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Low frequency ventilation during cardiopulmonary bypass for lung protection: A randomised controlled trial

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Low frequency ventilation during cardiopulmonary bypass for lung protection: A randomised controlled trial

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Key question

Does low frequency ventilation (LFV) during cardiopulmonary bypass (CPB) improve inflammatory markers and lung function compared to both lungs left collapsed in patients undergoing CABG?

Key findings

There were no significant differences between groups in inflammatory markers measured in the lung tissue and blood.

Take-home message

LFV during CPB when compared to both lungs left collapsed does not reduce inflammation in lung biopsies and blood.

Glossary of Abbreviations

Low frequency ventilation (LFV)

Cardiopulmonary bypass (CPB)

Coronary artery bypass grafting (CABG)

Acute lung injury (ALI)

Adult respiratory distress syndrome (ARDS)

Positive end-expiratory pressure (PEEP)

cardioplegic arrest (CA)

Pulmonary function tests (PFTs)

Respiratory index [(PAO_2-PaO_2)

Forced vital capacity (FVC)

Forced vital capacity ratio (FVCR)

forced expiratory volume in one second (FEV_1)

Forced expiratory volume after one second (FEV_1)

Continuous positive airway pressure (CPAP)

Geometric means (GM).

Means and standard deviations (SDs)

Ventilation/perfusion distribution (V/Q)

Adenine nucleotides (ATP, ADP, AMP)

Conventional mechanical ventilation (CV)

Open Lung Concept (OLC)

Partial pressure of oxygen (pO_2) (paO_2)

Nuclear Factor kappa-light-chain-enhancer of activated B cells **(NF-κB)**

Abstract

Objective: Pulmonary dysfunction is a common complication in patients undergoing heart surgery. Current clinical practice does not include any specific strategy for lung protection. To compare the anti-inflammatory effects of low frequency ventilation (LFV), as measured by NF-κB p65 pathway activation, for the entire cardiopulmonary bypass (CPB) versus both lungs left collapsed in patients undergoing coronary artery bypass grafting (CABG)

Methods: Two group parallel randomised controlled trial. Primary outcome was inflammation measured by NF-κB p65 activation in pre- and post-CPB lung biopsies. Secondary outcomes were additional inflammatory markers in both biopsy tissue and blood.

Results: 37 patients were randomly allocated to LFV (18) and to both lungs left collapsed (19). The mean concentration of NF-κB p65 in the biopsies before chest closure (adjusted for pre-CPB concentration) was higher in the LFV group compared to both lungs left collapsed group but this was not significant (0.102, 95% CI -0.022 to 0.226, p=0.104). There were no significant differences between groups in the other inflammatory markers measured in tissue and blood.

Conclusions: In patients undergoing elective CABG, the use of LFV during CPB when compared to both lungs left collapsed does not seem to reduce inflammation in lung biopsies and blood.

Abstract words count: 202

Keywords: Low frequency ventilation, cardiopulmonary bypass, lung protection, Lung biopsy, NF-kB

Introduction

Pulmonary dysfunction is a common complication for patients after cardiac surgery using cardiopulmonary bypass (CPB) [1]. Severity ranges from mild atelectasis to life threatening acute lung injury (ALI) or respiratory failure requiring prolonged postoperative ventilation [2] or adult respiratory distress syndrome (ARDS) [3-5]. Harmful effects of CPB on pulmonary function persist despite advances in anaesthetic techniques [6]. Pulmonary dysfunction after cardiac surgery also affects clinical outcomes with increasing morbidity, mortality and delaying discharge from hospital, leading to increase in the health care resources used and their associated cost [3, 7-13]

Presumed causative factors for atelectasis and ALI, include inflammation, prolonged lung collapse, pulmonary ischemia and related reperfusion injury, blood contact with the surface of the heart-lung machine, endotoxemia, surgical trauma, blood loss and transfusion [14, 15]. Inflammatory activation and cytokine release have been correlated with outcome after cardiac surgery [16]. Pulmonary function 24 hrs after CPB is associated with raised plasma levels of inflammatory cytokines and reduced levels of anti-inflammatory cytokines [17]. The inflammatory response from the lung during CPB and mechanical ventilation originates at the alveolar membrane as a result of collapse, ischemia, reperfusion injury and mechanical stress [18-20]. Suppression of activation of inflammatory mediators during CPB is associated with a reduction in pulmonary dysfunction [21].

