**Title:** Quantification of lipid filtration and the effects on cerebral injury during cardiopulmonary bypass.

**Authors:** Richard W. Issitt FCCP1,2, Ian Harvey LCCP3, Bronagh Walsh Ph.D2 and David Voegeli Ph.D2

1Perfusion Department, Great Ormond Street Hospital for Children, London, United Kingdom

2Faculty of Health Sciences, University of Southampton, Southampton, United Kingdom

3Perfusion Department, John Radcliffe Hospital, Oxford, United Kingdom

**Conflict of Interest Statement:** The study was funded by Eurosets s.r.l, Mirandola, Italy by way of a research grant to R.I for the biochemical analysis used in this study. The authors had full control of the design of the study, methods used, outcome measurements, analysis of data, and production of the written report.

**Corresponding Author:**

Richard Issitt

Perfusion Department,

Great Ormond Street Hospital

London, WC1N 3JH

United Kingdom

Email: Richard.Issitt@gosh.nhs.uk

International Standard Randomised Controlled Trial Number Register: ISRCTN56462370

Ethics Ref: 10/H0606/30

**Article Word Count: 4,869**

**Glossary of Abbreviations:**

CABG Coronary Artery Bypass Graft

CPB Cardiopulmonary Bypass

LME Lipid Microemboli

NSE Neuron Specific Enolase

PSB Pericardial Suction Blood

SCADs Small Capillary Arteriolar Dilatations

**Abstract**

**Objectives:** Lipid Microemboli (LME) are formed in pericardial suction blood which, when returned to the Cardiopulmonary Bypass (CPB) circuit, can pass through filter materials and are returned to the arterial cannula. LME have been observed to enter all major organs and have been associated with Small Capillary Arteriolar Dilatations (SCADs) in the brains of patients who have died following CPB. However, a causal relationship showing correlation between LME and organ dysfunction has not been demonstrated, or whether removal of LME results in improved organ function.

**Methods:** A prospective, single centre, randomised controlled trial examined 30 patients (15 per group) undergoing coronary artery bypass grafting using cardiopulmonary bypass with or without a lipid depleting filter. The effects of LME filtration on neurocognitive injury were assessed using Neuron Specific Enolase (NSE).

**Results:** The study group showed a significant reduction in LME after filtration of the pericardial suction blood [*p*<0.001] whilst the control group exhibited a significant rise in LME [*p*<0.001]. There was a significant reduction in peak NSE release [*p*=0.013] as well as significant attenuation throughout the postoperative period [*p*=0.002]. Correlation and regression analysis showed a significant relationship between the number of LME post-CPB and peak NSE release [*r*=0.42, *p*=0.02].

**Conclusions:** Several methods of LME filtration have been proposed but none provided a suitable, efficacious method for use within the clinical setting. The RemoweLL® CPB system removes significant numbers of LME from the cardiotomy suction. Furthermore, LME correlate to the release of a known marker of neurological injury.

**Introduction**

Major neurological injury following cardiac surgery using Cardiopulmonary Bypass (CPB) has an incidence of 1-5%, although select populations may have a stroke rate as high as 8-9% ([1](#_ENREF_1)). However, substantially more patients suffer with subtler forms of injury with studies observing postoperative cognitive and intellectual dysfunction in almost 50% of patients when examined by neuropsychological tests ([2](#_ENREF_2)). Furthermore, early postoperative neurological dysfunction correlates with progression of cognitive decline and impaired quality of life during later years ([3](#_ENREF_3)). Much of this dysfunction has been attributed to the presence of Lipid Microemboli (LME) from the surgical environment, passed through the CPB circuit into the aortic arch and onto the cerebral vessels. The reflection of lipid emboli in the microcirculation of the brain has been observed as Small Capillary Arteriolar Dilatations (SCADs) at the bifurcations of cerebral vessels ([4](#_ENREF_4), [5](#_ENREF_5)). Whilst work by Brooker and colleagues showed a direct relationship between the use of cardiotomy suction and SCADs, there has been no definitive proof that LME are responsible for neurological or neurocognitive dysfunction following CPB ([6](#_ENREF_6)). One possible reason for this is that ischaemic damage is attenuated by the surrounding capillary network, and dysfunction is thought only to occur when either several vessels in the same area are occluded, or occlusion occurs within the white matter which is less densely vascularised ([6](#_ENREF_6)).

