release	0.62±0.16	0.63±0.18	0.87
	End CPB	0.62±0.18	0.62±0.17	0.96
	1 Hr Post	0.64±0.17	0.66±0.17	0.75
	24 Hr Post	0.76±0.16	0.79±0.17	0.60
LDL (mmol/L)	Pre Op	1.41±0.4	1.68±0.73	0.22
	5 mins CPB	0.921±0.3	1.09±0.5	0.26
	30 mins CPB	0.89±0.32	1.1±0.54	0.22
	X Clamp Release	0.88±0.33	1.08±0.5	0.22
	End CPB	0.92±0.32	1.13±0.55	0.20
	1 Hr Post	0.89±0.33	1.17±0.63	0.15
	24 Hr Post	0.93±0.77	0.91±0.51	0.94
Cholesterol/HDL Ratio	Pre Op	3 (0.65)	3.2 (1.2)	0.25
	5 mins CPB	2.9 (1.1)	3.2 (1.2)	0.43
	30 mins CPB	2.9 (0.9)	3 (1.2)	0.34
	X Clamp Release	2.9 (0.8)	3 (1.3)	0.3
	End CPB	3.1 (0.8)	3 (1.1)	0.58
	1 Hr Post	3.2 (0.9)	3.2 (1.1)	0.47
	24 Hr Post	2.2 (0.7)	2.5 (0.9)	0.28
Triglycerides (mmol/L)	Pre Op	1.1 (0.7)	1.7 (0.7)	0.08
	5 mins CPB	0.7 (0.5)	0.9 (0.4)	0.18
	30 mins CPB	0.8 (0.3)	0.9 (0.5)	0.26
	X Clamp Release	0.7 (0.3)	0.9 (0.4)	0.37
	End CPB	0.7 (0.3)	0.8 (0.3)	0.67
	1 Hr Post	1.2 (0.4)	1.3 (0.5)	0.33
	24 Hr Post	0.8 (0.4)	0.8 (0.5)	0.44

Table 6. Lipid Profiles. HDL; High density lipoprotein, LDL; low density lipoprotein. Data presented as mean ±SD or median (IQR) depending on normality of distribution. A *p* value ≤0.05 was considered significant.

However, Dell'Amore and colleagues recently reported 2850 particles/dL, also using the RemoweLL system (Dell'Amore, Tripodi et al. 2010). There may be a number of reasons for this. Firstly, the study in question used a variety of procedures including CABG, and (the majority) isolated valve procedures, whereas this study and that of Eyjolfsson concentrated solely on CABG patients (Eyjolfsson, Scicluna et al. 2008). This is an important distinction as during CABG surgery, saphenous vein grafts from the leg and the Left Internal Mammary Artery (LIMA) from the chest wall needs to be harvested. This typically means that there is approximately one hour between the opening of the sternum and initiation of the cardiotomy suction. In valve surgery once sternotomy has been performed, initiation of cardiotomy suction takes place within 15 minutes. This discrepancy in time allows longer for fats to leach from the sternal bone marrow, therefore one would expect an increased number of LME produced. This is corroborated by the work of Brown *et al*., who discovered that the longer the CPB period (therefore the longer the sternal bone marrow exposure), the greater the number of SCADs (Brown, Moody et al. 2000). Furthermore, during preparation of the heart for valve surgery, there is very little bleeding before cardiotomy suction is required; other than the pericardium (which doesn't bleed) there are no cut surfaces, therefore the potential for PSB containing LME is low. During preparation for CABG surgery, the harvesting of the LIMA is associated with blood loss, and frequently the LIMA is left in the pericardial space where blood volume builds up, therefore the potential for LME is much higher. Secondly, the authors list the use of cell salvage, with mean volumes of 443±159mL (mean±SD, n=24) re-infused to the patient. Due to the mechanism of action of cell savers, significantly more volume is required for processing than is returned back for re-infusing; therefore one litre of blood may have been used as 70% of blood volume is plasma and cell savers only return red blood cells. This is a significant quantity of PSB containing LME. Furthermore, the samples were taken directly from the cardiotomy reservoir and so will not have taken into account this cell saved blood that has been returned to the patient. Lastly, the type of cell saver used (Latham bowl or continuous) is unclear so one cannot comment on the ability of the system to remove LME. As the Ejoylfsson study recruited similar demographic patients, and used a very precise method of LME measurement (Coulter counting) it is reasonable to suggest the number of LME particles obtained in this study is representative of this specific patient population (Eyjolfsson, Scicluna et al. 2008).

Lipid Microemboli removal was assessed by counting the numbers of LME present in the PSB once the cardiotomy suction had been initiated, and then again following re-infusion of PSB into the CPB circuit (see 3.1). The results show a highly significant efficacy for lipid removal compared to the control group. In the RemoweLL group 82.8% of the LME were removed [$p<0.0001$] with a

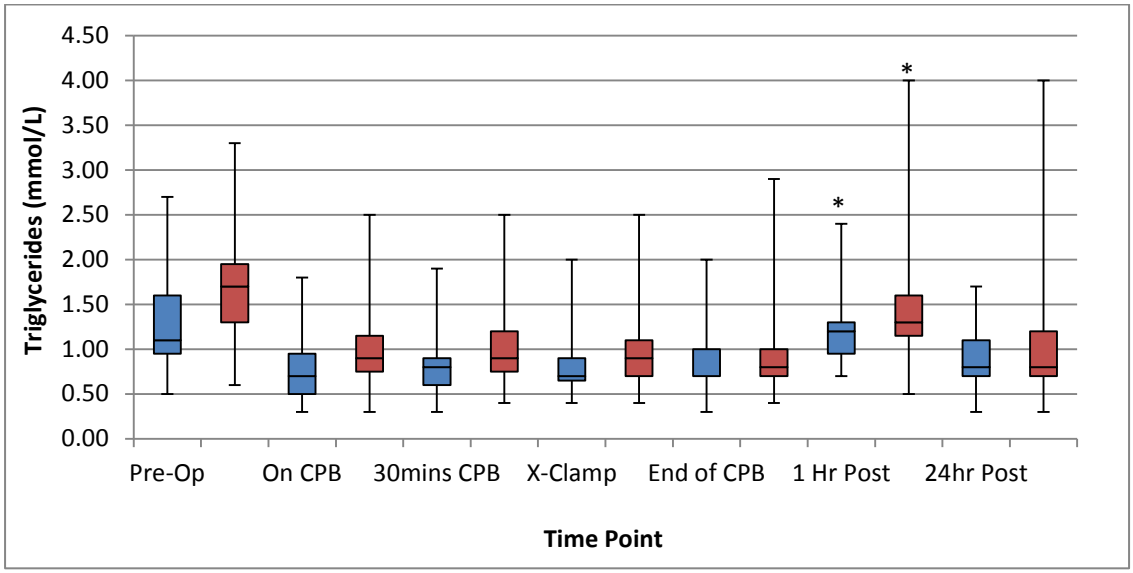


Figure 12. Perioperative Triglyceride changes during CPB. Samples taken pre-CPB, 5 and 30 minutes on CPB, 5 minutes before cross clamp removal, 5 minutes before end of CPB and 1 and 24 hours post-CPB. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values. *$p \leq 0.05$.

separation time of 67 minutes (time between cardiotomy suction initiation and re-infusion of PSB into the circulation). In the control group, there was a 115.7% increase in LME following re-infusion of PSB [p<0.0001], although the separation time was longer at 75 minutes this did not reach significance compared to the RemoweLL group [p=0.23].

The levels of circulating fats showed a consistent pattern, in that the concentrations of all the parameters dropped significantly from induction to the first sample during CPB. This is undoubtedly due to the haemodilutional effect of the CPB prime. Throughout the rest of the study period the concentrations of all parameters bar triglycerides (TGs) stayed stable showing that the RemoweLL filter does not affect HDL, LDL and Cholesterol concentrations. It is interesting to note increases in TGs as they have the potential to obstruct the micropores of oxygenators and cause myocardial ischemia upon reperfusion following removal of the aortic cross clamp. Both groups saw a significant increase in TGs in the immediate postoperative period [Admiral p=0.03; RemoweLL p=0.02; Figure 12] which had returned to baseline by the following day. There are a several possible hypotheses for this. Firstly, CPB is known to result in an acute phase response causing the hepatic synthesis of acute phase plasma proteins, although it is debatable whether triglycerides increase during this time (Hacquebard, Ducart et al. 2007). However, in a previous study there were significant differences to this one in that the sampling times were at the end of CPB and 2 days postoperatively, missing the key period of peak TG concentration seen by this and other studies (Oztekin, Gokdogan et al. 2007). Furthermore, the exclusion criteria of the Hacquebard study included patients with a BMI > 30 and patients with diabetes mellitus whereas this study included both those groups with a cut off of a BMI >35, altering the demographic and the array of TG profiles.

Cardiopulmonary bypass also utilises non-pulsatile flow and hypothermia, both of which are known to reduce splanchnic blood flow and cause peripheral shutdown and a reduction in lymphatic drainage. This not only affects hepatic and pancreatic blood flow, but also a reduction in the metabolic capacity of the organs. In the presence of hypertriglyceridaemia, this reduction in splanchnic blood flow and therefore lower oxygen delivery can lead to acute pancreatitis due to the high metabolic demands of the pancreas. Whilst the exact mechanism by which hypertriglyceridaemia causes pancreatitis is not fully understood, it is believed to be due to chylomicrons – the lipoprotein particles made up of triglycerides, phospholipids, cholesterol and proteins which transport dietary lipids from the intestines. Normally chylomicrons are formed 1-3 hours post prandially and are metabolised within 8 hours. If triglycerides are present in a concentration of >1g/dL , chylomicrons are constantly present. These large, low density particles can obstruct pancreatic capillaries causing acidaemia, exposing triglycerides to pancreatic lipases which facilitate their conversion to free fatty acids, which in turn induces cytotoxic injury,

inflammatory response and free radical release. Nys et al., has shown that there is a biphasic profile of pancreatic enzyme release – a substitute marker for pancreatic injury – occurring directly after surgery and 4-8 days postoperatively (Nys, Venneman et al. 2007). The first phase is likely to be initiated by ischemia or the perioperative inflammatory response to CPB and surgery, most probably the splanchnic hypoxia common with CPB. The second phase is likely to be a response to stimulation of the pancreas or reabsorption of digestive enzymes from the intestine after food intake, or an inflammatory reaction, perhaps from sequestrated neutrophils in the pancreas. It is, however, unlikely that this is the mechanism by which there is an increase in triglycerides in this study. Firstly, the definition of hypertriglyceridaemia is controversial, although a generally accepted limit is >150mg/dL or >1.7mmol/L, whereas pancreatitis normally only occurs once hypertriglyceridaemia has reached >1g/dL. None of the patients within this study reached this point. However, as this study was not designed to test this particular phenomenon, no markers of pancreatic function were assayed, therefore it is impossible to comment on the level of injury present, but it remains unlikely as no patients reported any symptoms suggesting pancreatitis.

The most likely source of the rise in triglyceride levels is due to the usage of propofol, an intravenous anaesthetic agent (Morgan, Campbell et al. 1990). All the patients in this study received a propofol infusion during cardiac surgery. Propofol is only slightly hydrophilic and is generally utilised in a 1% formulation in a 10% soybean fat emulsion (Rau, Roizen et al. 2001). General consensus suggests that the use of propofol is associated with elevated blood triglyceride concentrations including hypertriglyceridaemia and hypertriglyceridaemia-related pancreatitis. However, this has mainly been investigated in the intensive care setting after days of administration, not in the immediate postoperative hours (Theilen, Adam et al. 2002, Devlin, Lau et al. 2005). Soybean oil contains long chain triglycerides and it is hypothesised that plasma lipids change due to an interaction between propofol metabolism and proteins of the acute phase response of the inflammatory processes. It has also been postulated that serum lipids might increase as liver clearance is reduced during CPB. Myles et al., noticed changes in triglycerides (TG) following CPB but this did not reach significance. However, they did not include diabetic patients or those with pre-existing hyperlipidaemia. This study did, although as discussed above, the definition of hyperlipidaemia is slightly unclear (Myles, Buckland et al. 1995). The Myles study also observed a correlation between the increase in TGs and rise in plasma propofol concentration. Whilst the Myles study as well as work by Oztekin et al., have placed the elevation in TGs down to propofol, neither have presented an explanation of why the TGs increase postoperatively when propofol is administered at a constant infusion rate. Whilst this study was not designed to elucidate this

phenomenon, there are a number of factors that allow a hypothesis to be put forward. Due to the nature of sampling time points, the first 2 are during anaesthetic induction, and on CPB. Those studies that have examined at pre-induction have shown an increase upon induction that probably continues with the infusion up until the point at which CPB is commenced. Due to the dilutional aspect of CPB, the 2nd sample shows no or little difference to the induction sample, i.e., the increase has not been detected. During CPB, propofol continues to be infused at a constant rate, but the concentration of propofol and TGs do not change. This is likely to be due to the high lipid solubility of propofol which causes it to be sequestered into adipose tissue and the muscles. Whereas normally this would be cleared by the lymphatic drainage, during CPB, the non-pulsatile nature of the blood flow, coupled with the sedation and muscle relaxation attenuates this affect so that propofol is continuously absorbed into muscular tissue. Further reduction in blood flow to peripheral tissues due to hypothermia reduces washout of cells, and the reduction in splanchnic blood flow inhibits the metabolism of propofol in the liver (the systemic clearance of propofol is of the same order as the commonly accepted value for adult hepatic blood flow ≈1.5L/min; (Morgan, Campbell et al. 1990)). At the end of CPB, the patient has returned to normothermia and following cessation of CPB, pulsatile blood flow is re-established. This leads to a return to full hepatic blood flow and metabolism, and an increase in lymphatic drainage. Myles *et al*., noted a peak concentration of propofol and TGs at 4 hours post op, with increasing concentrations of both from the end of CPB until that point. As with the Myles study, the concentration of TGs returned to baseline by 24hours postoperatively, suggesting the systemic clearance of propofol, consistent with weaning from anaesthesia.

This study has demonstrated the efficacy of the RemoweLL oxygenation system at preventing LME from passing from the PSB into the systemic circulation without modifying the levels of circulating simple lipids.

6. Neutrophil Filtration and Activation

6.1. **Introduction**

Cardiopulmonary bypass is thought to induce a global systemic response through blood coming into contact with the non-physiological surfaces of the ECC leading to the release of (among others) complement factors C3a and C5a. Activation of C5a stimulates neutrophil adherence to endothelial cells, whilst neutrophil action can be mediated by the C3a molecule enabling local release of damaging substances upon adhesion. A second level of the RemoweLL filtration process involves a leucocyte-depleting filter. This filter removes activated leucocytes by an adherence mechanism that does not interact with inactivated leucocytes. This study tested the leucocyte filtration system (RemoweLL) against a normal ECC system with no leucocyte-depleting filter (Admiral) to determine the efficacy of the filtration system.

6.2. **Methods**

Patients and analytical methods are described in Section 3.3

Leucocyte and differential data showed positive skewness, which was confirmed as non-normal distribution using the Shapiro-Wilk test. None were able to show normal distribution when data were log transformed, therefore stepwise analysis using Mann-Whitney Test for two independent samples were used. C-reactive protein showed positive skewness that was confirmed as non-normal distribution using the Shapiro-Wilk test. Log transformation was unsuccessful so stepwise analysis with Mann-Whitney Test for two independent samples was used.

6.3. **Results**

The number of total leucocytes 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 leucocyte counts in the RemoweLL group at 1-hour post CPB that just failed to reach significance [Admiral 9.7 (4.5) x10^9/L vs. RemoweLL 13.4 (7.2) x10^9/L; p=0.06;**Error! Reference source not found.**]. Differential leucocyte analysis showed a similar pattern for neutrophils (Figure 13). There were no differences between the groups at any time point, and both groups showed significant increases in neutrophil numbers following CPB [p<0.001; Figure 13]. Lymphocytes were similar in both groups during the procedure but a significant difference was noted between the 2 groups in the immediate post op period [Admiral 1 (0.6) x10^9/L vs. RemoweLL 1.55 (0.73) x10^9/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) x10^9/L vs. 1 (0.6) x10^9/L; p<0.001, RemoweLL 1.6 (0.5) x10^9/L vs. 1.55 (0.73) x10^9/L; p=0.46].

	Time	Admiral	RemoweLL	*p*
WBC (x10^9/L)	Pre-Op	5.6 (3.1)	6.9 (2.8)	0.58
	Pre Release	5.8 (2.5)	7.45 (5.58)	0.46
	Post Release	7.7 (4.4)	9.1 (2.9)	0.28
	End CPB	9.4 (4.25)	11.9 (5)	0.2
	1Hr Post-Op	9.7 (4.5)	13.4 (7.15)	0.06
Neutrophils (x10^9/L)	Pre-Op	3.4 (2.45)	4.4 (3.2)	0.36
	Pre Release	4.55 (3.95)	5.7 (5.25)	0.46
	Post Release	6.85 (4.48)	6.9 (3.2)	0.39
	End CPB	7.6 (2.7)	10.6 (5.1)	0.18
	1Hr Post-Op	8.65 (3.88)	11.55 (6.23)	0.16
Lymphocytes (x10^9/L)	Pre-Op	1.6 (0.65)	1.6 (0.5)	0.75
	Pre Release	1.2 (0.8)	1.25 (0.7)	0.7
	Post Release	1.6 (0.7)	1.6 (0.9)	0.4
	End CPB	1.6 (0.7)	1.6 (0.8)	0.25
	1Hr Post-Op	1 (0.6)	1.55 (0.73)	0.02
Monocytes (x10^9/L)	Pre-Op	0.5 (0.4)	0.5 (0.2)	1
	Pre Release	0.25 (0.23)	0.3 (0.25)	0.49
	Post Release	0.3 (0.25)	0.3 (0.25)	0.83
	End CPB	0.4 (0.4)	0.4 (0.2)	0.66
	1Hr Post-Op	0.3 (0.3)	0.4 (0.25)	0.2

Table 7. White Blood Cell Differential. WBC; white blood cell. 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. Data presented as median (IQR). A *p* value ≤0.05 was considered significant.