Current clinical practice does not include any specific strategy for lung protection during CPB. When CPB is started, often both lungs are left collapsed for the entire CPB duration. We recently provided evidence in an experimental pig model that low frequency ventilation (LFV) during CPB reduces post-CPB lung injury [22].

Here, we report an evaluation of the effect of ventilating the lungs at low frequency during CPB comparing to collapsing the lungs in patients having coronary artery bypass graft surgery (CABG) with respect to inflammation measured by NF-κB p65 pathway activation and post-operative pulmonary dysfunction. We used a primary outcome measure that would give an early indication of inflammatory changes in the lungs and would allow detection of a large effect in a relatively small trial.

Materials and Methods

This study was a single-centre, two-group parallel randomised controlled trial. Patients were randomly assigned in a 1:1 ratio, using a secure concealed internet-based randomisation system (Sealed Envelope™, https://www.sealedenvelope.com/). Cohort minimisation was used to achieve balance between groups with respect to baseline lung function (≥60% predicted FEV_1). Patients returned to hospital for a follow up visit 6-8 weeks following the operation.

Study period between 07 January 2013 to 27 June 2014. Trial registration ISRCTN-34428459, protocol approved by the NRES London- Camden and Islington (REC-12/LO/0458). Protocol published at http://www.isrctn.com/ISRCTN34428459.

Trial Population

Patients having elective or urgent CABG with CPB and cardioplegic arrest (CA) at the Hammersmith Hospital. (Table 1)

Inclusion criteria

- Age ≥40 and <85 years
- Left ventricular ejection fraction >30%

Exclusion criteria

- Previous pulmonary embolism requiring long term warfarin for ≥3 months
- Previous cardiac surgery
- Current congestive heart failure (NYHA class IV)
- Chronic renal failure
- Emergency or salvage operation
- On corticosteroid or immunosuppressive treatment
- Severe chronic obstructive pulmonary disease, lung pathology, previous radiotherapy,
- Body mass index >35

Ventilation Protocol

Before starting CPB, for all participants, the lungs were ventilated with a tidal volume of 6-8 $ml.kg^{-1}$, I:E ratio of 1:2, positive end-expiratory pressure (PEEP) of 5cm H_2O and FiO_2 of 0.5 (range 0.45-0.55 O_2). The ventilatory rate was set to keep the $PaCO_2$ between 4.5 and 5.5 kPa.

In the comparator group (both lungs left collapsed), at the onset of CPB the lungs were disconnected from the ventilator and allowed to collapse completely for the duration of CPB.

In the treatment group (low frequency ventilation, LFV), the respiratory rate was maintained during CPB at 5 bpm with ($FiO_2 \cong 0.25$). PEEP was turned off during LFV but the tidal volume and inspiration to expiration (I:E) ratio were maintained at 6-8 $ml.kg^{-1}$ and 1:2. At the end of the CPB, patients in both groups had a lung recruitment manoeuvre using an FiO_2 of 0.5 and holding the lungs inflated for 15sec at 30cm H_2O, before the lungs were reconnected to the ventilator. The recruitment manoeuvre was repeated if necessary, though this event was not recorded on the data. No other variation in ventilation was permitted or necessary. The same ventilator protocol was used after CPB as before CPB with a PaO_2/FiO_2 >50 required on first post-bypass gas (i.e. PaO_2>25kPa). If PaO_2/FiO_2 <50 then the recruitment manoeuvre was repeated.

Anaesthetic protocol

Following premedication with temazepam (dose 20-30mg), anaesthesia was induced with propofol and remifentanil, using pancuronium 0.1 mg/kg for muscle relaxation. This was maintained by infusion of propofol and remifentanil (5mg remifentanil to 1g propofol), with isoflurane if required to keep the entropy value of the processed EEG below 55. At chest closure, 7μg/kg fentanyl was given in combination with plain propofol, which was switched to a propofol/remifentanil mixture for the transfer to the cardiac intensive care unit. It is important to mention that mixing remifentanil in propofol was at the time of our study common practice and was used in every subject of both groups. O'Connor's work [23] was published shortly after recruitment to our study had finished, and in any case may not be relevant because our syringes were kept horizontal throughout the procedure.