Previous attempts to remove LME have met with limited success; 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. 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 ([7](#_ENREF_7)) or using excessive fat in order to gain higher measurement resolution which leads to the saturation and decrease in efficacy of the filter. Therefore, at present there are few suitable filtration methods for efficacious LME removal in the clinical setting once LME have entered the systemic circulation.

There have been several reports of increased concentrations of markers of neurologic dysfunction correlating to the duration of CPB ([8](#_ENREF_8)). One such marker is Neuron Specific Enolase (NSE), 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 ([9](#_ENREF_9)). Damage to the neuron cell membrane causes leakage of NSE into the blood and cerebrospinal fluid where it may be detected. NSE exhibits an increased response to CPB and serum levels of NSE demonstrate a significant association with postoperative neurocognitive outcome ([10](#_ENREF_10)). Other markers, such as S100β, have shown non-specificity and an inability to correlate with neurological or neuropsychological outcome ([11](#_ENREF_11)).

The aim of the current study was to establish if a new lipid filtration system (RemoweLL, Eurosets s.r.l, Mirandola, Italy), which takes a unique approach and prevents the entry of LME into the systemic circulation using a siphon mechanism (rather than filtering once systemic infiltration has occurred), could remove LME from the Pericardial Suction Blood (PSB), and determine whether this could attenuate the release of Neuron Specific Enolase.

**Materials and Methods**

Following Institutional Review Board, and Research Ethics Committee approval (10/H0606/30), a prospective, single centre, single blind, randomised, controlled study was performed in 30 patients undergoing CABG with CPB assigned to either a control or intervention (RemoweLL) extracorporeal circuit at University Hospital Southampton (Southampton, United Kingdom). The study was conducted between March 2013 and December 2015. Exclusion criteria included emergency or previous cardiac surgery, morbid obesity, renal or pulmonary dysfunction and evidence of existing cognitive impairment, as adjudged at the pre-surgical assessment and after consultation with the patient’s General Practitioner. All patients provided written, informed consent for inclusion in this study.

Operative Details

Both intervention and control groups received the same anaesthetic regime. 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 a microporous hollow fibre membrane oxygenator (containing a heat exchanger) with integrated cardiotomy reservoir. This contained either a conventional cardiotomy filter (Control; Admiral, Eurosets s.r.l.) or a lipid/leucocyte filter (Intervention; RemoweLL, Eurosets s.r.l). The circuit was primed with 2L lactated Ringer’s solution (Baxter, Thetford, United Kingdom) and mannitol 20% w/v (2.5mL/kg; Baxter) that contained 5000 units of heparin (Wockhart, Wrexham, United Kingdom). 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 Getinge Group, Gothenburg, Sweden) at an indexed flow rate of 2.4L/m2/min. Alpha stat blood gas management was used to control acid-base balance. Mean arterial pressure was maintained between 50-60mmHg with pharmacological manipulation if necessary. All patients were systemically cooled to nasopharyngeal temperatures between 32-34°C. After aortic clamping, electromechanical diastolic arrest was induced with the delivery of cold (4°C) blood cardioplegic solution (IVEX Pharmaceuticals, Larne, Northern Ireland). Biochemical compatibility was maintained using sodium bicarbonate as a buffering agent. Distal anastomoses were completed during a single period of aortic clamping. Proximal anastomoses were performed on a beating heart using an aortic partial occluding clamp. CPB was terminated after the patient was re-warmed to a nasopharyngeal temperature of 36°C. The PSB was sent to the integrated cardiotomy reservoir where it was kept separated from the systemic circulation until immediately prior to the end of the CPB period, whereupon it was reintroduced via the venous reservoir back into the systemic circulation.