	Time	Admiral	RemoweLL	*p*
CD11b (MFC)	Pre-op	99 (40.8)	106.5 (37.5)	0.53
	CPB end	230.5 (126.5)	193.5 (53)	0.8
	1hr Post-op	154 (58)	169.5 (45.3)	0.68
	24hr Post-op	131 (40.5)	120.5 (63)	0.37

Table 8. Perioperative Neutrophil Activation. MFC; Mean fluorescence channel. Activation of neutrophil adhesion molecule CD11b used as an indicator of neutrophil activation as measured by FACs analysis (see Section 3.3). Samples taken pre-CPB, 5 minutes before the end of CPB and 1 and 24 hours post-CPB. Data presented as median (IQR). A *p* value ≤0.05 was considered significant.

The number of monocytes dropped significantly in both groups upon commencing bypass [Admiral 0.5 (0.4) x10^9/L vs. 0.25 (0.23) x10^9/L; *p*=0.004, RemoweLL 0.5 (0.2) x10^9/L vs. 0.3 (0.25) x10^9/L; *p*=0.005]. Post CPB the numbers of monocytes had recovered in the RemoweLL group [0.5 (0.2) x10^9/L vs. 0.4 (0.25) x10^9/L; *p*=0.15] but remained significantly suppressed in the Admiral group [0.5 (0.4) x10^9/L vs. 0.3 (0.3) x10^9/L; *p*=0.02; Appendix B – Leucocyte Differentials and FACS Data].

C-reactive protein showed no statistically significant differences between the 2 groups at any time point. There was a statistically significant rise in both groups at the 24-hour post CPB time point, but no differences between groups [Admiral 3 (3.75) vs. 122 (35.3); *p*<0.001, RemoweLL 2 (3) vs. 106.5 (29.3); *p*<0.001, Admiral vs. RemoweLL *p*=0.1]. Data shown in Appendix B – Leucocyte Differentials and FACS Data.

Neutrophil activation as measured by CD11b showed a significant increase in both groups (**Error! Reference source not found.**), 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) MFC vs. RemoweLL 193.5 (53) MFC; p=0.8; Figure 14]. 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 were not significant compared to baseline in the RemoweLL group [106.5 (37.5) MFC vs. 120.5 (63) MFC; p=0.24] but remained statistically elevated in the Admiral group [99 (40.75) MFC vs. 131 (40.5) MFC; p=0.016]. A patient example FACs plot is shown in Appendix B.

6.4. **Discussion**

The initial filtration step of the RemoweLL filter is designed to remove activated leucocytes. It is believed that activated leucocytes play a major role in the formation of 'post-pump' syndrome which can affect lungs, kidneys and the brain (Skrabal, Khosravi et al. 2006). Our results did not show any white cell reduction compared to the control group, in fact there was a tendency for the RemoweLL group to have greater numbers of leucocytes one-hour post CPB [*p*=0.06]. This is in line with the general consensus in studies that have investigated leucocyte-depleting filters that there is no effect on total or activated leucocytes following CPB (Chen, Tsai et al. 2002). Work by Lako and colleagues in patients undergoing CPB without leucocyte filtration have shown that leucocytes numbers peak on postoperative day 2 (Lako, Dedej et al. 2015). They observed a 128% increase in leucocytes compared with the 78% and 94% rise seen in the Admiral and RemoweLL groups respectively.

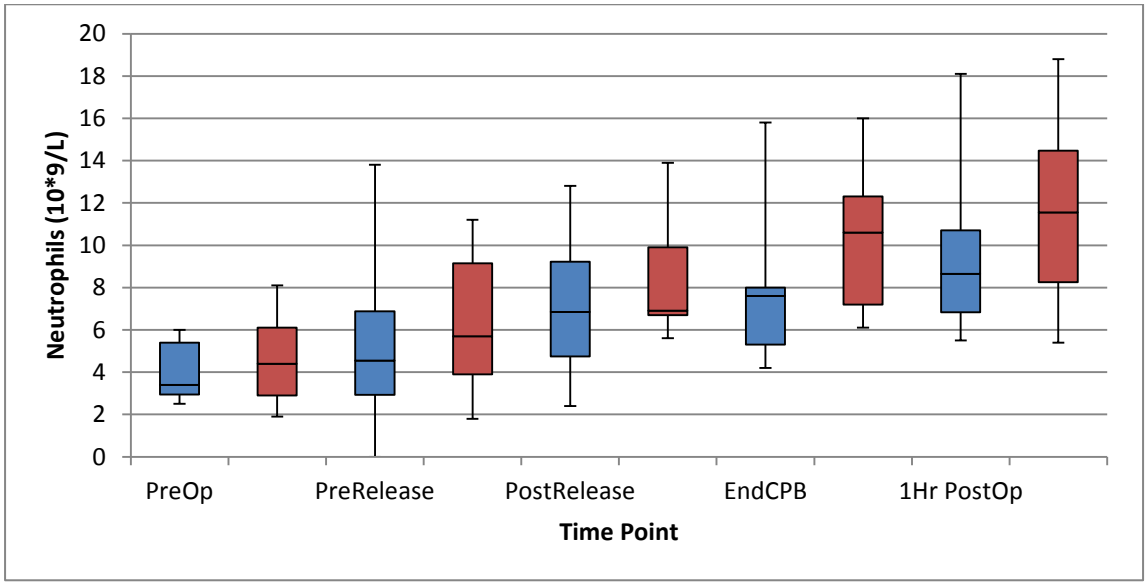


Figure 13. Neutrophil count during cardiopulmonary bypass. Samples taken pre-CPB, pre PSB release into the systemic circulation, post-release of PSB into the systemic circulation, 5 minutes before the end of CPB and 1 hour post-CPB. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values.

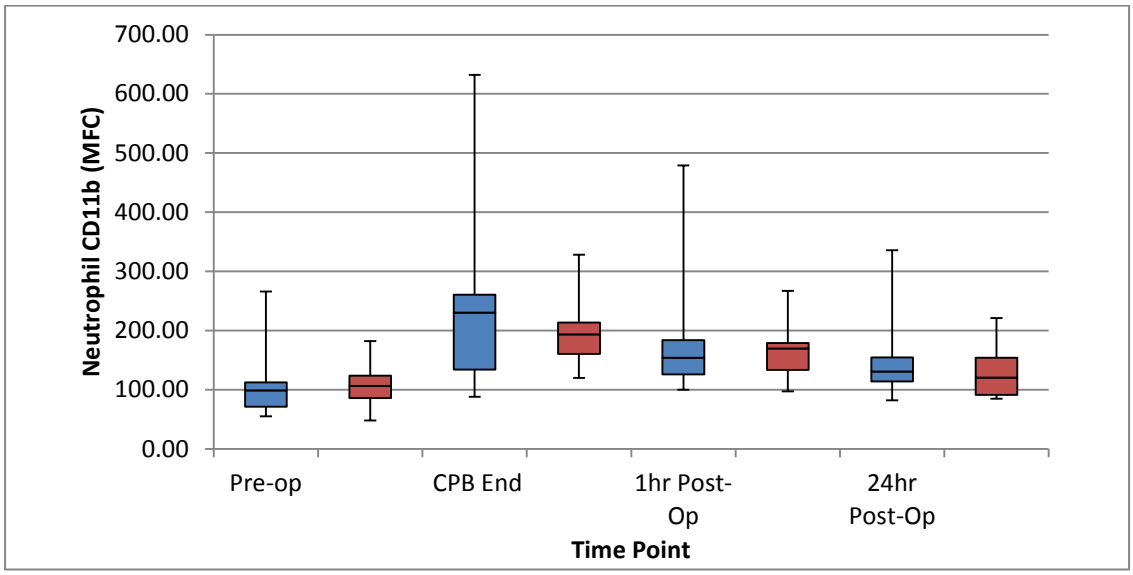


Figure 14. Neutrophil activation profile. MFC; Mean fluorescence channel. Activation of neutrophil adhesion molecule CD11b used as an indicator of neutrophil activation as measured by FACs analysis (see Section 3.3). Samples taken pre-CPB, 5 minutes before the end of CPB and 1 And 24 hours post-CPB. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values.

However, it should be noted that this was in the immediate post-op period and not at post-op day 2. Leucocyte differential data showed that the number of neutrophils rose significantly in both groups compared to baseline [3.4 (2.45) $x10^9$/L vs. 8.65 (3.88) $x10^9$/L p<0.001 Admiral; 4.4 (3.2) $x10^9$/L vs. 11.6 (6.23) $x10^9$/L p<0.001 RemoweLL]. The Lako group also observed an increase in neutrophil numbers, up 127% on post-op day 2. In the Admiral group, there was an increase of 154% and 164% in the RemoweLL group. However it should be noted that the Lako study further differentiated the leucocyte groups, whereas this study did not, therefore when these results are taken into account, the percentage increase observed was 198% (Lako, Dedej et al. 2015). This is in contrast to previous studies, which used leucocyte-depleting filers and have shown a decrease in neutrophil concentration. In particular, Dell'Amore and colleagues showed a decrease in neutrophil concentration from 76.2$x10^3$/mL to 69.7$x10^3$/mL using the same RemoweLL system (Dell'Amore, Tripodi et al. 2010). However, the 25–75 Percentiles were 56-78$x10^3$/mL in the control group and 55-77$x10^3$/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 taken in this study were from the cardiotomy reservoir, and not the systemic circulation (discussed further below). Other studies have shown consistent removal of activated leucocytes, but this is in studies that have placed the leucocyte depleting filters within the arterial limb of the CPB circuitry; i.e. all of the circulating volume is passing through the filter and so it is exposed to numerous passes of leucocyte containing blood (Alexiou, Tang et al. 2004). Santa Ursula Tolosa *et al*., (1991), previously reported a 'neutrophilic leucocytosis' after cardiac surgery which persists for 2-3 days. This is associated with immature forms of neutrophils being released from the bone marrow. Leucocytosis represents the acute response to stress, the mobilisation of marginalised neutrophils and the release of new neutrophils from the bone marrow and pulmonary circulation (Santa Ursula Tolosa, Criado et al. 1991). In the Lako study, 90.2% of all patients exhibited a neutrophil leucocytosis (Lako, Dedej et al. 2015). A common observation following cardiac surgery is the acute reduction in circulating lymphocytes. In the Admiral group, a 37.5% reduction was noted between induction and during CPB, whilst the RemoweLL only showed a 3% reduction. It should be noted though that there was a tendency for a higher pre-operative lymphocyte count in the RemoweLL, which may affect the interpretation of this result. Work by Lako *et al*., showed a 52% decrease on post op day 1. It is difficult to comment on this difference as the Lako group did not test perioperatively so potential lymphocyte numbers in this study may have dropped further during the immediate postoperative period (Lako, Dedej et al. 2015). Rinder *et al*., have suggested that the loss of lymphocytes might be due to numerous factors including haemodilution, redistribution of cells to the tissues, loss due to bleeding and adhesion to CPB circuitry (Rinder, Bonan et al. 1992). Further work by the group proposed that cellular activation causes adhesion

activation of lymphocytes and endothelial cells promoting exit from the circulation (van Kooyk and Figdor 1993, Rinder, Mathew et al. 1997).

This study also noticed a significant decrease in monocytes from induction to during CPB. Rinder *et al*., has previously shown that monocytes have increased expression of activation-dependent adhesion receptors immediately post-CPB (Rinder, Mathew et al. 1997). In contrast to this study, they report observing an increase in monocytes to almost double baseline levels. However, this was on the 1st 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 drop 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]. Rinder *et al*., focussed on the expression of the MHC class II surface receptor, Human Leucocyte Antigen – D Related (HLA-DR) on monocytes which constitutes a ligand for T-cell receptors and is known to be responsible for initial graft rejection in HLA-mismatched donors (Rinder, Mathew et al. 1997). They saw a decrease of 67% in HLA-DR expression at day 1, which remained depressed (47%) on day 3. Work by Polk *et al*., has previously shown that reduced HLA-DR expression correlates to major post-op bacterial and fatal infections (Polk, George et al. 1986). 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 (Clarkson and Ory 1988).

In order to assess the level of neutrophil activation that occurred during the procedure CD11b, a surface adhesion molecule that regulates the adhesion of activated leucocytes, was measured using FACs analysis. The CD11b integrin is a key factor in allowing the transendothelial migration of neutrophils through the modulation of endothelial cytoskeleton. When inappropriate activation occurs, this is a primary cause of tissue oedema (Vestweber 2000). Of particular interest to this study is the observation of Mastrangelo *et al*., who observed a direct interaction between the CD11b integrin and oleic acid, the major fatty acid component of bone marrow derived fat emboli (Mastrangelo, Jeitner et al. 1998). This phenomenon, they reported, caused the neutrophil to aggregate and adhere with CD11b/CD18 to endothelial membranes. Moreover, previous work by the group demonstrated that free-fatty acids caused and amplified the mobilisation of myeloperoxidase-containing granules within the neutrophil (Qian and Eaton 1994). Previous studies have demonstrated reduced expression of CD11b in patients undergoing CABG surgery with continuous arterial line leucocyte depletion (Hurst, Johnson et al. 1997, Chen, Tsai et al. 2002). In contrast to inline leucocyte filtration, this study showed no statistical difference in peak CD11b expression between the Admiral and RemoweLL groups [Admiral 230.5 (126.5) MFC vs. RemoweLL 193.5 (53) MFC; p=0.8]. However, overall there was reduced activation in the

RemoweLL group, with a significant decrease between the 1-hour post-op and 24 hour post-op time points [169.5 (45.3) MFC vs. 120.5 (63) MFC; *p*=0.04]. The Admiral group also saw a decrease but this was not significant [154 (58) MFC vs. 131 (40.5) MFC; *p*=0.43]. The 24-hour post CPB samples returned to baseline in the RemoweLL group [106.5 (37.5) MFC vs. 120.5 (63) MFC; *p*=0.24] whilst the Admiral group retained an elevation that proved significant compared to baseline [99 (40.75) MFC vs. 131 (40.5) MFC; *p*=0.016]. There are a number of factors that might explain these observations. Firstly, the majority of research published on leucocyte filtration uses continuous inline filtration; the filter is placed directly into the arterial line of the CPB circuit. In this situation there will be continuous circulation of systemic blood through the circuit and the patient; i.e. there is more exposure to not only the artificial surfaces of the CPB circuit, but to pro-inflammatory cytokines and damaged cell membranes resulting in continuous activation of circulating leucocytes. In contrast, the filtration step in the RemoweLL is isolated to PSB in the cardiotomy suction. Therefore, not only is less volume being filtered (and thus fewer leucocytes), but the systemic circulation continues to pass unfiltered into the patient. As the post-filtration measurement was obtained 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 activation would be similar in both groups as this displays systemic activation. Whether there is a different level of activation in the cardiotomy suction with the RemoweLL compared to the Admiral group has not been established. However, one might argue that as this appears to bear no significance to overall peak leucocyte activation, it is of little importance, especially given evidence that global leucocyte activation is a risk factor for ischaemic events one year post-cardiac surgery (Rashidi, Jamshidi et al. 2012). Conversely, the quicker reduction in activation in the RemoweLL group is intriguing. As the 2nd time point (End CPB) would have been shortly after the release of cardiotomy 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, which has been shown to be a potent activator of neutrophil CD11b expression (Mastrangelo, Jeitner et al. 1998). However, there appears to have been little, if any research on the length of action of oleic acid on neutrophils. Mastrangelo *et al*., saw increasing expression of CD11b with time following the addition of oleic acid to neutrophils, 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, neutrophil activation does not occur solely from oleic acid interactions; exposure to artificial surfaces, anaesthesia, even the surgery itself can cause neutrophil activation (Matheis, Scholz et al. 2001). Ultimately, given the sample size of this study, it may be underpowered to detect differences of this size, and any conclusions based upon such results should be interpreted with caution.

This study has not been able to demonstrate any reduction in leucocyte activation by the RemoweLL oxygenation system. Furthermore, in confirmation of other studies, this had no impact on postoperative leucocyte counts.

7. Cerebral Function

7.1. **Introduction**

Studies have observed postoperative cognitive and intellectual dysfunction in almost 50% of patients when examined by neuropsychological tests (Murkin 2000). Much of this has been attributed to the presence of LME from the surgical environment, passed through the CPB circuit into the aortic arch and onto the cerebral vessels. These particles have been observed as SCADs at the bifurcations in cerebral vessels (Moody, Bell et al. 1990, Moody, Brown et al. 1995, Brooker, Brown et al. 1998). However, due to the lack of clinically applicable LME removal methods, a direct causal link has yet to be made. The aim was to establish if a new lipid filtration system (RemoweLL) could attenuate the release of a neurological marker, Neuron Specific Enolase (NSE), against a normal ECC system with a 40µM cardiotomy filter (Admiral) and provide evidence of a direct link between LME and cerebral injury/decreased function.

7.2. **Methods**

Patients and analytical methods are described in Section 3.4.

Descriptive statistics showed positive skewness that was confirmed as non-normal distribution using the Shapiro-Wilk test. Log transformed data showed normal distribution, therefore two factor ANOVA for repeated measures was used with differences in peak concentration analysed using the TTest for two independent samples. NSE and post-CPB LME data were tested for correlation and regression using Pearson's product moment of correlation coefficient (*r*) to determine the relationship between the two parameters.

7.3. **Results**

Two-factor ANOVA revealed a significant interaction between groups and NSE release (p=0.002). There were no differences between groups at baseline and NSE release peaked in both groups at the end of CPB with significantly lower concentrations in the RemoweLL group [Admiral 23 (6.5) µg/L vs. RemoweLL 16 (7) µg/L; p=0.013; Table 9]. Subsequent reductions were seen towards baseline in both groups, although those patients in the Admiral group continued to show elevated NSE levels compared to those in the RemoweLL group [p=0.01 6hr post-CPB; p=0.005 24hr post-CPB; Figure 15]. Compared to baseline values both groups remained statistically elevated at 24hr post-CPB [Admiral 10 (3.5) µg/L vs. 14 (4) µg/L, p=0.003; RemoweLL 10 (1) µg/L vs. 11 (1.5) µg/L, p=0.03].