Perfusion protocol

A standard CPB was used, primed with 1400 ml of Hartmann's solution and 10000 IU of heparin. Systemic temperature was between 32°C and 35°C. Cardioplegic arrest was achieved with intermittent antegrade cold blood cardioplegia.

Lung biopsy protocol

To measure inflammatory markers in the lung, two lung biopsies (1cm x 1cm) were taken using the LigaSure Impact™ instrument (LF4318, Covidien, Minneapolis, USA). The first (pre-CPB) biopsy was taken from the left upper lobe immediately after sternotomy and the second from the left lower lobe prior to weaning from CPB after lung recruitment manoeuvre (see above).

The criteria for extubation were: Normothermia (a core temperature range of 36.0°C to 37.0°C); haemodynamical stability and blood loss <50mls/h; comfortable breathing with good bilateral air entry (RR 10-20/min, tidal volumes 8-10mls/kg, minimal tracheal suction) and arterial blood gases with parameters PaO_2 >10kpa on FiO_2 <0.5, PCO_2<7 kPa, BE -5 to +5.

Blood samples were taken: i) after anaesthetic induction and pre-sternotomy, ii) 10 minutes after the end of CPB, and iii) 2, 6 and 24 hours after the end of CPB. No blood sample were taken during CBP as our end points was to look into the effect before and after CPB.

Outcomes

The primary outcome was inflammation measured by NF-κB p65 activation in pre- and post-CPB lung biopsies. This outcome measure was chosen because exposure of a cell to a cytokine or an infectious agent leads to binding to a cell surface receptor and activation of a kinase cascade resulting in the nuclear translocation of the master pro-inflammatory transcription factor NF-κB. NF-κB is known to drive the expression of most inflammatory genes [24, 25].

Secondary outcomes included additional inflammatory markers in both biopsy tissue and blood. Namely, *p38 MAPK* phosphorylation, expression of *TNFα, IL1β, IL18, IL6, IP10, IL8, IL10, chemokine receptor CXCR3 and Caspase 3* measurements of apoptosis in biopsies. ROS levels, phosphorylation of p38 and NF-κB p65 in blood.

Laboratory analysis

Blood samples were taken: i) after anaesthetic induction and pre-sternotomy, ii) 10 minutes after the end of CPB, and iii) 2, 6 and 24 hours after the end of CPB.

Leukocytes were fixed and lysed with BD Phosflow Lyse /Fix buffer, (BD Biosciences, Oxford, UK). Samples were stained using a redox-sensitive fluorescent probe 3'-(*p*-aminophenyl) and stained with antibodies raised against phosphorylated p38 (Thr180/Tyr182) (Cell Signaling ♯6908, Danvers, MA, USA) and NF-κB p65 (Ser529) (Cell Signaling ♯4887). Samples were analysed by flow cytometry compared to unstained controls.

Biopsies:

The pre-CPB biopsy provided a within-subject control for the second, post-CPB biopsy.

NF-κB p65 nuclear localization and activation was assessed by immunofluorescent staining followed by confocal microscopy and by testing nuclear lysates by DNA-binding ELISA (TransAm Assay, Carlsbad, USA).

Measurements of *p38 MAPK* phosphorylation in biopsies were carried out using Western blotting and were analysed and expressed as ratio of phosphorylated p38 and total p38. Expression of

TNFα, IL-1β, IL-18, IL-6, IP-10, IL-8, IL-10 and chemokine receptor CXCR3 were done using ELISA and qPCR.

For RNA extraction biopsies were homogenised in RLT buffer (Qiagen, Hilden, Germany), containing beta mercaptoethanol (Sigma-Aldrich, St Louis, USA). RNA was quantified using nanodrop and reverse transcribed to (ThermoFisher Scientific, Waltham, USA).

Gene expression was measured using Taqman qPCR and normalised to *18S* rRNA using the ΔΔct method.