Lipid Analysis

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 homogenise. 10µL was placed onto a Thoma Chamber and lipids counted under light microscopy with 40/0.65 optics. The lipids could be seen as spherical non-nucleated cells (Figure 1). 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.

Cerebral Injury

Blood samples were taken for analysis of NSE at pre-CPB, 5 minutes before the end of CPB, and 6 and 24 hours post-CPB as described previously by Bonacchi *et al*., ([12](#_ENREF_12)). Commercially available ELISA assays were performed at King’s College Hospital, London. Briefly, 25µL of sample was added to each well of prepared antibody solution (HRP Anti-NSE and Biotin Anti-NSE; 100µL) and incubated at room temperature for 1 hour. Following washing the sample was added to a TMB HRP-substrate and incubated for a further 30 minutes before absorbance was read at 620nm.

Statistics

In vivo data (unpublished) showed that the numbers of LME in the RemoweLL system post filtration compared to a standard circuit was 1095±579 vs. 2970±1405.29 particles/mL giving an average percentage removal of 63±8.4% and effect size of 1.745. Previous studies have shown that the lower the serum concentrations of NSE, the better the outcome of patients after CPB. For this reason a tentative a priori power calculation was undertaken based on Bonacchi’s work ([12](#_ENREF_12)) where the average postoperative peak serum NSE concentration was 17.7±6.5µg/L. An assumption was made that for a significant, clinically relevant, difference in peak circulating NSE, a minimum reduction of 33% 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 was calculated. The number of patients in each study group (25 per group) 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 the primary objective (LME removal) and a power (1-β) of 80% and α=0.05 for the secondary objective (peak NSE concentration). However, no data were available to indicate the direct relationship that LME filtration will have on biochemical markers of organ injury; for this reason, and following consultation with a Statistician, an Interim Assessment using the Haybittle-Peto boundary was prospectively planned after 20 patients. This showed an effect size of 3.5 for the primary objective and 1.2 for the secondary objective. Therefore, a revised power calculation showed a cohort of 30 patients (15 per group) was required for the same assumptions as above.

Primary and secondary endpoints were analysed using the SPSS statistical package (SPSS, Chicago, USA) and a post hoc power analysis was used to compute achieved power for the secondary objective. This was independently verified. Assessment of normal distribution was carried out using the Shapiro-Wilk Test, and confirmed using a QQ Plot. 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. Two Factor ANOVA tests were undertaken to examine repeated measures. All tests were considered to be Two Tailed. Correlation and Regression analysis using Pearson’s Coefficient were used to examine any relationships between LME numbers and NSE concentrations. A *p* value <0.05 was considered significant. Normally distributed data are presented as mean±standard deviation whilst non-normally distributed data are presented as median (IQR). Both data are graphically displayed as box and whisker plots.

**Results**

Thirty patients successfully completed the study. Demographic data are shown in Table 1. There were no significant differences between the 2 patient groups. However, there was a trend towards shorter cross clamp time in the intervention (RemoweLL) group (*p*=0.08). Both groups were evenly matched for sex, age, comorbidities, pre-operative statin regime and number of grafts. There were no differences in transfusion rates or haemoglobin levels between the 2 groups at any time point (*p*>0.05), and no patients exhibited any gross neurological deficits.

Both groups processed similar volumes of PSB [control 776.67±632.14mL vs. intervention 780.00±567.20mL; *p*=0.99;] whilst the sedimentation time (the time PSB left in the cardiotomy reservoir) was similar in both groups [control 74.93±19.27mins vs. intervention 67±17mins; *p*=0.23]. Baseline LME counts (n/µL; Table 2) 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 control group [1,200 (200); *p*<0.001] (Figure 2). Post op differences between the control and intervention group were significant [1,200 (200) vs. 100 (75) respectively; *p*<0.001].