	Time	Admiral	RemoweLL	*p*
NSE (µg/L)	Pre-Op	10 (3.5)	10 (1)	0.32
	End of CPB	23 (6.5)	16 (7)	0.01
	6hr Post-op	18 (6)	14 (4.5)	0.01
	24hr Post-op	14 (4)	11 (1.5)	0.004

Table 9. Neuron Specific Enolase Release. NSE; neuron specific enolase. Samples taken pre-CPB, 5 minutes before end-CPB and 6 and 24 hours post-CPB. Data are presented as median (IQR). A *p* value ≤0.05 was considered significant.

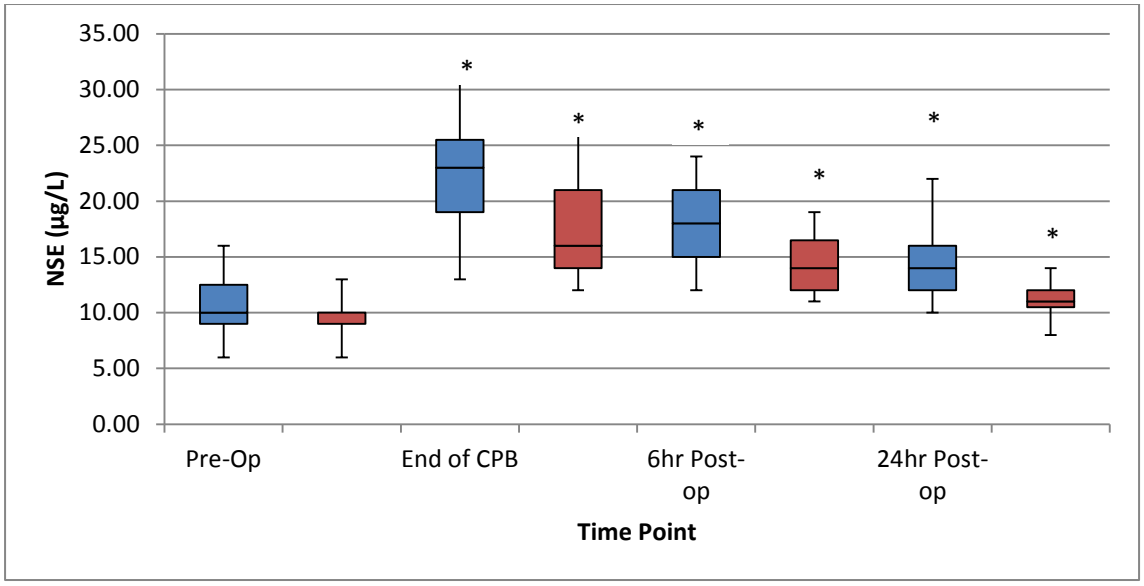


Figure 15. Neuron Specific Enolase changes. NSE; neuron specific enolase. Samples taken pre-CPB, 5 minutes before end-CPB and 6 and 24 hours post-CPB. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values. *$p \leq 0.05$

Analysis of correlation between NSE and post-CPB LME data showed that at the post-CPB, 6hr post-CPB and 24hr post-CPB time points, there was a significant positive correlation between NSE release and the number of LME observed [r=0.42, 0.41 and 0.4 respectively]. Further regression analysis showed a significant positive relationship between the two variables at each of the time points [p=0.02, 0.02 and 0.03 respectively; Figure 16].

7.4. **Discussion**

Due to the position of the aortic cannula, arterialised blood from the CPB circuit is directed towards the head and neck vessels, which might explain why patients undergoing open heart procedures are believed to be most at risk of embolization from vegetations, LME and gaseous emboli (Wolman 1999). This study focussed on CABG patients in order to better separate the effects of lipid vs. gaseous emboli. Despite advances in Perfusion technology, current estimates of neurological dysfunction following CPB indicate that >50% of patients have neuropsychological deficits during the first week after surgery, 10-30% have long-term or permanent deficits and 1-5% experience severe disability or die (Brown, Moody et al. 2000). Current CPB circuitry does not prevent the passage of LME from the cardiotomy suction and into the patient's circulating volume and previous work has shown the distribution of LME throughout the major organs (Brondén, Dencker et al. 2006). Of particular concern are the possible effects upon neurological function that LME pose; thousands of microemboli have been observed distributed throughout the brain (Moody, Brown et al. 1995). Many studies have failed to provide a correlation between lipids and neurological function for 2 reasons. Firstly, a number of the detection techniques used are inadequate for LME detection (e.g. TCD does not allow detection of particles under 40µm or the distinction between gaseous and particulate emboli) and secondly, there has never previously been a control cohort in order to legislate for other factors (Issitt, Crook et al. 2015). The aetiology of neurocognitive dysfunction is highly complex, multifactorial and the major mechanisms behind it are yet to be elucidated (Rasmussen, Christiansen et al. 2000). Furthermore, imaging techniques such as Computerised Tomography (CT) and Magnetic Resonance Imaging (MRI) have failed to show clear evidence of infarction or neuron loss in a substantial proportion of patients (Muraoka, Yokota et al. 1981, Sellman, Hindmarsh et al. 1992, Harris, Bailey et al. 1993).

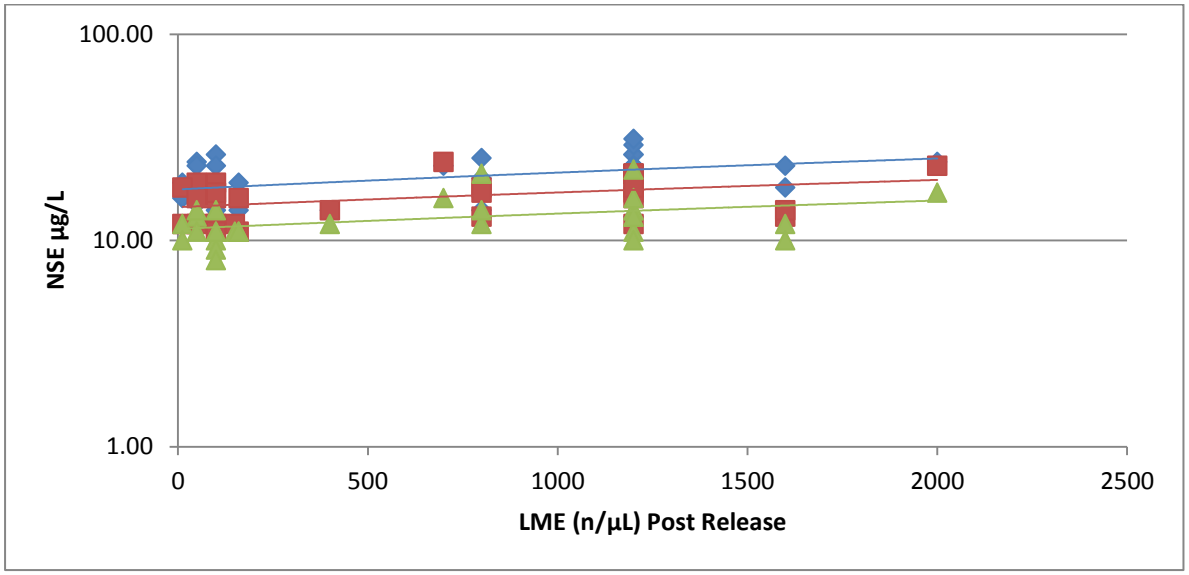


Figure 16. Neuron Specific Enolase vs. Post Filtration Lipid Microemboli Correlations. NSE; neuron specific enolase, LME; lipid microemboli. Data analysed using Pearson's correlation coefficient. Blue; End-CPB NSE level. Red; 6 Hour post-CPB NSE level. Green; 24 Hour post-CPB level.

Neuron specific enolase (NSE) was chosen as a surrogate marker of neurological function as serum concentrations exhibit a significant association with postoperative neurocognitive outcome (Ramlawi, Rudolph et al. 2006) whereas other markers such as S100β have shown non-specificity and an inability to correlate with neurological or neuropsychological outcome (Westaby, Saatvedt et al. 2000, Whitaker, Green et al. 2007). Damage to the neuron cell membrane results in leakage of cellular proteins into the blood and cerebrospinal fluid where they may be detected. NSE is unique to neurons and neuroendocrine cells, but is observed in blood following cerebral infarction, head trauma, cardiac arrest and more recently cardiac surgery (Cunningham, Young et al. 1991, Dauberschmidt, Zinsmeyer et al. 1991, Isgro, Schmidt et al. 1997). Rasmussen *et al*., found that there was a significant correlation between the increase in NSE following CPB and the change in cognitive function at the time of discharge (Rasmussen, Christiansen et al. 2000). They noted that patients with neurocognitive dysfunction had a significantly elevated mean NSE level [4.9 µg/L higher] than those that did not at the point of discharge, and 3µg/L higher in patients with neurocognitive dysfunction 3 months post-surgery (although this did not reach significance). However, further work by the authors speculated that this might be due to insufficient sample size to detect differences of this magnitude (Rasmussen, Christiansen et al. 2002). This study observed a peak reduction in NSE release between Admiral and RemoweLL groups at the end of CPB [Admiral 23 (6.5)µg/L vs. RemoweLL 16 (7)µg/L; p=0.013], and further significant differences at both the 6 and 24 hours post CPB sample times [Admiral 18 (6)µg/L and 14 (4)µg/L vs. RemoweLL 14 (4.5)µg/L and 11 (1.5)µg/L; p= 0.01 and 0.005 respectively]. This is the first study to show a difference between groups of patients that have had LME filtered and those undergoing standard CPB. Whilst it wouldn't be prudent to extrapolate these results to long-term neurological outcome, the results are suggestive that further work would be warranted. It should be highlighted that these results are in patients whose surgical intervention poses a lower risk of other embolic events i.e. the patients were not having valve repairs/replacements and therefore the cardiac cavities are kept intact and not opened to atmosphere. Furthermore, it could be speculated that those patients with a previous history of Transient Ischaemic Attack (TIA) and Cerebrovascular Accident (CVA), who might be at higher risk of neurological insult would benefit more from this technology. It is interesting to note that the rise in NSE observed in this study was significantly higher than in the study by Bonacchi *et al*. (Bonacchi, Prifti et al. 2006). They reported peaks of 17.7±6.5µg/L (mean±SD, n=42) with and IQR (9.8-25) in the CPB group, which is similar to the peak concentrations seen in the RemoweLL group [16 (7)µg/L] but much lower than those in the Admiral group [23 (6.5)µg/L]. However, there are 2 explanations for this observation. Firstly, the group of patients in Bonacchi's work were younger than those within this study [Bonacchi range 52-67 years, Admiral 57-85 years, RemoweLL 59-82 years). Previous data from Nygaard *et al*., have shown a

clear progression in increasing NSE concentrations with age from 24-84 years, therefore a higher overall concentration in a more elderly group is to be expected (Nygaard, Langbakk et al. 1998). Secondly, and more importantly, the CPB group of Bonacchi *et al*., did not have cardiotomy blood returned to them. The rationale was that the study was investigating the use of S100β which has been reported to be unspecific (discussed in section 1.3). This inadvertent observation from Bonacchi provides a further control for this study; the magnitude of NSE increase seen in the RemoweLL group is equivalent to discarding the PSB. As discussed earlier in Chapter 2, however, there are major drawbacks in the discarding of PSB, not least is the increase in blood transfusion requirements and increase in postoperative haemorrhage (Rubens, Boodhwani et al. 2007). Whilst this study was not designed to evaluate this, or blood product usage, one may confidently infer that using the RemoweLL system reduces the release of neurological injury markers to concentrations seen when cardiotomy suction is not used, but without the associated risks to haemostatic integrity. When one considers the volume of cardiotomy suction routinely seen in cardiac surgery [Admiral 776.67±632.14mL (mean±SD, n=15); RemoweLL 780.00±567.20mL (mean±SD, n=15)] the benefits to being able to reinfuse this volume of autologous blood back to the patient are obvious.

A major finding of the present study is the direct correlation between the degree of NSE release at each of the post-op time points and the number of LME post reinfusion of PSB. Previous work by the group at Wake Forest University, North Carolina has shown that the number of SCADs are proportional to the time spent on CPB, with each 1-hour increase correlating to a 90.5% increase in SCAD numbers observed in the brain sections of patients that had died within 3 weeks of cardiac surgery with CPB (Brown, Moody et al. 2000). The numbers of LME and peak concentrations of NSE observed in this study showed no correlation with the time spent on CPB, which might suggest conflicting evidence to that of Brown *et al*. However, there may be 2 reasons for this. Firstly, the length of CPB investigated in the study by Brown was significantly longer than this study (mean 172mins range 82-278mins vs. mean 95mins range 49-140mins). As previously discussed in section 5.4 the longer sternal bone marrow is left exposed (a function of the time taken to complete the surgery; therefore CPB time), the greater the lipid release. Brown looked only at the numbers of SCADs and did not report circulating numbers of LME or LME present in other organs such as the kidneys (discussed in the following section). Therefore Brown's study population may simply have had far greater numbers of LME present. Secondly, Brown studied those patients that died within 3 weeks following surgery, who are therefore more likely to have had more extensive cerebral insults. None of the patients in this study died. Furthermore, it is difficult to reconcile the two studies in terms of neurological data as a physical relationship between SCADs and NSE has not been demonstrated, so such supposition is purely theoretical. One would expect NSE to show a more

global response to LME as many cerebral membranes might be compromised in some form due to small SCADs, but more severe insults will depend on the areas affected and how densely vascularised they are (discussed in section 1.6.1). Ultimately, the number of patients may be too small and the population too homogeneous in this study to determine a link between LME release and CPB time.

Critically, this study reports a methodology that can be used to remove LME and which has provided the first biochemical basis to demonstrate a significant direct link between LME and neurological dysfunction.

8. Renal Function

8.1. **Introduction**

Brondén and colleagues have demonstrated a high concentration of LME in the kidneys following the transfusion of shed mediastinal blood containing lipid microparticles (Brondén, Dencker et al. 2006). It is believed that LME may therefore be pivotal in the postoperative renal dysfunction observed after cardiac surgery with CPB. Acute kidney injury is often defined in terms of serum creatinine increase, although its testing is subject to a number of flaws. Cystatin C is a well-documented marker of renal injury as it is exclusively excreted by the kidneys and is highly correlated to glomerular filtration. As yet there are no data providing a link between LME and renal dysfunction due to the variability of current methods of LME removal. The aim was to establish if a new lipid filtration system (RemoweLL) could attenuate the release of Cystatin C and lead to smaller increases in serum creatinine, against a normal ECC system with a 40µM cardiotomy filter (Admiral).

8.2. **Methods**

Patients and analytical methods are described in Section 3.6

Descriptive statistics showed a positive skewness to Cystatin C and serum creatinine data, which was confirmed as non-normal distribution using the Shapiro-Wilk test. Log transformed data showed normal distribution, therefore two factor ANOVA for repeated measures was used with differences in peak concentration analysed using the TTest for two independent samples. Serum creatinine increases and changes in glomerular filtration rates were analysed after subtraction of baseline values and reported according to the RIFLE criteria. Serum sodium and potassium showed normal distribution, whilst urea, creatinine were non-normally distributed but were log transformed and therefore all analysed using two factor ANOVA. Urinary osmolarity was normally distributed whilst creatinine was log transformed to give normalised distribution, therefore two factor ANOVA was used. Urea, potassium, sodium and albumin:creatinine ratio were all non-normally distributed and so analysis using the Mann-Whitney test for two independent samples was used.

8.3. **Results**

Baseline concentrations of Cystatin C were greater in the Admiral group compared to the RemoweLL group although this was not significant [Admiral 1.14 (0.49) mg/L vs. RemoweLL 0.96 (0.22) mg/L; p=0.11; Table 10]. Two factor ANOVA revealed an association towards interaction but this failed to reach significance [p=0.06].

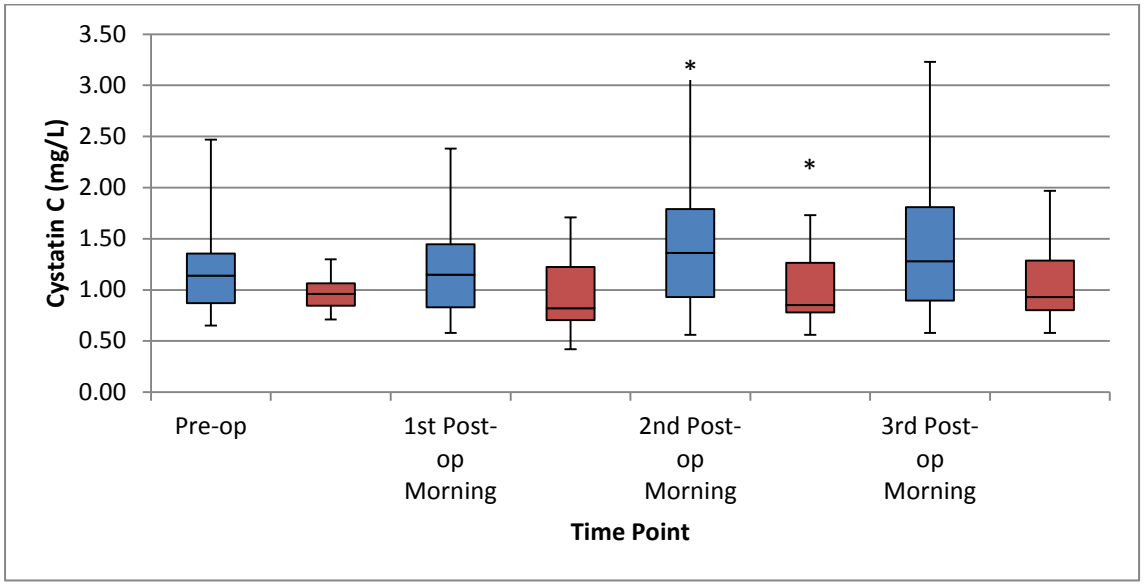


Figure 17. Cystatin C changes. Samples taken pre-CPB, 1st, 2nd and 3rd postoperative mornings. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values. *$p \leq 0.05$.