For protein extraction biopsies were homogenised in either radioimmunoprecipitation assay (RIPA) (Sigma Aldrich) buffer or Lysis buffer AM1 (Active Motif).

Protein concentration was determined using the bicinchoninic acid assay (ThermoFisher Scientific). ELISA was used to measure expression of TNFα, IL-1β,IL-18, IL-6, IP-10, CXCL-8, and IL-10 in the cytoplasmic fraction. Caspase 3 activity was measured by colormetric assay kit (Abcam, Cambridge, UK), p38 phosphorylation by ELISA and Western Blot [26].

Other secondary outcomes included pulmonary function tests (PFTs), pulmonary gas exchange and adverse events

Pulmonary gas exchange was assessed by the respiratory index [(PAO_2-PaO_2) / (PaO_2)] measured i) post-induction and pre-sternotomy, ii) 10 minutes following CPB weaning, iii) 2 hours post CPB, iv) 4 hours post CPB, v) first gas post extubation, vi) 12 hours post CPB, and vii) before removal of the arterial line.

Pulmonary function tests (PFTs) were carried out pre-operatively and at 6-8 weeks post-surgery. PFTs included forced vital capacity (FVC), forced vital capacity ratio (FVCR), forced expiratory volume in one second (FEV_1), forced expiratory volume after one second (FEV_1) and FEV to FEV_1 ratio. Pulmonary function was assessed by a combination of the following tests: spirometry, gas diffusion and thoracic gas volume.

During hospital stay and at the follow up visit patients underwent a pulmonary function test and any documented occurrence of adverse events were recorded.

Statistical Analysis

Without taking the baseline biopsy into account, it was calculated that a sample size of 32 patients would be able to detect a standardised difference of 1.0 with 80% power and 5% significance (2-tailed). If the baseline biopsy improves the relative efficiency of the comparison (with an estimated correlation of 0.5 between measures of the primary outcome for the pre/upper lobe and post/lower lobe), the trial either had more power (88%) or was able to detect a smaller target difference (0.9SD). These standardised differences (1.0 or 0.9) represent large differences between groups; we justified the plausibility of this large target difference on the grounds that the primary outcome was chosen to assess the biomarker and site (i.e. the lungs) which we hypothesised would be most directly influenced by the intervention.

This target sample size was able to detect a standardised difference of 0.75 in biomarkers measured in the serum with 80% power and 5% significance (2-tailed), assuming a correlation of

0.5 between pre and post-intervention measures and a correlation of 0.7 between the four repeated post-intervention measures.

The study was not powered to detect differences between the groups in pulmonary function or adverse events. Specific adverse events were too infrequent to be able to detect differences between groups. Frequencies of these adverse outcomes are tabulated, in line with guidelines for reporting adverse events in trials. The trial was an "early phase" and it aimed at identifying an intervention worth taking forward to late phase 3 trials quickly and relatively inexpensively, hence the choice of a primary outcome and an effect size that would allow a small sample size.

Primary analyses were by intention-to-treat. The final analyses were performed after the database had been locked and the statistical analysis plan approved. The statistical software STATA (version 13.2) was used to analyse the data as well as to generate tables, figures, and listings.

Most of the data measured continuously scaled outcomes which are summarised as means and SD, at each time point if measured more than once, in each treatment group. If distributions were non-normal, appropriate transformations were used. Analyses were carried out on the transformed data and the findings were transformed back to the original scale where possible, e.g. if a logarithmic transformation was used then the results are presented as geometric means (GM).

For inflammatory markers in biopsy samples, where only one post-intervention measure was collected, models were fitted using linear regression to adjust for the baseline level. Each model estimated the main effect of group allocation (LFV vs. conventional management) and the baseline covariate.

For inflammatory markers in monocytes and polymorphonuclear cells from peripheral blood samples and pulmonary gas exchange expressed as *A-a gradient*, mixed regression models were fitted. These models estimated coefficients for group allocation and the interaction term for group allocation by time. If the interaction was significant ($p<0.05$), then a group comparison is reported at each time point. All results are presented as differences between, or ratios of, the means for the two groups with 95% CIs.