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 intervention group [control 23 (6.5) µg/L vs. intervention 16 (7) µg/L; *p*=0.012; Figure 3]. Subsequent reductions were seen towards baseline in both groups, although those patients in the control group continued to show elevated NSE levels compared to those in the intervention group [*p*=0.01 6hr post-CPB; *p*=0.004 24hr post-CPB]. Compared to baseline values both groups remained statistically elevated at 24hr post-CPB [control 10 (3.5) µg/L vs. 14 (4) µg/L, *p*=0.003; intervention 10 (1) µg/L vs. 11 (1.5) µg/L, *p*=0.03]. Post-hoc power analysis showed an effect size (d) of 1.1 for the secondary objective, demonstrating an achieved power (1-β) of 0.83.

Analysis of correlation showed no relationship between CPB time, volume of PSB or sedimentation time and the numbers of LME (Table 3). However, comparison 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].

**Discussion**

The role of LME in organ injury has been postulated but so far no definitive causal evidence exists. For this reason, this study set out to establish the efficacy of a new LME filtration system that is situated in the cardiotomy reservoir of a CPB circuit, as this circuit component has been shown to be the major source of LME in patients undergoing CPB ([5](#_ENREF_5)). The RemoweLL cardiotomy reservoir consists of 2 filtering mechanisms, as opposed to the traditional 1 used in standard cardiotomy reservoirs. The first uses a 40µm membrane, similar to cardiotomy reservoirs, but specifically coated to provide multilayer filtration for leucocytes and lipids. The blood then passes into the sedimentation chamber where it is kept separate from the circulating volume to obtain a supernatant. The supernatant, rich in lipid particles, is blocked by the siphon (the second filtration method) at the base of the reservoir, which is then discarded following re-infusion of the lipid-filtered PSB (Figure 4). Lipid Microemboli removal was assessed by counting the number of LME present in the PSB once the cardiotomy suction had been initiated, and then again in the systemic circulation following re-infusion of PSB into the CPB circuit. The results show a significant efficacy for lipid removal compared to the control group. In the intervention group 82.8% of the LME were removed [*p*<0.001] with a 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*<0.001]. Although the separation time was longer at 75 minutes this did not reach significance compared to the intervention group [*p*=0.23]. Whilst a propofol infusion was used for maintenance of anaesthesia, it is unlikely this influenced LME numbers as both groups had the same dosage regime and there was no difference in triglyceride levels between the two groups (data not shown).

Despite advances in Perfusion technology, current estimates of neurological injury following CPB show 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 ([13](#_ENREF_13)). Current CPB circuitry does not prevent the passage of LME from the cardiotomy suction and into the patient’s systemic circulation and previous work has shown the distribution of LME throughout the major organs ([14](#_ENREF_14)). Of particular concern are the possible effects upon neurological function that LME pose; thousands of microemboli have been observed distributed throughout the brain ([4](#_ENREF_4)).

Neuron specific enolase was chosen as a surrogate marker of neurological function as serum levels of NSE exhibit a significant association with postoperative neurocognitive outcome ([15](#_ENREF_15)) whereas other markers such as S100β have shown non-specificity and an inability to correlate with neurological or neuropsychological outcome ([11](#_ENREF_11)). 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 ([16](#_ENREF_16)). 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 these authors speculated that this may be due to insufficient sample size to detect differences of this magnitude ([17](#_ENREF_17)). This study observed a peak reduction in NSE release in the filtration group at the end of CPB [Control 23 (6.5)µg/L vs. Intervention 16 (7)µg/L; *p*=0.013], and further significant differences at both the 6 and 24 hours post CPB sample times [Control 18 (6)µg/L and 14 (4)µg/L vs. Intervention 14 (4.5)µg/L and 11 (1.5)µg/L; *p*= 0.01 and 0.005 respectively]. Moreover, we observed a direct correlation between the number of LME and NSE release. This is the first study to show a difference in a known neurological injury marker between groups of patients that have had LME filtered and those undergoing standard CPB. Whilst it would be imprudent to extrapolate these results to long-term neurological outcome, the results are suggestive that further work would be warranted and provide biochemical evidence for a role of LME in neurological dysfunction. 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. ([12](#_ENREF_12)). They reported peaks of 17.7±6.5µg/L with and IQR (9.8-25) in the CPB group, which is similar to the peak concentrations seen in the filtration group [16 (7)µg/L] but much lower than those in the control group [23 (6.5)µg/L]. However, there are 2 possible 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 ([18](#_ENREF_18)). Secondly, and more importantly, the CPB group of Bonacchi et al., did not have cardiotomy blood returned to them. The rationale behind this was that the study was investigating the use of S100β which is also contained within the heart, aorta and mediastinal tissues which are disrupted during cardiac surgery causing potential contamination from non-cerebral sources ([8](#_ENREF_8)). This inadvertent observation from Bonacchi provides a further control for this study; the level of NSE increase seen in the filtration group is equivalent to discarding the PSB. However, there are major drawbacks in the discarding of PSB, not least is the increase in blood transfusion requirements and increase in postoperative bleeding ([19](#_ENREF_19)).