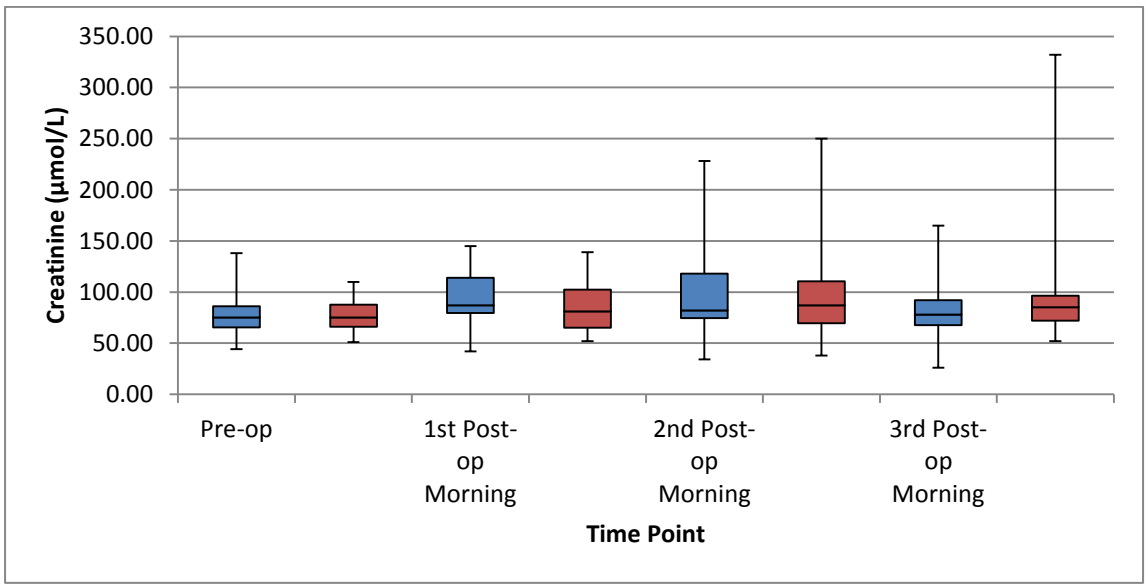


Figure 18. Serum Creatinine Concentrations. Samples taken pre-CPB, 1st, 2nd and 3rd postoperative mornings. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values.

Analysis of peak concentrations demonstrated significantly less Cystatin C in the RemoweLL group on the 2nd postoperative morning [Admiral 1.36 (0.86) mg/L vs. RemoweLL 0.85 (0.49); *p*=0.04]. The subsequent postoperative morning showed no difference between the two groups [*p*=0.08] with both groups returning to baseline values (Figure 17). There were no differences throughout the study period in serum creatinine concentrations [ANOVA *p*=0.35]. Analysis of serum creatinine increases and reduction in GFR (according to RIFLE criteria) showed 8 patients in total that suffered some form of AKI. Four patients (26% - 1 in Admiral Group, 3 in RemoweLL group) were classed as at "Risk" whilst one was defined as having sustained "Injury" (1 in RemoweLL group). Three further patients (all Admiral group) were classed as having renal "Failure". Serum creatinine concentrations were analysed using log transformed TTests and presented no significance at any time points [*p* range 0.41-0.66; Table 11, Figure 18]. Stepwise analysis of GFR reported no differences at any time point [*p* range 0.61-1;
Figure 19]. There were no differences in any of the serum or urinary electrolytes (Table 12 and Appendix C – Renal Profiles).

8.1. **Discussion**

Acute renal injury affects approximately 1-5% of patients undergoing cardiac surgery and is a major cause of morbidity and mortality (Abu-Omar, Mussa et al. 2005). Whilst there are many factors indicated in its causation, such as perioperative renal hypoperfusion and the presence of endogenous and exogenous nephrotoxins (such as free radicals, anaesthetic agents etc.) which result in glomerular and tubular injury, there is evidence that suggests LME might facilitate ischaemic damage rather than altered blood flow profiles (Abu-Omar, Mussa et al. 2005). During CPB the synergy of blood pressure and flow are uncoupled; the output of the heart-lung machine provides the flow whilst pharmacological agents will adjust the vascular tone and therefore the pressure, especially if the mean blood pressure is under 50mmHg (where pressure autoregulation is not possible). Brondén *et al*., observed an extremely high uptake of a radioactive tritium-labelled triolein shed blood phantom (to replicate LME) into the kidneys of pigs (Brondén, Dencker et al. 2008). When one considers the highly vascularised nature of the kidneys, and the double capillary network of the glomeruli and tubuli, and high blood flow to the organs, it is highly suggestive that LME may contribute to renal complications postoperatively.

	Time	Admiral	RemoweLL	*p*
Cystatin C (mg/L)	Pre-op	1.14 (0.49)	0.96 (0.22)	0.1
	1st Post-op Morning	1.15 (0.62)	0.82 (0.52)	0.12
	2nd Post-op Morning	1.36 (0.86)	0.85 (0.49)	0.04
	3rd Post-op Morning	1.28 (0.92)	0.93 (0.49)	0.08

Table 10. Cystatin C Release. Samples taken pre-CPB, 1st, 2nd and 3rd postoperative mornings. Data are presented as median (IQR). A p value ≤0.05 was considered significant.

	Time	Admiral	RemoweLL	*p*
Sodium (mmol/L)	Pre	137.33±2.35	137.6±2.06	0.74
	Day 1	137.2±3.78	136.53±3.66	0.63
	Day 2	135.53±4.29	134.27±3.15	0.36
	Day 3	136±2.93	133.67±3.66	0.06
Potassium (mmol/L)	Pre	3.88±0.36	4.31±0.64	0.03
	Day 1	4.57±0.5	4.5±0.34	0.68
	Day 2	4.43±0.51	4.37±0.3	0.74
	Day 3	4.41±0.23	4.29±0.41	0.33
Urea (mmol/L)	Pre	5.5 (2.2)	5.4 (1.5)	0.9
	Day 1	6 (3.6)	5.2 (2.1)	0.22
	Day 2	5.8 (5.8)	5.7 (3.5)	0.61
	Day 3	6.5 (4.9)	6.3 (3.8)	0.88
Creatinine (µmol/L)	Pre	75 (20.5)	75 (21.5)	0.67
	Day 1	87 (34.5)	81 (37.5)	0.43
	Day 2	82 (43.5)	87 (41)	0.83
	Day 3	78 (24.5)	85 (24.5)	0.41
Estimated GFR (mL/min/1.73m2)	Pre	89 (13.5)	89 (19)	0.91
	Day 1	76 (33)	79 (30)	0.61
	Day 2	82 (38)	72 (30)	0.67
	Day 3	85 (32)	81 (24)	1

Table 11. Serum Electrolytes. Samples taken pre-CPB, 1st, 2nd and 3rd postoperative mornings. Data are presented as median (IQR) or mean±standard deviation. A p value ≤0.05 was considered significant.

Two mechanisms have been proposed that LME might act through to facilitate renal dysfunction. The first is a mechanical obstruction; Appelblad and colleagues demonstrated the ability of mediastinal fat to impair capillary-pore blood flow (Appelblad and Engström 2002). The second is a possible toxic effect; oleic acid (the major component of LME) is a known initiator of neutrophil activation that induces ARDS-type clinical symptoms in animal models (Grotjohan, van der Heijde et al. 1993) whilst free fatty acids and triglycerides have toxic properties, as demonstrated in a feline model where charged oleic acid caused cytotoxic cerebral oedema (Kim, Lee et al. 2002). This implies that lipid material cannot only cause mechanical obstruction but chemical interactions may also play a negative role in the capillaries of the organs.

There are several methods of measuring renal function, Cystatin C was chosen as a surrogate marker of renal function as it is exclusively excreted via glomerular filtration and has shown good correlation with glomerular filtration rate, without the meticulous collection of urine, and is more specific and less susceptible to methodological interference than serum creatinine testing. Furthermore, its use has been validated in CABG patients (Abu-Omar, Mussa et al. 2005). The Acute Kidney Injury Network defines AKI as an abrupt increase in absolute serum creatinine ≥3mg/dL (26.4μmol/L) or a percentage increase greater than 50% (1.5 fold from baseline). Therefore in this study we used both Cystatin C and increases in serum creatinine to investigate renal dysfunction during and in the post-CPB period.

Baseline measurements showed similar concentrations of serum Cystatin C in both groups [p=0.11], with a significant increase occurring at the second postoperative morning. There was an association towards significance [p=0.06] between the groups overall but this failed to reach significance. No patient received any postoperative support for renal failure, which is reflected in the similarity in GFR and increases in serum creatinine between both groups. Normal serum Cystatin C is considered to be in the region of 0.6 to 1mg/L (Villa, Jimenez et al. 2005). Given that these patients whilst being otherwise relatively fit and healthy, are being treated for atherosclerosis, it is perhaps unsurprising that their Cystatin C concentrations are on the upper limits of normal at baseline [Admiral 1.14 (0.49) vs. RemoweLL 0.96 (0.22) mg/L], as it is unlikely that the coronary arteries are the only vessels that exhibit signs of narrowing. The results reported here mimic those described in the study by Abu-Omar *et al*., who also demonstrated an increase in serum Cystatin C levels at postop day 2.

	Time	Admiral	RemoweLL	*p*
Albumin Urine (mg/L)	Pre	10.4 (14.5)	11 (11.5)	0.61
	Day 1	26 (18.5)	40 (11)	0.11
	Day 2	39 (40.5)	38.2 (45.5)	0.58
	Day 3	41 (38)	22 (11)	0.44
Creatinine (mmol/L)	Pre	7.4 (6.8)	11 (8.9)	0.38
	Day 1	9.9 (9.3)	10.5 (3.3)	0.84
	Day 2	9.4 (4.3)	9.5 (5.8)	0.57
	Day 3	6.1 (2.8)	4.5 (6.2)	0.69
Albumin/Creatinine Ratio (mg/mmol)	Pre	1.96 (1.84)	1.01 (1.71)	0.90
	Day 1	2.76 (2.33)	3.45 (1.43)	0.28
	Day 2	3.8 (3.47)	3.2 (3.15)	0.50
	Day 3	5.71 (4.63)	4.37 (3.73)	0.24
Creatinine Urine (mmol/L)	Pre	10 (8.8)	11 (8.8)	0.51
	Day 1	9.4 (5)	9.6 (3.2)	0.62
	Day 2	10 (10.8)	11.9 (5.7)	0.38
	Day 3	7.6 (2.6)	8.5 (2.4)	0.39
Potassium Urine (mmol/L)	Pre	41 (14)	52 (40)	0.28
	Day 1	76 (54)	104 (41)	0.59
	Day 2	73 (37)	54 (38)	0.20
	Day 3	49 (33)	33 (12)	0.21
Sodium Urine (mmol/L)	Pre	64 (46)	69 (47)	0.69
	Day 1	39 (56)	72 (61)	0.71
	Day 2	53 (52)	60 (42)	0.77
	Day 3	52 (23)	78 (33)	0.18
Osmolality Urine (mosm/kg H_2O)	Pre	474.13±162.5	475.93±201.1	0.98
	Day 1	617.25±259.52	665.33±100.66	0.51
	Day 2	531.33±138.27	562.53±176.96	0.59
	Day 3	487.93±148.41	526.93±120.28	0.44
Urea Urine (mmol/L)	Pre	64 (45.5)	69 (47)	0.93
	Day 1	39 (55.5)	72 (61)	0.70
	Day 2	53 (51.5)	60 (41.5)	0.43
	Day 3	52 (22.5)	78 (33)	0.20

Table 12. Urinary Electrolytes. Samples taken pre-CPB, 1st, 2nd and 3rd postoperative mornings. Data are presented as median (IQR) or mean±standard deviation. A p value ≤0.05 was considered significant.

As patients in both groups can be considered low risk, it is debatable whether any changes in their Cystatin C levels would be similar or relevant to those patients with preoperative renal dysfunction. However, it is known that those patients with chronic renal failure are more at risk of developing an AKI on top of their already diminished renal function; therefore it seems reasonable to suggest that the prevention of LME uptake in the renal vasculature may be of greater benefit in this particular cohort. It should be noted however, that as a smaller number of patients were entered into the trial (due to the interim analysis) than previously planned, one might question whether the study is powered to detect any significant differences. Post hoc analysis based upon peak Cystatin C concentrations reveals an effect size of 0.77 was achieved, therefore a patient cohort of 58 patients (29 per group) would be needed to achieve a power (1-β) of 0.8 with α=0.05. Therefore this study was underpowered to make significance claims on the ability of LME filtration to attenuate renal dysfunction.

This study has shown that there is a weak association towards improved Cystatin C removal, which is a surrogate marker of glomerular filtration and therefore renal function. Whilst this study was ultimately underpowered to claim any significant findings, there is data enough to warrant further investigation of this technology.

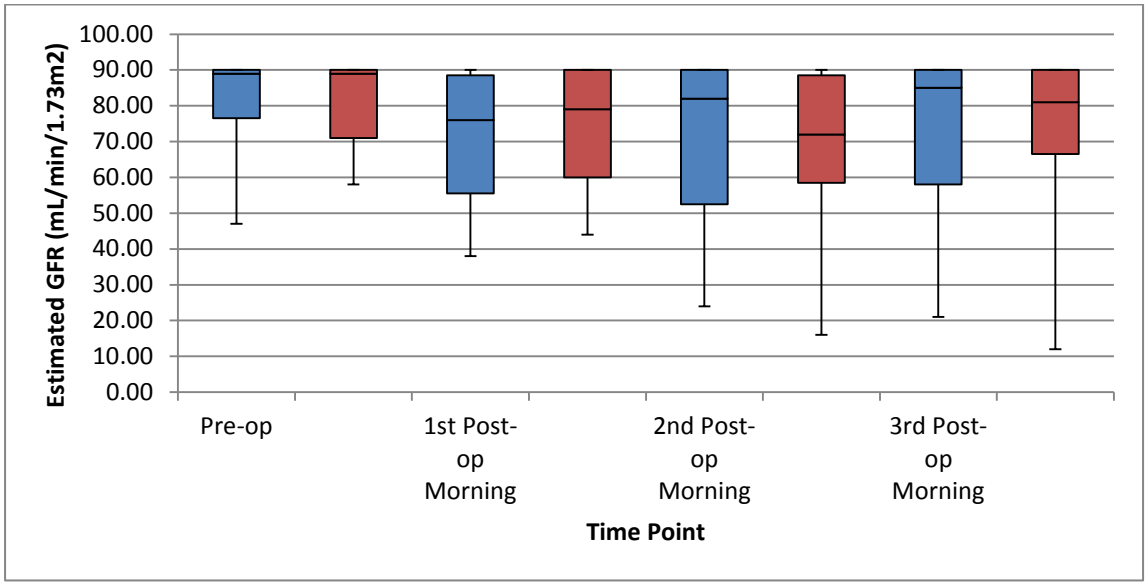


Figure 19. Glomerular Filtration Rate. Samples taken pre-CPB, 1st, 2nd and 3rd postoperative mornings. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values.

9. Pulmonary Dysfunction

9.1. **Introduction**

Pulmonary dysfunction is one of the most frequent complications associated with CPB (Taggart, el-Fiky et al. 1993), ranging from mild postoperative dyspnoea to acute respiratory distress syndrome (ARDS), which although only affecting 2% of patients, has a 50% mortality rate (Clark 2006). Dreyer and colleagues (Dreyer 1995) have previously reported that activated neutrophils may be responsible for pulmonary injury. As discussed in Section 6, oleic acid is known to activate neutrophils, through a direct interaction between the neutrophil CD11b integrin and oleic acid, therefore the filtration of LME and removal of activated leucocytes has the potential to ameliorate pulmonary dysfunction following CPB. Calculation of the Alveolar-Arterial Oxygenation Index (AaOI) is an established method for the assessment of peri-operative changes in lung function, which can be classified using the Berlin definition of ARDS, and is invariably elevated after cardiac surgery (Alexiou, Tang et al. 2004). The aim was to determine whether there were any differences in postoperative pulmonary function between those patients who had LME and activated leucocyte filtration (RemoweLL) and those that did not (Admiral).

9.2. **Methods**

Patients and analytical methods are described in Section 3.5.

Descriptive statistics showed a positive skewness for AaOI and pO_2 measurements. Non-normality was confirmed using Shapiro-Wilk. Log transformation revealed normally distributed data, confirmed using Shapiro-Wilk test. pCO_2 measurements showed normal distribution therefore this and log transformed data were tested with Two-Factor ANOVA with Repeated Measures. Ratios of F_iO_2/pO_2 were used to create ARDS categories as described in Section 3.5. These were analysed using the Chi Squared Test.

9.3. **Results**

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 20]. ANOVA results showed weak evidence for lower AaOI in the RemoweLL group although this did not reach significance [p=0.075; Table 13]. Further examination using pairwise analysis with TTest for 2 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].