Results

Study Population

Forty-nine patients were eligible and were invited to take part in the trial between January 2013 and June 2014; 38 gave written informed consent, 8 declined for personal reasons and 3 preferred the standard procedure. One patient became ineligible (had off-pump CABG) and was withdrawn (see consort diagram, Fig 1); available data for the remaining 37 patients were analysed (18 in the LFV group and 19 in both lungs left collapsed CPB group). Baseline characteristics, pre-operative co-morbidities, operation details and baseline respiratory measurements were balanced (Table 1). Means and standard deviations (SDs) for all the markers are tabulated in (TableA1 and TableA2). Adverse events during hospital stay and at follow up are tabulated in (Table 2), Figure 2 illustrates the treatment effect and (Figure 3) illustrates the raw data on a log scale (for y axis).

Primary outcome

The mean concentration of NF-κB p65 in the biopsies before chest closure (adjusted for pre-CPB concentration) was higher in the LFV group compared to both lungs left collapsed CPB group but

this was not significant (Table A1, Table A2a), (Figure 2a, Figure3a); (0.102, 95% CI -0.022 to 0.226 p=0.104).

Secondary outcomes

Biopsy markers analysis results are summarised in (Table A2) and (Figure 2a).

The mean concentration of p38 MAPK in the biopsies before chest closure (adjusted for pre-CPB concentration) was lower for the LFV group compared to both lungs left collapsed CPB group but this was not significant. Expression of *IL18* and *IL10* were also lower in the LFV group compared to both lungs left collapsed CPB group but not by statistically significant amounts.

Gene expression of *TNFA*, *IL1B*, *IL6*, *IP10*, *IL8* and *CXCR3* as well as caspase activity were higher in the LFV group compared to the standard CPB group. These differences were statistically significant for *IL1B* and *IL6*. There were no significant differences in cytokine levels between each group.

Blood markers analysis results are summarised in (Table A1b, Table A2) and (Figure 2b).

Leukocytes were separated into monocytes, granulocytes and lymphocytes by forward and side scatter. The permeabilised monocytes and granulocytes, when measuring p38 MAPK and NF-κB p65, showed significant overlap by this method and were therefore treated as a single group.

There were no statistically significant differences in NF- κB, MAPK, ROS, or A-a gradients between groups.

NF-κB p65 and p38 MAPK levels in combined monocytes and granulocytes were lower in the LFV group compared to both lungs collapsed CPB group, but these differences were not statistically significant. NF-κB p65 levels in lymphocytes were higher in the LFV group compared to both lungs left collapsed CPB group but not significantly so. ROS levels were not significantly lower in all cell types in the LFV group compared to both lungs left collapsed group.

When time was fitted in the model, we found that p38 MAPK in lymphocytes levels were significantly lower in the LFV group at 2, 6 and 24 hours compared to 10 min. The interaction of intervention x time was not significant at any time point for any of the blood markers.

The A-a gradient was higher in the LFV group compared to both lungs left collapsed CPB group, but this was not significant Table A3. When time was fitted in the model, we found that there was a significant reduction in A-a gradient in time compared to 10 min in the LFV group. However, the interaction of intervention x time was not significant at any time point. No difference was found in lung functions between the two groups (Table A3b)

Frequency of adverse events are summarised in (Table 2).

Mask CPAP was necessary for one patient in the LFV group and 2 in both lungs left collapsed CPB group. Arrhythmia occurred in 50% of patients in the LFV group and 37% of patients in both lungs left collapsed CPB group. The biggest difference in groups for occurrence of adverse events was in relation to the need for haemodynamic support, 89% of patients in the LFV group vs 47% in both lungs left collapsed CPB group. Hospital and post discharge infective complications were similar between groups.

Discussion

This trial investigated the possibility of reducing the inflammatory response associated with CABG by a technique of ventilating of the lungs at low frequency during CPB. The change in

inflammatory response was measured in both tissue biopsy and blood using NF-κB p65 in lung biopsies and other inflammatory markers in both tissue and blood.