*Limitations*

This study has several limitations. Whilst contemporary literature has been provided that identifies a relationship between LME, NSE, and cognitive function, whether LME filtration would attenuate adverse cerebral events cannot be answered, as the actions of LME will be dependent upon which vessels are affected, and whether they are located in areas of low or high vascularisation. The aim of this study 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 current study is the first demonstration that LME filtration is not only possible, but that prevention of LME from entering the patient’s systemic circulation can attenuate the release of a known marker of neurological injury.

The patient cohort under investigation was CABG-only patients. One reason for this 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. However, one might argue that this was unnecessary, as gaseous emboli would be equal in both groups and this study was not focussing on clinical outcomes. Testing of the LME removal system in complex cases (such as redo, valves, aortic surgery etc.), that require longer bypass times and mandate the PSB to be recycled during CPB, were not examined. Further work into clinical outcomes should involve more complex cases and involve patient groups that were excluded in this study, especially as there is evidence that suggests those with pre-existing neurocognitive or renal impairment might benefit more from LME filtration.

*Summary*

This study has shown the efficacious filtration of LME in the clinical setting using the RemoweLL lipid filtration system, and the subsequent attenuation of NSE release, a known marker of neurological injury. Furthermore, our data suggest a direct correlation exists between the number of LME and the level of NSE release. Further work is now planned to determine if this translates into longer-term neurocognitive protection.

**References**

1. Redmond JM, Greene PS, Goldsborough MA et al. Neurologic injury in cardiac surgical patients with a history of stroke. The Annals of thoracic surgery 1996;61(1):42-47.

2. Murkin JM. Neurological outcomes after opcab: How much better is it? The heart surgery forum 2000;3(3):207-210.

3. Newman MF, Kirchner JL, Phillips-Bute B et al. Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery. The New England journal of medicine 2001;344(6):395-402.

4. Moody DM, Brown WR, Challa VR, Stump DA, Reboussin DM, Legault C. Brain microemboli associated with cardiopulmonary bypass: A histologic and magnetic resonance imaging study. The Annals of thoracic surgery 1995;59(5):1304-1307.

5. Brooker RF, Brown WR, Moody DM et al. Cardiotomy suction: A major source of brain lipid emboli during cardiopulmonary bypass. The Annals of thoracic surgery 1998;65(6):1651-1655.

6. Brown WR, Moody DM, Challa VR. Cerebral fat embolism from cardiopulmonary bypass. Journal of Neuropathology & Experimental Neurology 1999;58(2):109-119.

7. Chen YF, Tsai WC, Lin CC et al. Leukocyte depletion attenuates expression of neutrophil adhesion molecules during cardiopulmonary bypass in human beings. The Journal of thoracic and cardiovascular surgery 2002;123(2):218-224.

8. Anderson RE, Hansson LO, Nilsson O, Liska J, Settergren G, Vaage J. Increase in serum s100a1-b and s100bb during cardiac surgery arises from extracerebral sources. The Annals Of Thoracic Surgery 2001;71(5):1512-1517.