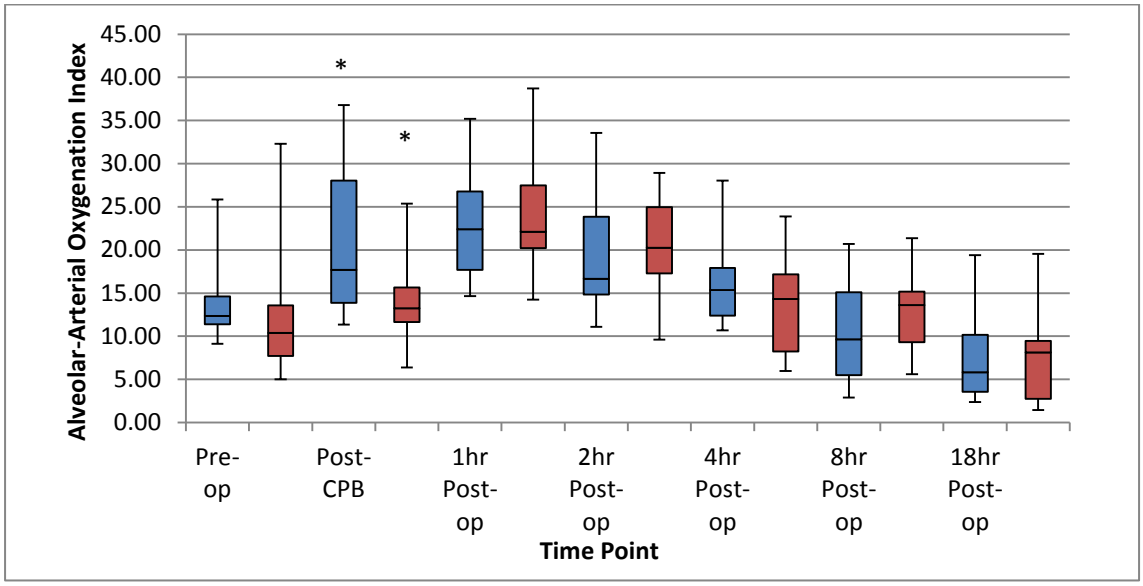


Figure 20. Alveolar-Arterial Oxygenation Index. Samples taken pre-CPB, immediately post CPB and then 1, 2, 6, 12, 24 and 48 hours post-CPB. For the 48 hours sample the patient breathed room air for 10 minutes to allow for equilibration and then samples of arterial blood were taken. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values. *$p \leq 0.05$.

There were no differences between the two groups at any other time points. Pre CPB vs. 18hr-post CPB showed significant differences in both groups [Admiral 12.4 (3.12) vs. 5.81 (6.6); 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] but this was not reflected in the lowest P_aO_2/F_iO_2 ratios and ARDS definitions (Table 14), which showed no differences between the 2 groups [p=0.33; Table 14]. Although data were not collected on length of ventilation, as the study was not powered to detect such differences, all patients were extubated within 24 hours.

9.1. **Discussion**

Pulmonary dysfunction following surgery is not unique to cardiothoracic surgery involving cardiopulmonary bypass, with multiple factors indicated such as general anaesthesia and surgery (Cox, Ascione et al. 2000). However, due to the nature of the extracorporeal circuit, its non-endothelial material, the opening of the pleural space for harvesting of the mammary artery, and the deflation of the lungs during CABG surgery, the incidence of pulmonary dysfunction associated with CPB-facilitated cardiac surgery is more common and severe (Clark 2006). 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. Westerberg *et al*., (2006) have previously cited studies that have shown both increases and decreases in PVR following reinfusion of PSB (see section 1.4). This study is unable to comment on this particular issue as none of the patients had PVR calculated during the CPB period. An increase in AaOI was observed in all patients following CPB. This has been demonstrated in previous studies and is thought to arise from several factors; the disruption of pulmonary endothelial membranes, haemodilution and the subsequent sudden decrease in colloid osmotic pressure and mechanical ventilation (Alexiou, Tang et al. 2004).

However, one of the key components believed to facilitate changes in pulmonary function is the inflammatory response. As discussed in Section 6, the artificial surfaces of the ECC cause and upregulate neutrophil activation, increasing expression of CD11b surface adhesion integrins, promoting the adhesion to ICAM-1 on the pulmonary endothelium allowing the transmigration into the lung parenchyma under the influence of IL-8 (Asimakopoulos 1999), which mediates lung parenchymal damage through cellular and tissue injury (Clark 2006). 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 leucocytes and LME should provide significant protection from lung injury. This study was not designed to examine differences in length of ventilation, as far greater number of patients would be required, so cannot comment on the findings of Alexiou *et al*., who demonstrated a lowered requirement for the duration of ventilation with leucocyte filtration (Alexiou, Tang et al. 2004).

	Time	Admiral	RemoweLL	*p*
F_iO_2	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_aO_2 (kPa)	Pre-Op	33.5 (3.2)	36.3 (4.6)	0.223
	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_aCO_2 (kPa)	Pre-Op	5.32±0.42	5.42±0.56	0.63
	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-Op	5.12±0.39	5.59±0.43	0.02
	18 Hr Post-Op	5.03±0.33	5.34±0.22	0.01
AaOI (kPa)	Pre-Op	13.8±4.2	11.7±6.7	0.1
	Post-CPB	20.6±8.6	14±4.4	0.01
	1 Hr Post-Op	23.4±6.4	23.7±6.4	0.89
	2 Hr Post-Op	19.6±6.5	20.5±5.4	0.63
	4 Hr Post-Op	15.9±5	14.1±5.9	0.24
	8 Hr Post-Op	10.5±5.8	12.5±4.5	0.16
	18 Hr Post-Op	7.5±4.9	7.9±5.7	0.85

Table 13. Ventilation Settings and Alveolar-Arterial Oxygenation Index. F_iO_2; fraction of inspired oxygen. p_aO_2; arterial partial pressure of oxygen. p_aCO_2; arterial partial pressure of carbon dioxide. AaOI; Alveolar-Arterial Oxygenation Index. Samples taken pre-CPB, immediately post CPB and then 1, 2, 6, 12, 24 and 48 hours post-CPB. A *p* value ≤0.05 was considered significant.

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

Table 14. ARDS Classification. Classifications based upon Berlin Definitions; None PaO_2/FiO_2 >300, Mild PaO_2/FiO_2 = 200 – 300, Moderate PaO_2/FiO_2 = 100 – 200, Severe PaO_2/FiO_2 < 100. A *p* value ≤0.05 was considered significant.

This study focussed on the calculation of the Alveolar-Arterial Oxygenation Index (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 2 groups in terms of AaOI [p=0.075]. Stepwise analysis of log transformed data using unpaired TTests showed a significant difference in the immediate post-op period [p=0.01] but there were no further differences at any other time point. Furthermore, analysis revealed that the fraction of inspired oxygen showed lower values at this time point which may explain the differences in the post-op AaOI [0.58 (0.03) vs. 0.57 (0.04); p=0.06]. Chi-square testing of the ARDS classification showed no significant differences between the two groups [p=0.33]. Whilst the lack of a difference might seem counter intuitive, there are a number of reasons why this might be explained. Firstly, there are issues with the Berlin definitions of ARDS, in particular that they don't 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 PaO_2:F_iO_2 ratio. Secondly, it does not allow the early identification of patients who may be amenable to therapies before ARDS becomes established. Finally, as none of the patients exhibited signs of severe ventilation requirements, the usefulness of this parameter in the current study could be questioned.

As discussed above, the numbers and activation statuses of leucocytes was shown to be similar in both groups, therefore the opportunity for parenchymal damage might be also considered similar. If an increase in alveolar-endothelial permeability did occur, the fluid balance of both groups were similar [1678.60±842.38ml (mean±SD, n=15) vs. 1562.27±867.16ml (mean±SD, n=15); p=0.71] and so this study would not be powered to detect any differences in this situation. Furthermore, whether oleic acid in the LME contributes to this effect has not been demonstrated in humans. Whilst none of the patients exhibited severe symptoms of ARDS (Table 14), there were significant increases in AaOI in the immediate postoperative period in the Admiral group [p=0.007] but not in the RemoweLL group [p=0.1]. Comparison between the 2 groups showed significance [0.012] but any functional difference had resolved by 1 hour following surgery [p=0.9] and remained insignificant for the remaining period of observation. As there are no differences between groups, and yet there is a highly significant difference in LME counts between groups, one might suggest that there is little interaction. However, there are confounding factors to this. During fracture of the long bones, bone marrow as well as cut capillaries and veins are exposed taking up LME which, due to the venous circulation, reach the lungs as the first densely vascularised structure. It is important to note that the proportions of fat in Yellow bone marrow

are much greater than Red bone marrow (85% vs. 5%). Furthermore, unless an arterial-venous malformation is present, there is no route by which LME may pass to the arterial system (Eyjolfsson, Plaza et al. 2009). This would explain why patients undergoing hip and knee reconstructions rarely exhibit neurological complications but are known to suffer pulmonary fat embolism. This is in stark contrast to patients undergoing CPB, where the LME are placed back into the arterial system (Brooker, Brown et al. 1998). However, it should be noted that the same bone marrow veins and capillaries are still present in the sternum so it would be entirely plausible for some LME to pass into the venous system, although this is unlikely to reach the lungs due to the presence of the venous cannula which drains blood from the right atrium before the blood enters the pulmonary circulation. The bronchial circulation may provide the lung with some LME rich blood, but this is greatly diminished during CPB so the potential for this to influence pulmonary function post CPB is negligible. Therefore this study cannot demonstrate the ability for LME to influence pulmonary function in this group of patients.

In a similar way to the Cystatin C data discussed in section 8.1, due to the early cessation of the trial, it was necessary to carry out a post hoc analysis based upon peak AaOI (1hr-post CPB) data to ascertain what statistical power was obtained. This revealed an effect size of 0.71, therefore a patient cohort of 36 patients would be needed to achieve a power ($1-\beta$) of 0.8 with α=0.05. Therefore this study was underpowered to make significance claims on the ability of LME filtration to attenuate pulmonary dysfunction.

This study was unable to show any differences in terms of pulmonary function between the two groups, or any evidence that would suggest that further investigation was warranted. As described above, it is possible that the “pump syndrome” is not a weaker form of fat embolism syndrome seen in patients with breaks to long bones, but the direct effects of surgery and the large haemodilution/inflammatory response to CPB.

10. Conclusions

The role of LME in "pump syndrome" and multi-organ dysfunction following cardiac surgery using CPB has been long suggested and debated. Whilst is it generally believed that LME are the causal factor behind these events, there has never been definitive evidence to confirm this. There are a number of reasons for this; firstly LME are destroyed in the fixation process of cerebral autopsy samples, leading to suppositions that SCADs are generated by LME based upon the high levels of fat in the surrounding tissues of cerebral vessels, rather than their direct observation within the vessels themselves (Brown, Moody et al. 1999). Secondly, much of the data regarding LME are founded upon animal studies that employ surrogate emboli that will not only function differently to cardiotomy derived LME, but dependent upon the animal under investigation, end up in different organs dictated by the blood flow characteristics of that animal (Brondén, Dencker et al. 2006). Thirdly, in order to demonstrate a difference in organ function between those who are subjected to LME and those who are not, requires a clinically efficacious method of LME removal; to date this has not been produced (Issitt and Sheppard 2011). The RemoweLL oxygenation system is the first ECC that has an integrated cardiotomy reservoir with a lipid and activated leucocyte filter, providing a treatment group that can be compared to its sister product, the Admiral, which is identical other than having a standard 40µm filter in the cardiotomy reservoir (Issitt, Cumberland et al. 2008). Therefore this study was the first to be able to compare two groups of patients undergoing CABG surgery using CPB in order to look at the direct effects of LME filtration on biochemical markers of organ function, whilst keeping all other surgical variables constant, i.e. the surgical correction taking place. The biochemical markers would then act as substitute indices of end organ function. This has 2 advantages; firstly the actions of LME are dependent upon which vessels are affected, and whether they are located in areas of low or high vascularisation. Therefore studying neurocognitive changes may not detect subtle damage affecting cerebral cellular membranes; something that neuron specific enolase is capable of assessing. Secondly, by confining the study to CABG only patients, it is possible isolate to the effects of LME from gaseous emboli present once cardiac chambers are opened to atmosphere. This would prevent any interaction and keep the group homogenous. The aim of this study, therefore, was to observe any biochemical data that would give an indication of benefit or indeed, provide any evidence that there was a link between LME and neurological dysfunction.

The key findings of this study were that there were significantly more LME removed from the PSB in the RemoweLL group than the Admiral group. This represents a major advance in PSB management capabilities. As predicted, the LME load increased during CPB in the control group due to the continued exposure of sternal bone marrow to open atmosphere. LME continued to be produced via fats leaching

from the bone marrow, which then entered the circulation via the cardiotomy suction. As expected, circulating fats (such as cholesterol) were not affected by the fat filtration process as both groups had consistently similar and statistically insignificant differences between them, again demonstrating the targeted efficiency of the cardiotomy filter. However, this study could not demonstrate the efficacy of the 2nd stage of the filtrations process, the activated leucocyte removal. There was an increase in the inflammatory response in both groups with greater numbers of leucocytes seen in the RemoweLL group. This was also observed in the total number of neutrophils, although this did not reach significance. There was no difference in terms of neutrophil activation between the 2 groups, although there was a return to pre-CPB status in the RemoweLL group, which remained elevated in the Admiral group.

Both groups showed a significant increase from baseline in a known marker of neurological dysfunction, Neuron Specific Enolase, shortly after commencing CPB. However, concentrations in the Admiral group were significantly higher still compared to the RemoweLL group at all time-points. Peak concentrations above 15 µg/L have been linked to neurological dysfunction following CPB. RemoweLL had significantly less peak NSE release compared to Admiral. This study is the first to have demonstrated the efficacious filtration of LME in the clinical setting, and the subsequent attenuation of NSE release. Furthermore, the data suggest a direct correlation exists between the number of LME and the magnitude of NSE release.

Kidneys have exhibited the highest uptake of LME in animal models, and so the capacity of LME in renal dysfunction was investigated using Cystatin C as a surrogate maker of AKI. There was a significantly lower peak concentration of Cystatin C in the RemoweLL group in the postoperative period, suggesting a renoprotective role for LME filtration, although no other parameters confirmed prevention of renal injury/dysfunction, and analysis using ANOVA for repeated measures concluded that Cystatin C data over the 3 days post-surgery period failed to reach significance overall.

Analysis of lung function by the arterial-alveolar oxygenation index, and categorisation using the Berlin definitions of ARDS revealed no significant differences between the Admiral and RemoweLL groups, although a difference was noted in the immediate post-operative period. However, this could be explained by differences in F_iO_2 settings in the immediate post-CPB period. This study, therefore could not demonstrate any evidence that LME are responsible for ARDS-type symptoms post-CPB.

As with all studies there are important limitations that should be noted. Whilst a randomised controlled trial was undoubtedly the correct method of assessment for the study of LME, one might question the patient group under investigation. Coronary artery bypass grafting does not routinely produce large amounts of blood to be shed into the pericardium, and often this is managed without the

cardiotomy suction (i.e. with the use of cell salvage). Therefore it might be considered more appropriate to examine LME removal in more complex cases (such as redo, valve, or aortic surgery) that requires longer bypass times and would invariably require the use of cardiotomy suction to be recycled during the procedure. One reason for carrying out this procedure in CABG-only patients was to isolate the effects of LME from gaseous emboli present once cardiac chambers are opened to atmosphere. This would prevent any interaction and keep the group homogenous. Ultimately further work into clinical outcomes should involve more complex cases and involve patients groups that were excluded in this study, especially as there is evidence that suggests those with pre-existing neurocognitive and renal impairment might benefit more from LME filtration.

The major limitation of this study is the lack of a true measure of cognitive function, especially in the pre-operative assessment. Whilst the patient, any family present and the patient's General Practitioner were all consulted to check for changes in mental state and any documented or family history of seizures, strokes or neurological disease, no formal analysis of cognitive function was undertaken. Whilst the contemporary literature identifies a relationship between LME, NSE and cognitive function, it is hard to know what levels of LME and NSE are clinically significant for cognitive function in reality. One might suggest that what appear to be major differences in the data presented in this Thesis may actually produce marginal, if not clinically undetectable effects in the post-CPB patient. However, LME in the form of SCADs are not dependent upon number *per se* to cause stroke, it is dependent upon which vessels are affected and whether they are in areas of low or high vascularisation. Given that the rate of stroke following cardiac surgery is reported to be 2%, the cohort required to show clinical benefit would be enormous. Even examining diffuse damage which is thought to affect 50% is open to interpretation from the analysing neurologist. Therefore the study aimed to try and observe any biochemical data of a link between LME and neurological dysfunction. This study has provided that initial data, which might be used to further pursue the subject; to investigate neurocognitive outcomes in a larger, multi-centre trial.

A second limitation of this study was brought about by the early cessation of the trial due to the statistical stopping rules. Whilst these were inserted for justifiable reasons, the knock on effects of finishing the trial with 30 rather than 50 patients impacted on the outcome measures of the renal and pulmonary function data. The kidneys have exhibited the highest uptake of LME in animal models, and so an objective of this study was to determine the role of LME in renal dysfunction using Cystatin C as a surrogate maker of AKI. Whilst there was a significantly lower concentration of Cystatin C in the RemoweLL group in the postoperative period, suggesting a renoprotective role for LME filtration, no other parameters confirmed prevention of renal injury/dysfunction, and due to the small sample size, further investigation is necessary to corroborate the Cystatin C data and to elucidate any long term

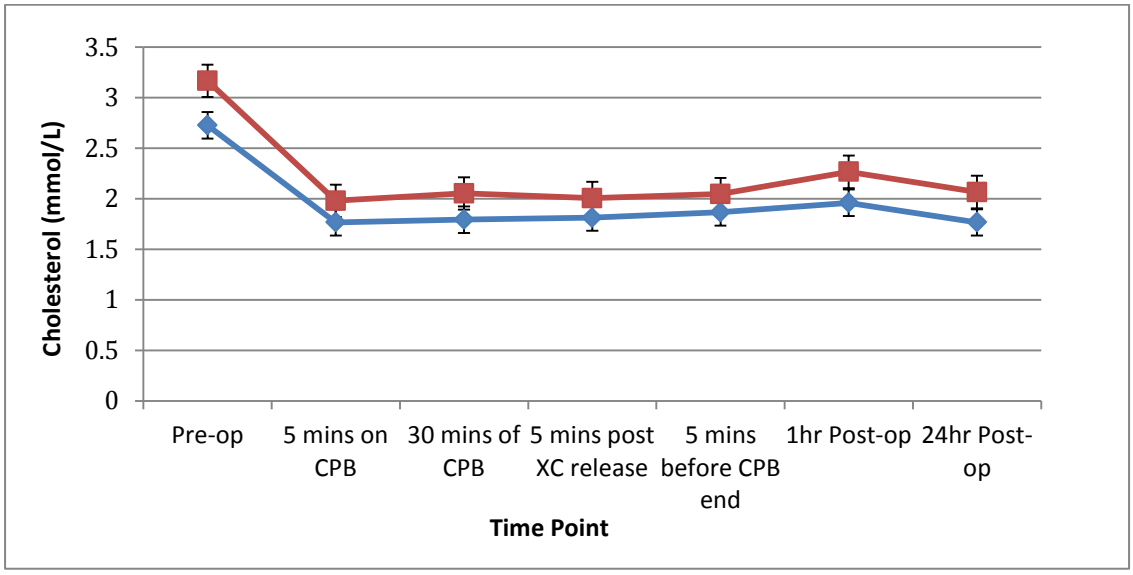
benefits of LME filtration in patients undergoing cardiac surgery. Similarly, when considering the pulmonary function data, there are very wide confidence intervals demonstrating the effects of having a small sample size, therefore these data should be interpreted with caution.