9. Karkela J, Bock E, Kaukinen S. Csf and serum brain-specific creatine kinase isoenzyme (ck-bb), neuron-specific enolase (nse) and neural cell adhesion molecule (ncam) as prognostic markers for hypoxic brain injury after cardiac arrest in man. Journal of the neurological sciences 1993;116(1):100-109.

10. Ramlawi B, Rudolph JL, Mieno S et al. Serologic markers of brain injury and cognitive function after cardiopulmonary bypass. Ann Surg 2006;244(4):593-601.

11. Westaby S, Saatvedt K, White S et al. Is there a relationship between serum s-100beta protein and neuropsychologic dysfunction after cardiopulmonary bypass? The Journal of thoracic and cardiovascular surgery 2000;119(1):132-137.

12. Bonacchi M, Prifti E, Maiani M, Bartolozzi F, Di Eusanio M, Leacche M. Does off-pump coronary revascularization reduce the release of the cerebral markers, s-100beta and nse? Heart, Lung & Circulation 2006;15(5):314-319.

13. Brown WR, Moody DM, Challa VR, Stump DA, Hammon JW. Longer duration of cardiopulmonary bypass is associated with greater numbers of cerebral microemboli. Stroke; a journal of cerebral circulation 2000;31(3):707-713.

14. Brondén B, Dencker M, Allers M, Plaza I, Jönsson H. Differential distribution of lipid microemboli after cardiac surgery. The Annals of thoracic surgery 2006;81(2):643-648.

15. Ramlawi B, Rudolph JL, Mieno S et al. Serologic markers of brain injury and cognitive function after cardiopulmonary bypass. Annals of surgery 2006;244(4):593-601.

16. Rasmussen LS, Christiansen M, Rasmussen H, Kristensen PA, Moller JT. Do blood concentrations of neurone specific enolase and s-100 beta protein reflect cognitive dysfunction after abdominal surgery?Ispocd group. British journal of anaesthesia 2000;84(2):242-244.

17. Rasmussen LS, Christiansen M, Eliasen K, Sander-Jensen K, Moller JT. Biochemical markers for brain damage after cardiac surgery -- time profile and correlation with cognitive dysfunction. Acta anaesthesiologica Scandinavica 2002;46(5):547-551.

18. Nygaard O, Langbakk B, Romner B. Neuron-specific enolase concentrations in serum and cerebrospinal fluid in patients with no previous history of neurological disorder. Scandinavian journal of clinical and laboratory investigation 1998;58(3):183-186.

19. Rubens FD, Boodhwani M, Mesana T, Wozny D, Wells G, Nathan HJ. The cardiotomy trial: A randomized, double-blind study to assess the effect of processing of shed blood during cardiopulmonary bypass on transfusion and neurocognitive function. Circulation 2007;116(11):I-89-i-97.

**Tables**

|  |  |  |  |
| --- | --- | --- | --- |
|   | Control | Intervention |   |
|   | Mean | SD | Mean | SD | *p* |
| Male (n) | 12.00 |   | 11.00 |  |   |
| Diabetes (n) | 2.00 |   | 3.00 |   |   |
| Statin (n) | 8.00 |   | 8.00 |   |   |
| Age (years) | 69.93 | 7.54 | 69.33 | 7.29 | 0.83 |
| Height (m) | 1.76 | 0.10 | 1.71 | 0.08 | 0.10 |
| Weight (kg) | 87.51 | 13.37 | 82.84 | 14.90 | 0.37 |
| Body Mass Index | 28.15 | 3.56 | 28.31 | 4.15 | 0.91 |
| Body Surface Area | 2.07 | 0.20 | 1.98 | 0.21 | 0.24 |
| Calculated Flow (l/min) | 4.96 | 0.47 | 4.74 | 0.50 | 0.24 |
| Bypass Time (min) | 101.40 | 22.01 | 88.47 | 23.51 | 0.13 |
| X-Clamp Time (min) | 62.67 | 17.67 | 51.20 | 17.11 | 0.08 |
| Procedure (CABG x N) | 3.33 | 0.49 | 3.13 | 0.83 | 0.43 |
| Fluid Balance (mL) | 1678.60 | 842.38 | 1562.27 | 867.16 | 0.71 |
| Time of Cardiotomy Release (min) | 74.93 | 19.27 | 67 | 17 | 0.23 |
| Volume in Cardiotomy Reservoir (mL) | 776.67 | 632.14 | 780.00 | 567.20 | 0.99 |