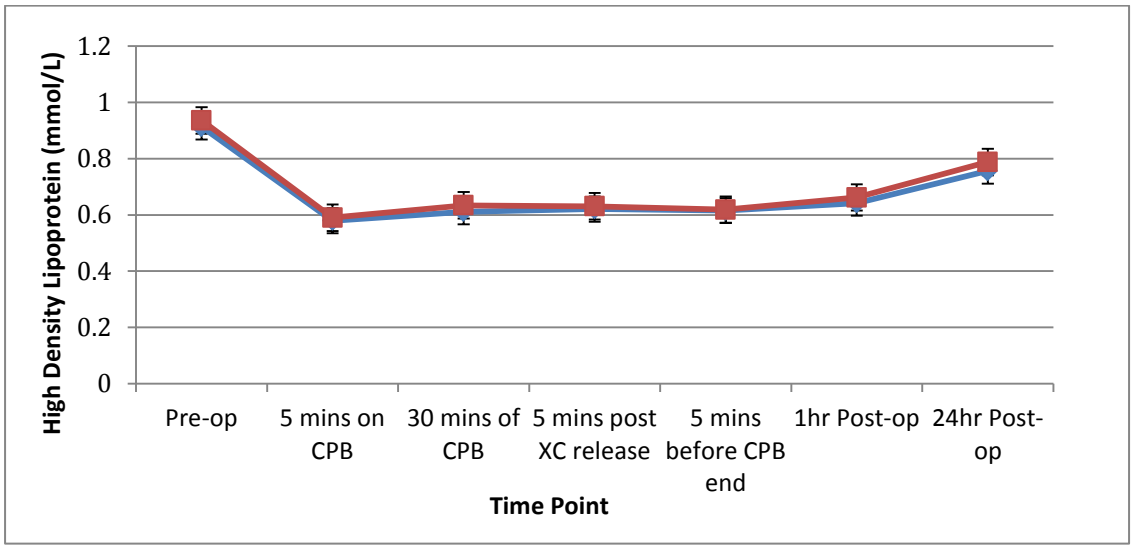
The final "limitation" of the study might be the methodology of LME sampling. The sampling from the arterial limb of the circuit for the post-filtration time point might be considered to have a dilutional effect; i.e. the parameters have been diluted from the PSB into the systemic circulation. However, LME will separate due to density if left for any length of time, and adhere to plastic surfaces. Therefore to ensure that the results were accurate and not a result of the separation from the systemic circulation, the cardiotomy blood was returned to the circulation first, and then tested for numbers. Whilst there is an argument for a dilutional effect, the number seen in this study is similar to other studies that investigated LME, so it is doubtful that the effect is particularly great. Furthermore, it would be equal for both groups. As the results clearly show a significant rise in one, and a significant decrease in the other, it would appear doubtful that this bears any relevance on the outcome.

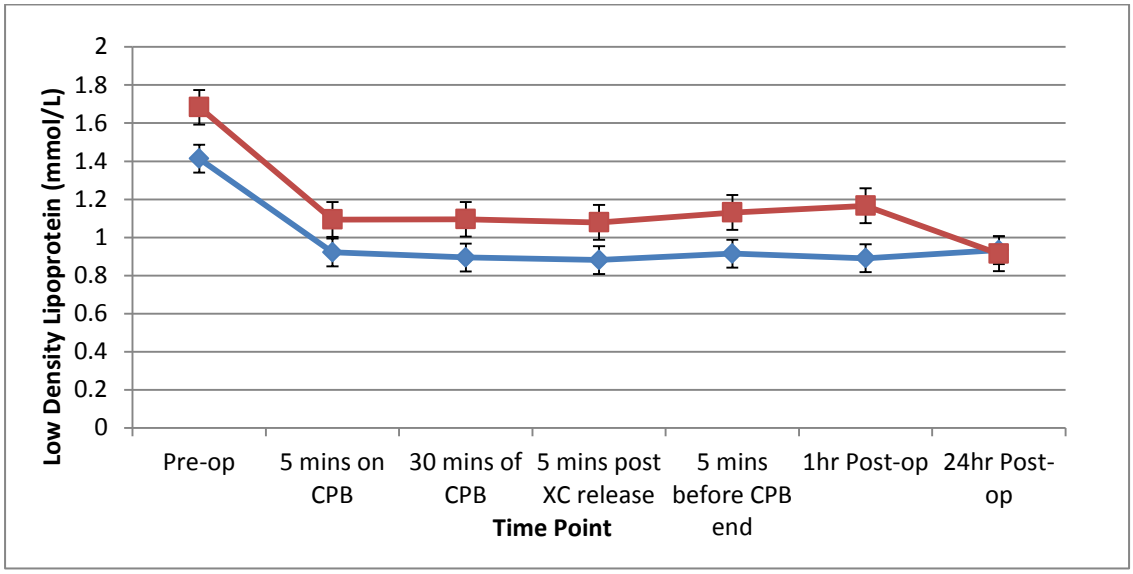
In summary, the RemoweLL CPB system removes significant numbers of LME compared to the current ECC technology. Significant differences in the release of Neuron Specific Enolase, a marker of neurological function in the immediate postoperative period have been demonstrated. Furthermore, there is limited evidence towards improved clearance of Cystatin C, a surrogate marker of glomerular filtration. Based upon the results of this Thesis, the following recommendations for practice can be made: LME filtration of the cardiotomy suction should be undertaken in all CABG cases to reduce the embolic load to the patient. The RemoweLL system should not be used however, as a leucocyte-depleting mechanism, as no evidence was observed that the device removes leucocytes or indeed attenuates systemic leucocyte activation. Further research is necessary to investigate the effects of LME on cognitive outcome and how this relates to the long term progression of neurocognitive decline. Any future investigations ought to include subsets of patients considered at risk from LME (such as those with pre-existing neurocognitive impairment or renal failure).

Appendix A – Lipid Profiles

Data for Cholesterol, HDL and LDL. Samples taken pre-CPB, 5 and 30 minutes on CPB, 5 minutes before cross clamp removal, 5 minutes before end of CPB and 1 and 24 hours post-CPB. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as mean with error bars representing standard error of the mean.

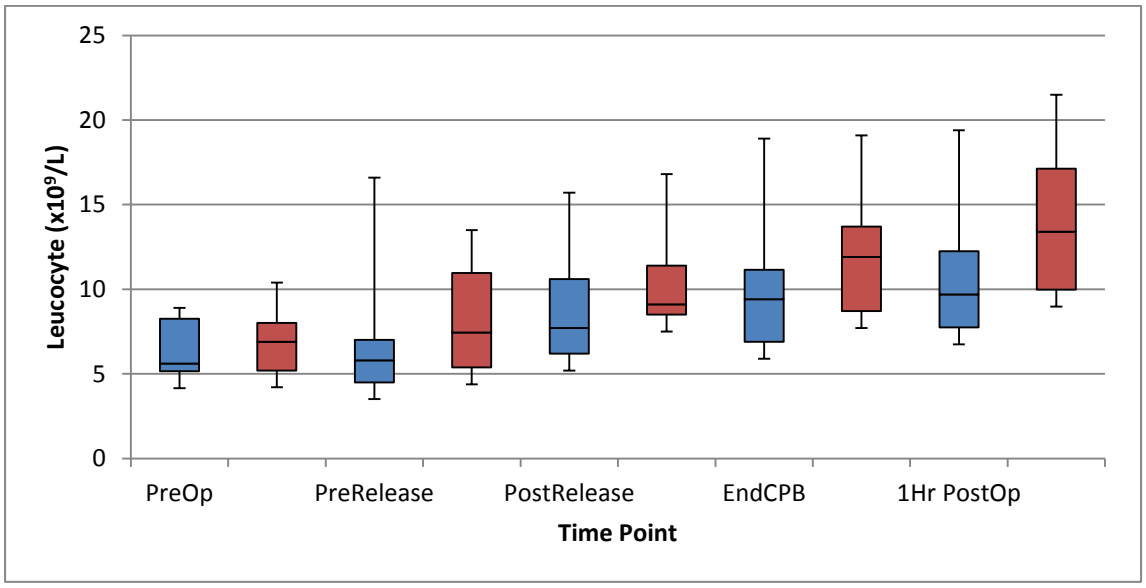


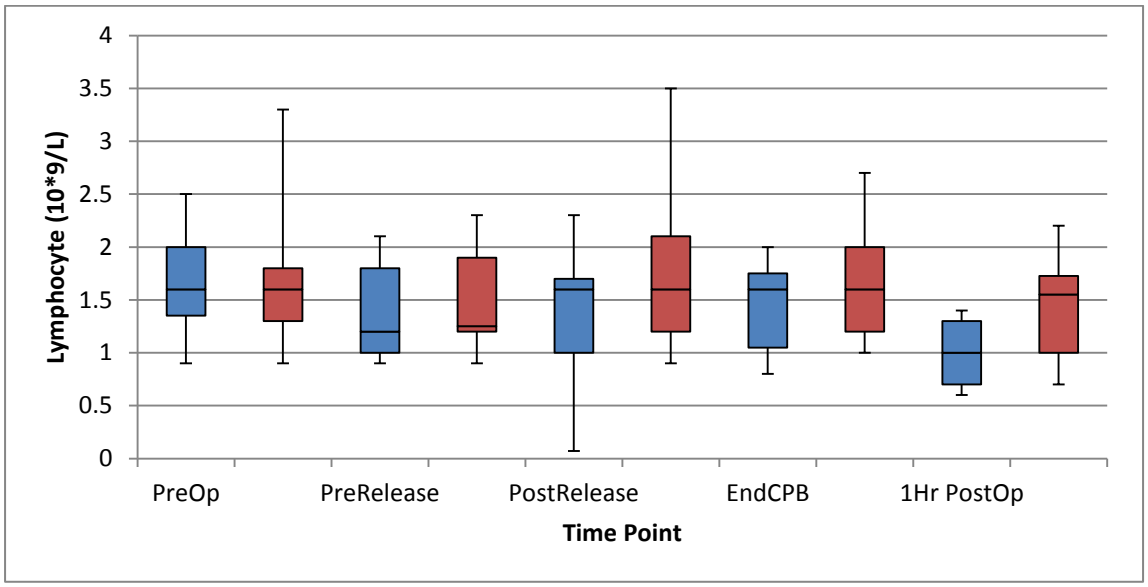


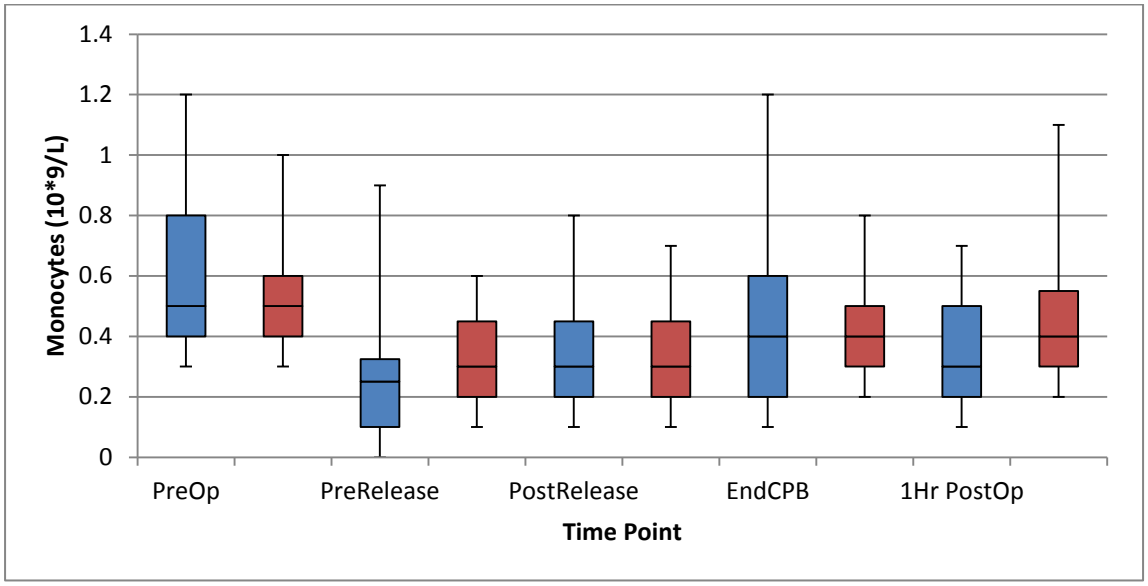

Low Density Lipoprotein (mmol/L)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Pre-op
5 mins on CPB
30 mins of CPB
5 mins post XC release
5 mins before CPB end
1hr Post-op
24hr Post-op
Time Point

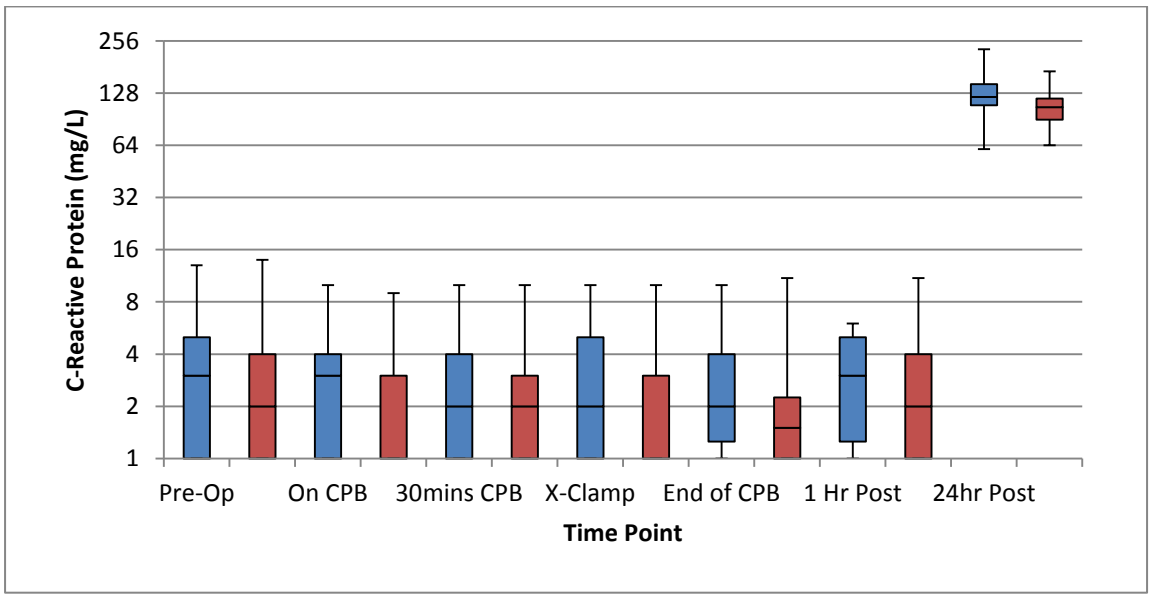
Appendix B – Leucocyte Differentials and FACS Data

Data for total leucocyte count, lymphocytes, and monocytes. 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. Data for C-Reactive Protein. Samples taken pre-CPB, 5 and 30 minutes on CPB, 5 minutes before cross clamp removal, 5 minutes before 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-75 centiles with median and whiskers as maximum and minimum values. FACS data taken from patient number 3 showing peak concentration vs. Control sample.