**Table 1. Patient Demographics**

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.

|  |  |  |  |
| --- | --- | --- | --- |
|   | Control | Intervention |   |
|   |   |   | *p* |
| Pre-CPB LME Count (n/µL) | 400 (200) | 400 (400) | 0.47 |
| Post-Release LME Count (n/µL) | 1200 (200) | 100 (75) | *<0.001* |
| Pre-CPB NSE (µg/L) | 10 (3.5) | 10 (1) | 0.32 |
| End-CPB NSE (µg/L) | 23 (6.5) | 16 (7) | *0.012* |
| 6-Hour Post-CPB NSE (µg/L) | 18 (6) | 14 (4.5) | *0.01* |
| 24-Hour Post-CPB NSE (µg/L) | 14 (4) | 11 (1.5) | *0.004* |

**Table 2. Perioperative Data**

Data presented as median (IQR). LME counts taken from the start of cardiotomy suction (pre-CPB) and from the arterial sampling port following the re-introduction of PSB from the cardiotomy reservoir. NSE was taken following induction of anaesthesia and placement of central venous and arterial catheters. A significant difference was observed in the post-release LME counts (*p*<0.001) as well as the end-CPB, 6-hour post-CPB and 24-hour post-CPB NSE levels (*p*<0.05).

|  |  |
| --- | --- |
| Measure  | Correlation |
|   | Pre-CPB NSE | End-CPB NSE | 6-Hour Post-CPB NSE | 24-Hour Post-CPB NSE |
| Bypass Time | 0.25 (0.19) | 0.28 (0.13) | 0.02 (0.93) | 0.29 (0.12) |
| Time of Cardiotomy Release | 0.26 (0.17) | 0.24 (0.19) | 0.17 (0.36) | 0.15 (0.44) |
| Cardiotomy Volume | 0.01 (0.96) | 0.15 (0.42) | 0.02 (0.93) | 0.21 (0.26) |
| Pre-CPB LME Count | 0.13 (0.48) | 0.16 (0.4) | 0.05 (0.79) | 0.13 (0.5) |
| Post-Release LME Count | 0.27 (0.16) | 0.42 (*0.02*) | 0.41 (*0.02*) | 0.4 (*0.03*) |

**Table 3. Correlation Analysis**

Data expressed as correlation coefficient, *r*, with *p* values in parentheses. All tests used Pearson’s correlation. Critical *r* for 28 patients (d.f. = n-2) were 0.374 (0.05) and 0.588 (0.001).

**Figures**

**Figure 1. Lipid Microemboli under Microscopy**

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

**Figure 2. Pre vs. Post LME Counts**

Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values. Blue; control, Red; intervention. Both groups showed significant changes in total LME counts compared to baseline and to each other (*p*<0.001). Control group showed a mean increase of 115.7% in LME whilst filtration group saw an 82.8% decrease.

**Figure 3. Neurone Specific Enolase Release**

Data presented as box and whiskers plots with boxes representing 25th-75 centiles with median and whiskers as maximum and minimum values. Blue; control, Red; intervention. Both groups saw a significant increase with peak concentrations at the end of CPB (*p*<0.001); however, there was significantly attenuated peak in the filtration group compared to the control (*p*=0.012). Repeated measures ANOVA analysis showed significantly less NSE release in the filtration group throughout the sampling period (*p*=0.002).

**Figure 4. The RemoweLL Lipid Filtration System**

The RemoweLL® ECC system comprising 2 filtering mechanisms: a leucocyte filter and lipid microemboli siphon. A 40µm membrane provides multilayer filtration for leucocytes and lipids, whilst the siphon, at the base of the cardiotomy reservoir, prevents the reinfusion of the lipid rich supernatant which is then discarded.