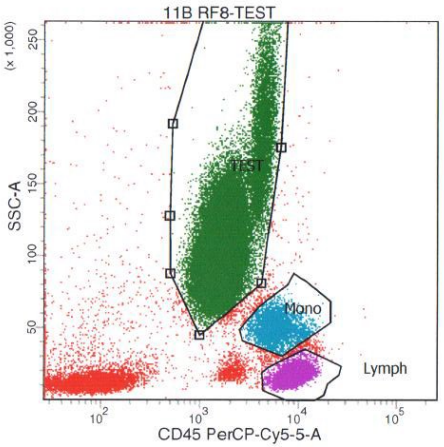


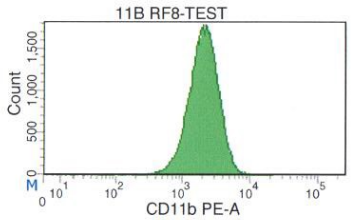


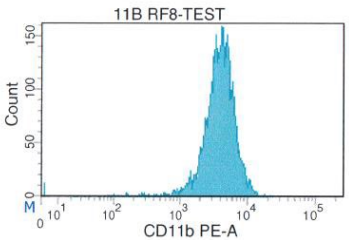

1.4
1.2
1
0.8
0.6
0.4
0.2
0
Monocytes (10*9/L)
PreOp
PreRelease
PostRelease
EndCPB
1Hr PostOp
Time Point

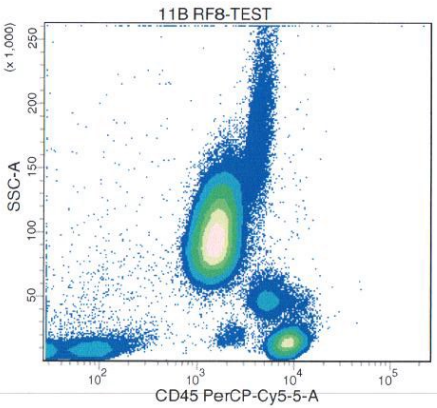

256
128
64
32
16
8
4
2
1
C-Reactive Protein (mg/L)
Pre-Op
On CPB
30mins CPB
X-Clamp
End of CPB
1 Hr Post
24hr Post
Time Point

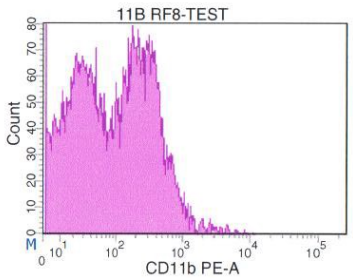
BD FACSDiva 7.0











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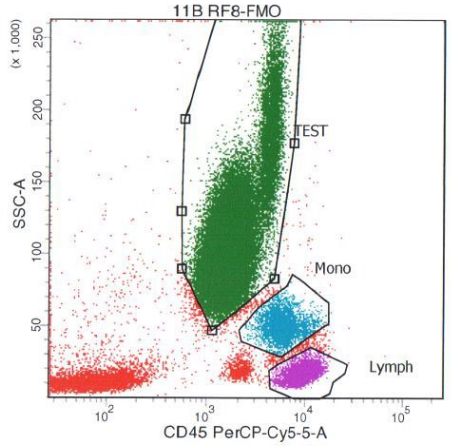
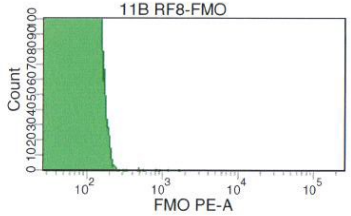
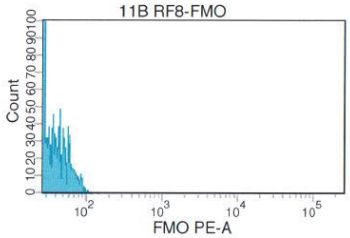
Cytometer:	**V96300612**
Sample ID:	**11B RF8**
Operator:	**Cells**
PANEL NAME:	**11B**
SAMPLE NAME:	**TEST11B TEST11B**
Analysed by:	
Display Range	**Linear Scale (0-10,000)**

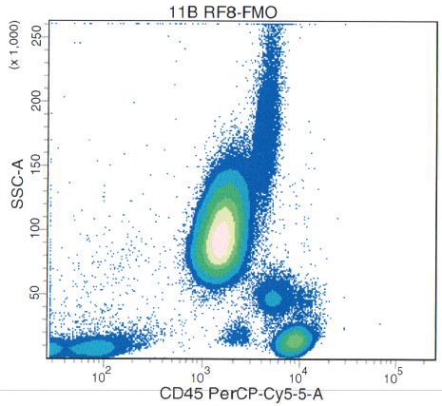
Population	CD11b PE-A Mean
TEST	82
Mono	152
Lymph	6

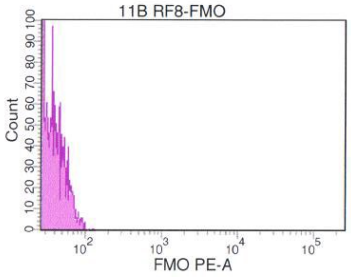
BD FACSDiva 7.0











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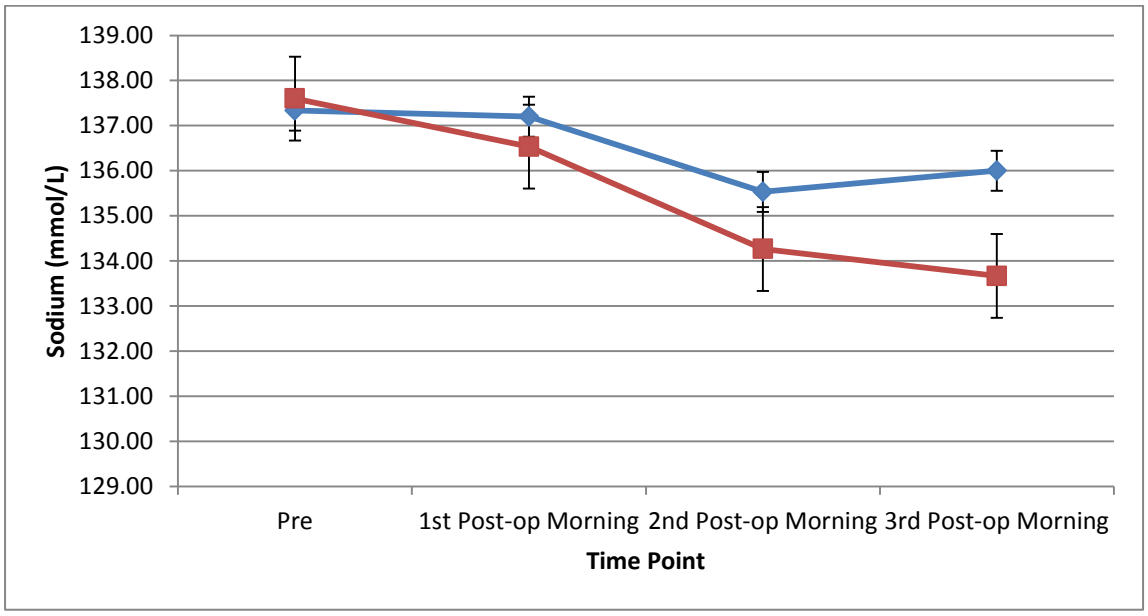
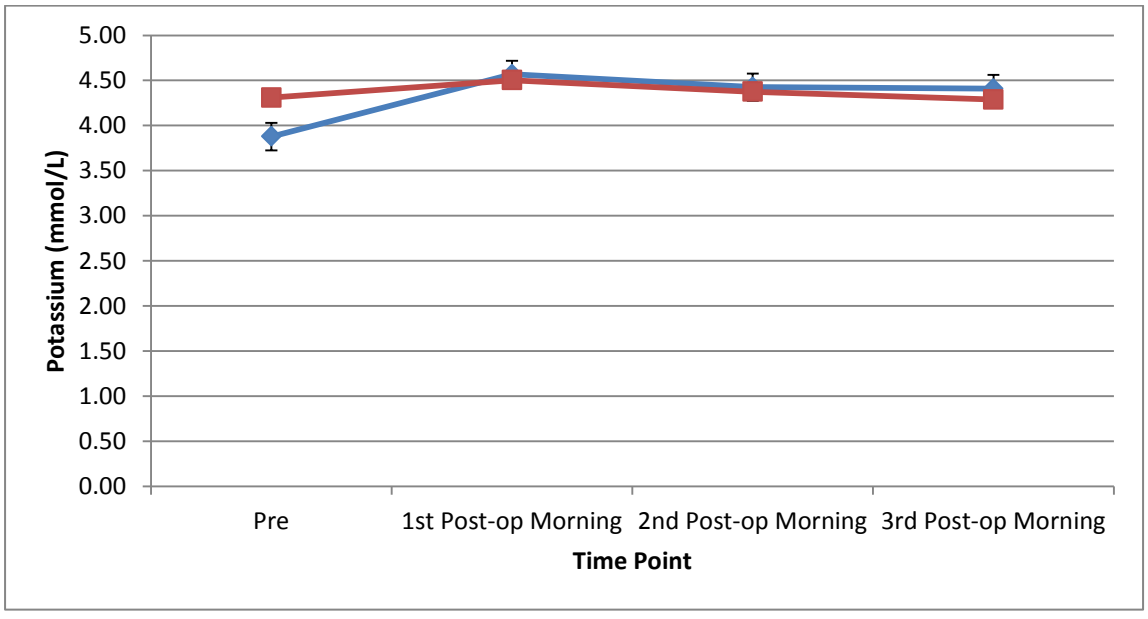
Sample ID:	**11B RF8**
Operator:	**Cells**
PANEL NAME:	**11B**
SAMPLE NAME:	**TEST11B TEST11B**
CYTOMETER:	**V96300612**
ANALYSED BY:	
Display Range	**Linear Scale (0-10,000)**

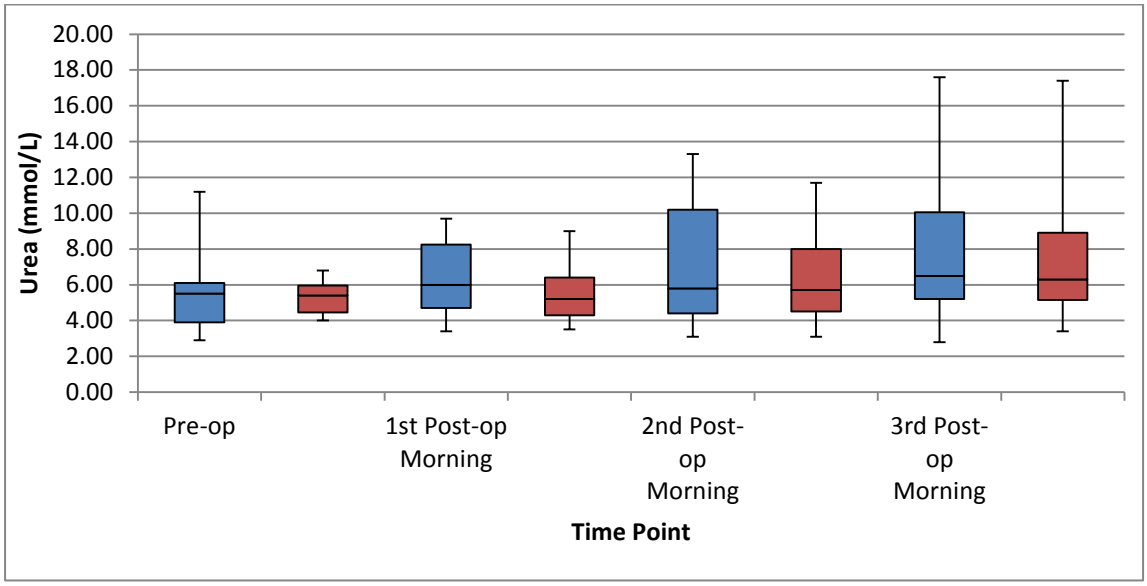
Population	FMO PE-A Mean
TEST	1
Mono	1
Lymph	1

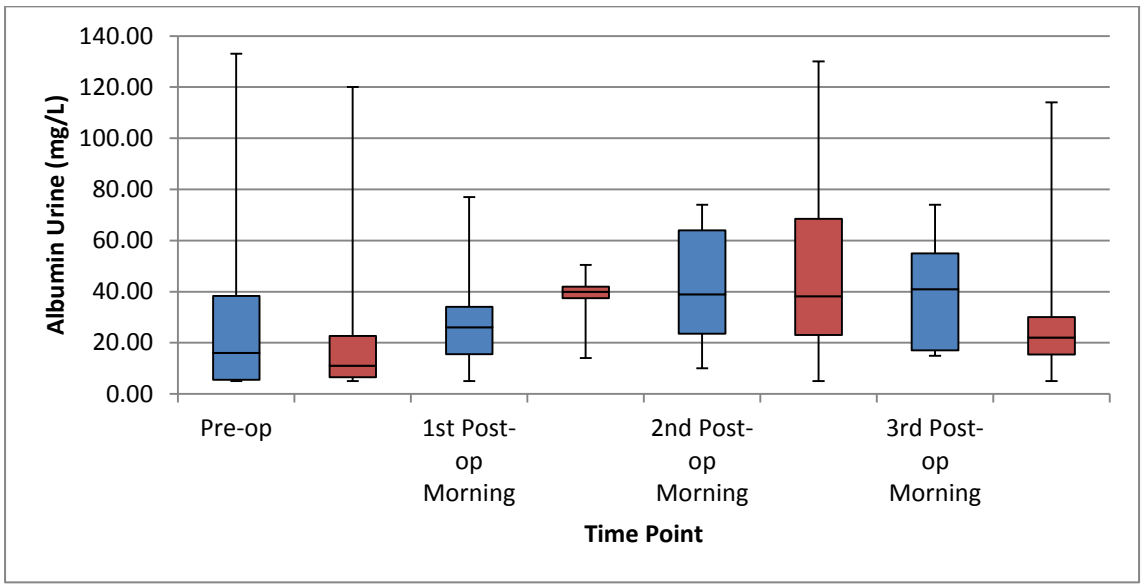
Appendix C – Renal Profiles

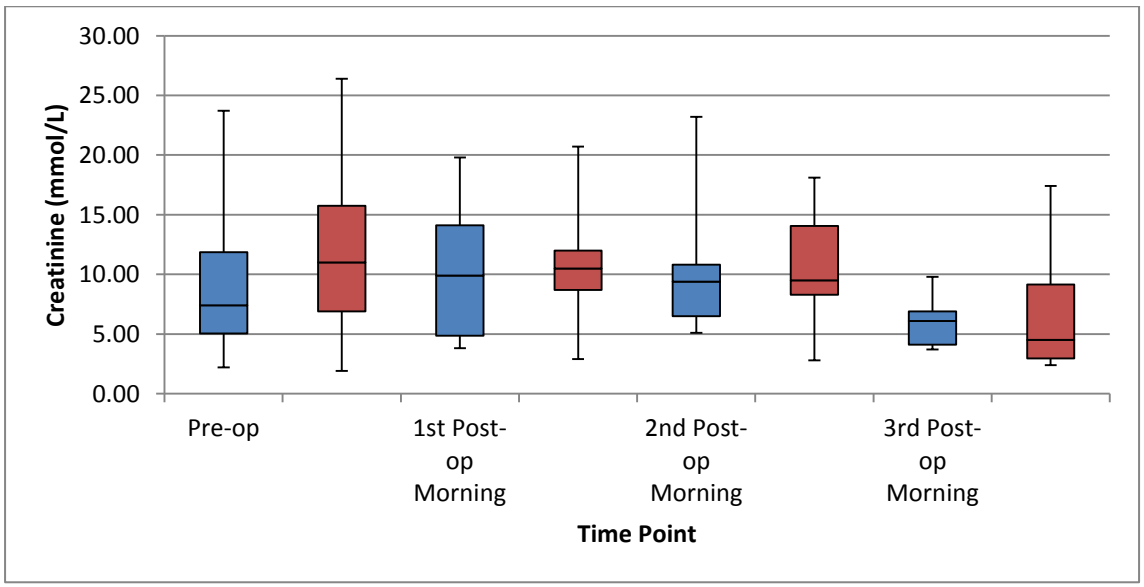
Data for urinary and serum electrolytes. Samples taken pre-CPB, 1st, 2nd and 3rd postoperative mornings. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as either mean with error bars representing standard error of the mean or as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values.

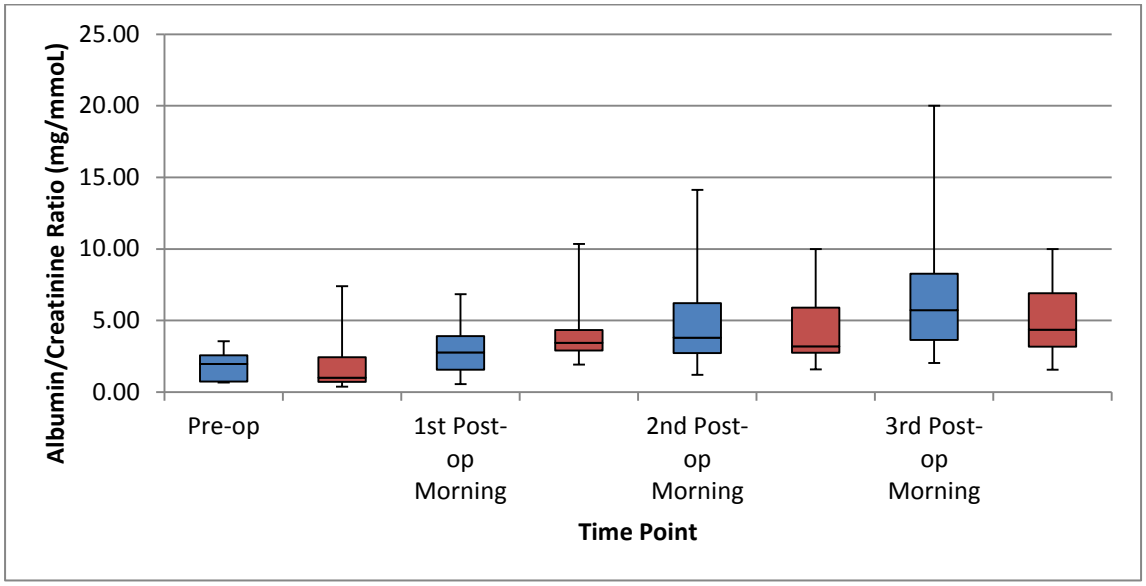


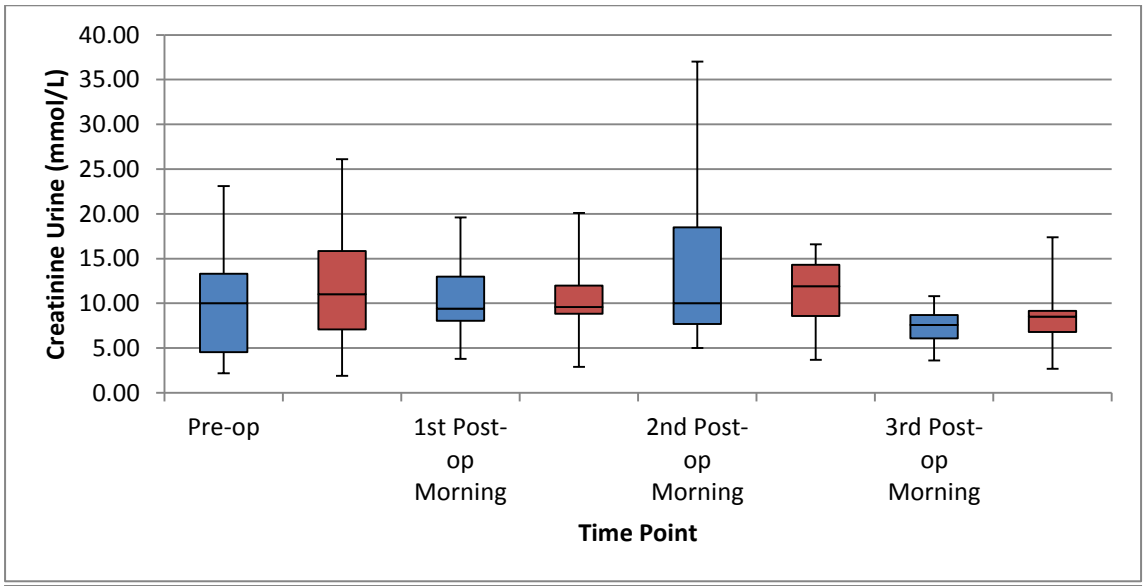


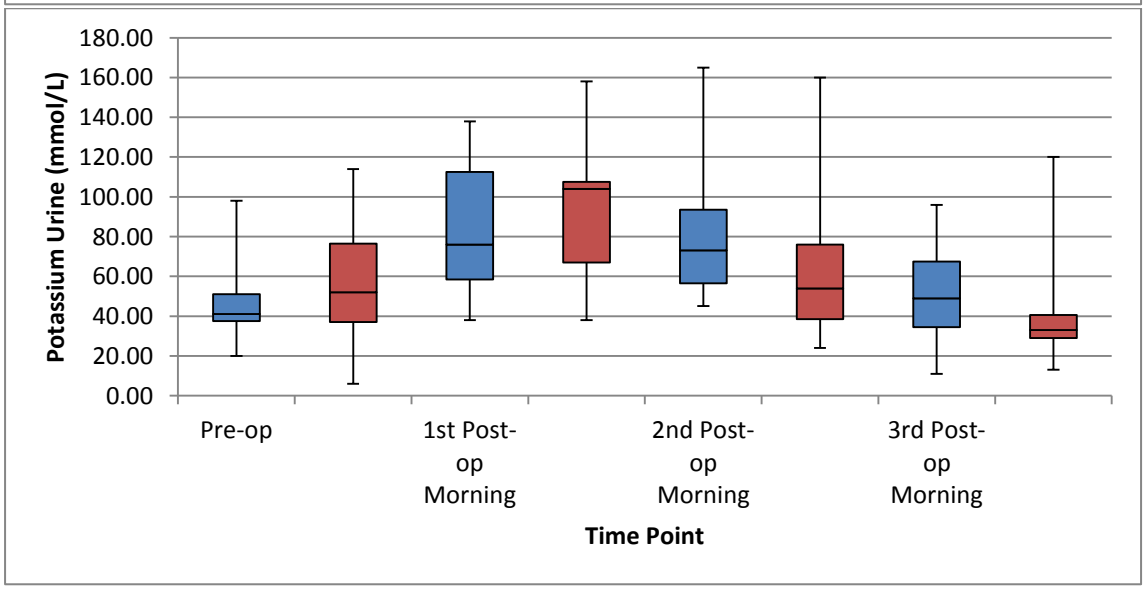

20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Urea (mmol/L)
Pre-op
1st Post-op Morning
2nd Post-op Morning
3rd Post-op Morning
Time Point

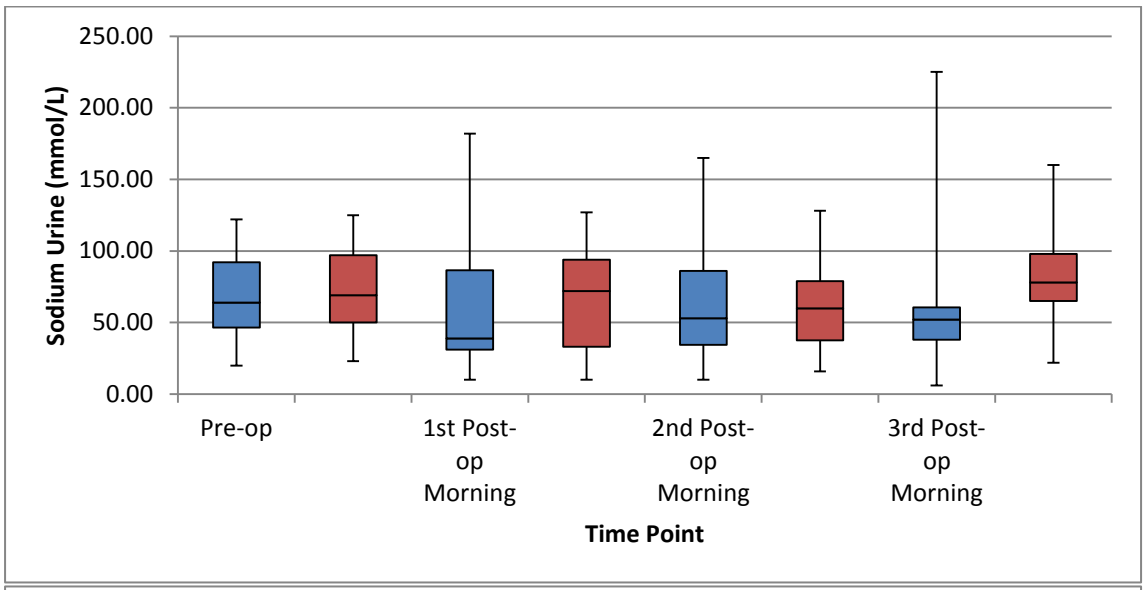

140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Albumin Urine (mg/L)
Pre-op
1st Post-op Morning
2nd Post-op Morning
3rd Post-op Morning
Time Point

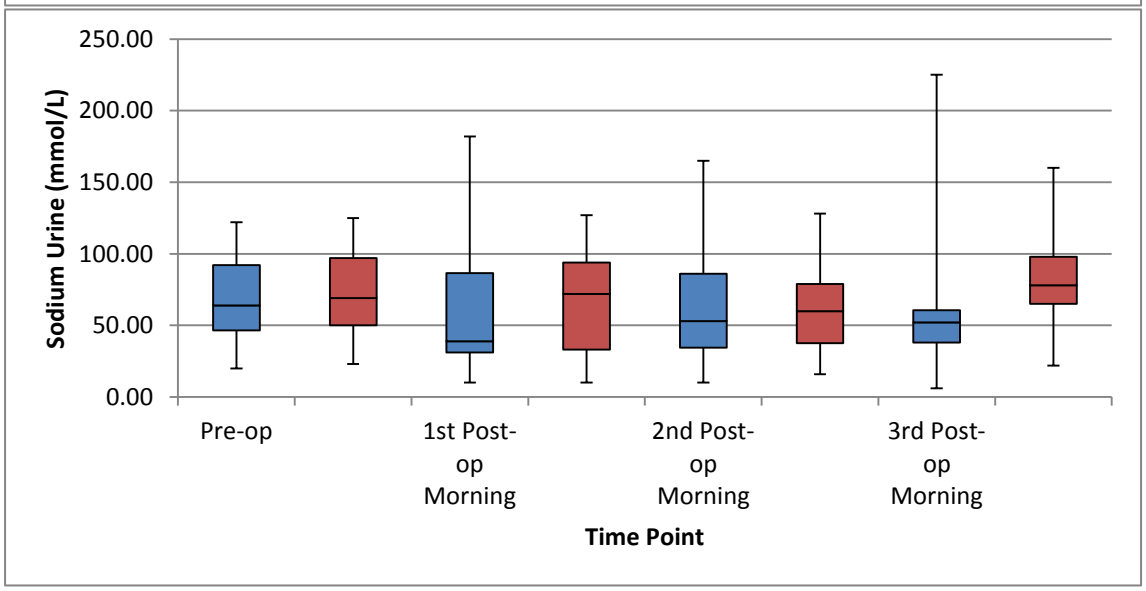

30.00
25.00
20.00
15.00
10.00
5.00
0.00
Creatinine (mmol/L)
Pre-op
1st Post-op Morning
2nd Post-op Morning
3rd Post-op Morning
Time Point

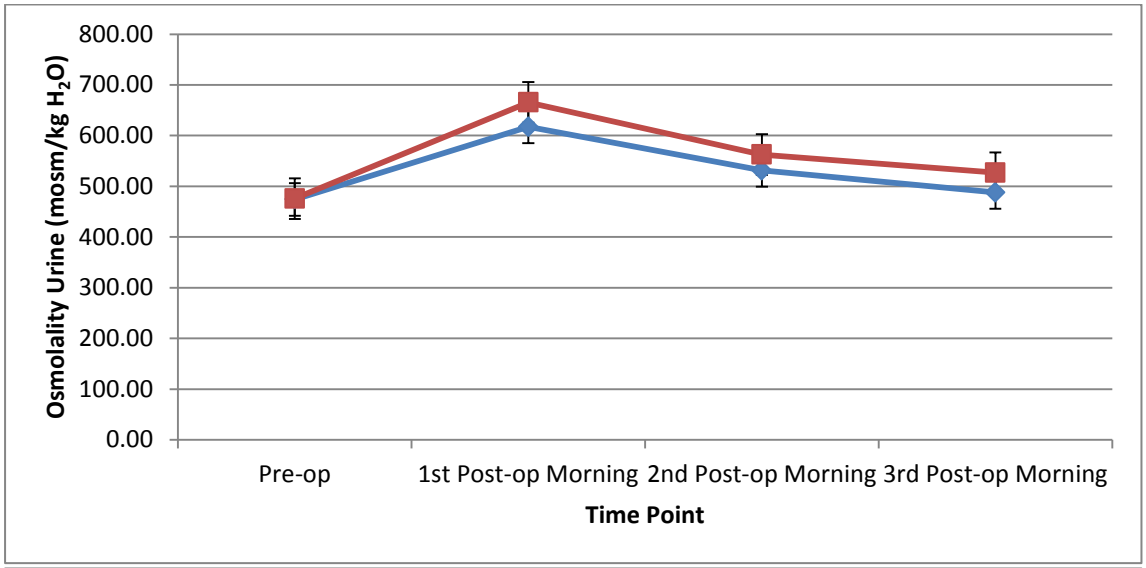

Albumin/Creatinine Ratio (mg/mmoL)
25.00
20.00
15.00
10.00
5.00
0.00
Pre-op
1st Post-op Morning
2nd Post-op Morning
3rd Post-op Morning
Time Point

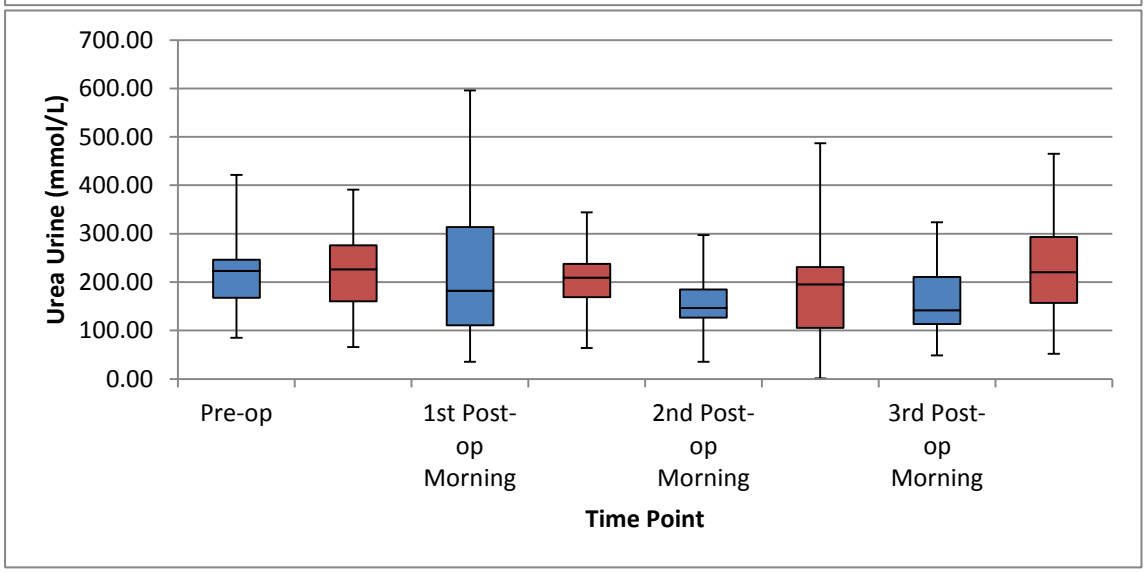

Creatinine Urine (mmol/L)
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Pre-op
1st Post-op Morning
2nd Post-op Morning
3rd Post-op Morning
Time Point


Potassium Urine (mmol/L)
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Pre-op
1st Post-op Morning
2nd Post-op Morning
3rd Post-op Morning
Time Point


250.00
200.00
150.00
100.00
50.00
0.00
Sodium Urine (mmol/L)
Pre-op
1st Post-op Morning
2nd Post-op Morning
3rd Post-op Morning
Time Point

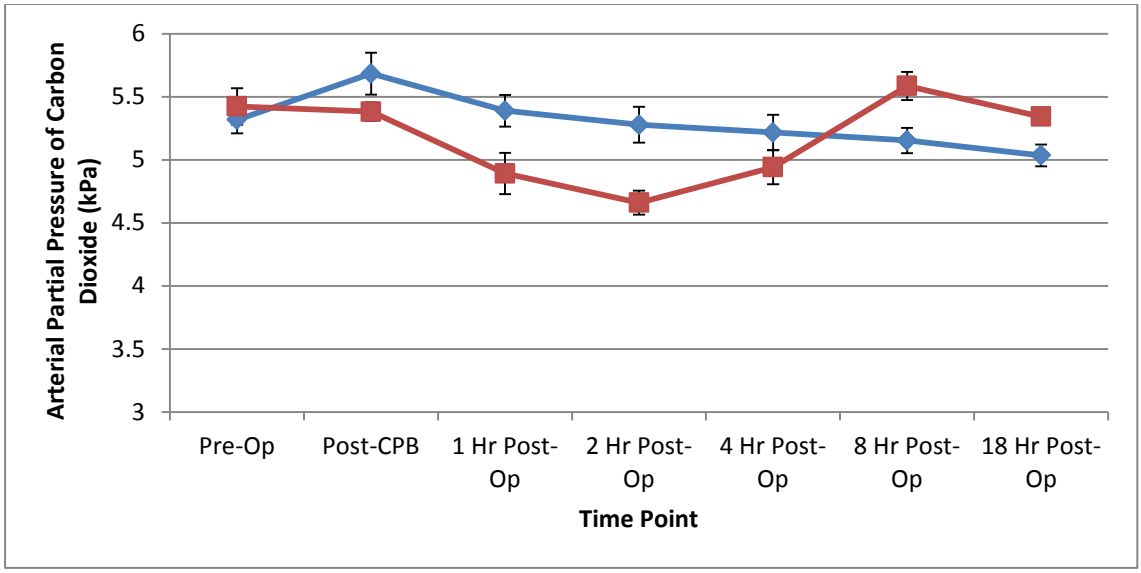

250.00
200.00
150.00
100.00
50.00
0.00
Sodium Urine (mmol/L)
Pre-op
1st Post-op Morning
2nd Post-op Morning
3rd Post-op Morning
Time Point

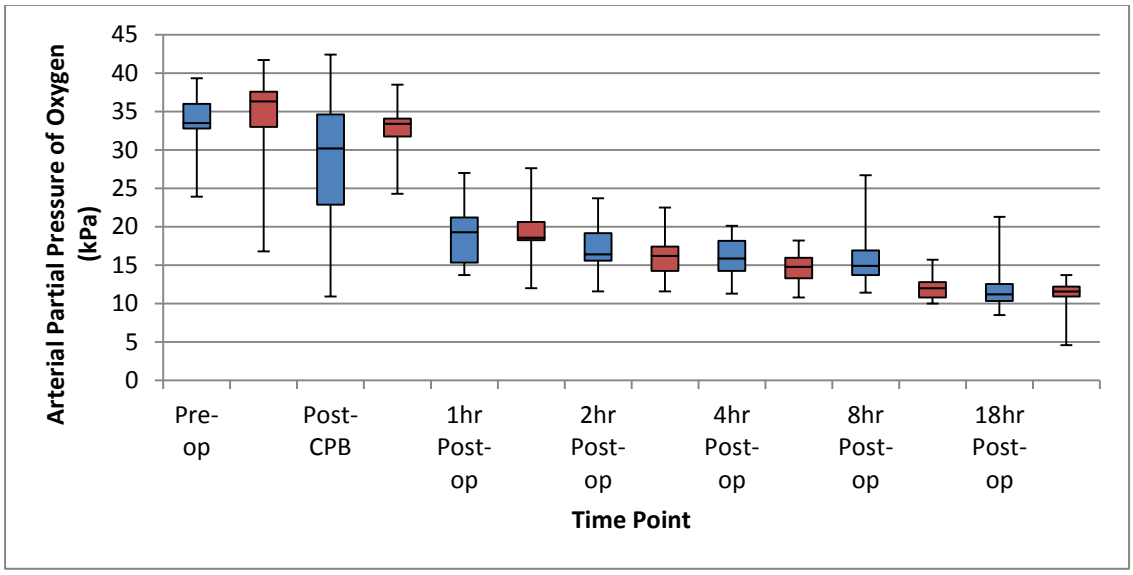

800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
Osmolality Urine (mosm/kg H_2O)
Pre-op
1st Post-op Morning
2nd Post-op Morning
3rd Post-op Morning
Time Point


700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
Urea Urine (mmol/L)
Pre-op
1st Post-op Morning
2nd Post-op Morning
3rd Post-op Morning
Time Point

Appendix D – Pulmonary Function Data

Data for arterial blood gas pressures. Samples were taken pre-CPB, immediately post CPB and then 1, 2, 6, 12, 24 and 48 hours post-CPB. Blue; control (Admiral), Red; intervention (RemoweLL). Data presented as either mean with error bars representing standard error of the mean or as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values.





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