Our selection for NF-κB as a primary end point was because its activation can be induced upon physical or oxidative stresses resulting from cardiac surgery using CPB [27]. NF-κB seems to be a reasonable end point reflecting the adverse effect of the inflammation on the lungs. We could argue that it might not be very specific to lung tissue but rather systemic inflammatory status as it can be also produced by, physiological changes including ischemia and hyperosmotic shock, or by numerous inflammatory cytokines and chemokines [27]. Our choice to measure NF-κB in the lung tissue with a control sample as the primary end-point can be justified as CPB enhanced lung and systemic inflammation. Samples have been collected for future transcriptomic and proteomic analysis and this may help provide insight into possible pathways driving CPB.

Results showed levels of all markers apart from p38 MAPK, *IL18* and *IL10* measured in the lung biopsy tissue were higher in the LFV group compared to both lungs left collapsed CPB group. However only the increases in *IL6* and *IL1B* gene expression were statistically significant and these differences were counter to our working hypothesis that LFV would reduce inflammation following surgery. We observed a marked increase in the expression of a number of NF-κB-induced inflammatory markers at both the protein and mRNA level in lung tissue following CPB. The failure to observe any change in NF-κB p65 activation in tissue may reflect the rapid early nature of NF-κB activation compared to the time under CPB (in the LFV group median of 71min, IQR 63.5-93.5 and in both lungs left collapsed group median of 80min, IQR 60-92). Gene stimulation can lead to waves of NF-κB activation over time [28] and we may have missed a second round of NF-κB activation at the time points analysed chosen. However, we detected a marked effect on p38 MAPK activity in both lung tissue and in peripheral blood following CPB and the effect in blood reached significance over the time series and may have been greater if subsets of cells were analysed. It is possible that CPB is a greater activator of inflammation in response to oxidative stress than NF-κB and further studies would be required in disease models to test this hypothesis. Overall, there was no significant difference in the inflammatory markers measured in the blood between LFV and both lungs left collapsed CPB group.

A clinical study reported the use of continuous positive airway pressure (CPAP) during CPB in 14 elective cardiac surgery patients. Seven patients received CPAP at 10cm H_2O during CPB, and in the other seven patients, the lungs were open to the atmosphere (control). CPAP at 10cm H_2O resulted in significantly more perfusion of lung areas with a normal ventilation/perfusion distribution (V/Q) and significantly less shunt and low V/Q perfusion 4h after CPB in comparison with the control group. The authors concluded that CPAP at 10cm H_2O during CPB is a simple manoeuvre that improves postoperative gas exchange and resulted in significantly more perfusion of lung areas with a normal ventilation/perfusion distribution (V/Q) and significantly less shunt [29].

The effect of both low frequency ventilation (LFV) and continuous positive airway pressure (CPAP) during CPB to reduce post-CPB lung injury have been evaluated in an established experimental pig model [22]. This study strongly suggested that the use of LFV is associated with significantly better pulmonary gas exchange, higher adenine nucleotide, lower lactate dehydrogenase levels and reduced histological damage in lung biopsies as well as lower DNA levels in bronchoalveolar lavage compared to the control group. The rationale for this experimental study was to maintain some degree of ventilation during CPB to prevent persisting lung collapse and complete loss of gas exchange by passive diffusion at the blood–gas barrier. A similar concept was used by Reis Miranda and colleagues who studied 62 patients post cardiac

surgery, randomly assigned to three groups: (1) conventional mechanical ventilation (CV), (2) Open Lung Concept (OLC) started after arrival on the ICU and (3) OLC started directly after intubation. They observed an increase in functional residual capacity, reduced risk of hypoxemia and lower levels of IL-10 and IL-8 release, hence concluded that OLC ventilation leads to an attenuated inflammatory response, presumably by reducing additional lung injury after cardiac surgery [30].

A recent study was undertaken to examine the effect of maintaining ventilation during bypass compared with discontinued ventilation upon several parameters that may be indicative of lung injury. Twenty-three elective patients for CABG were randomised to either ventilation (VB) (n=12) or non-ventilation on bypass (NVB) (n=11). The post-bypass extravascular lung fluid was significantly smaller in the VB group compared to the NVB group and extubation time was significantly shorter [31], hence this study has shown the benefits of maintaining ventilation during CPB on post-CPB oxygenation and included shorter mechanical ventilation [31]. On the other hand, a small study of fifty-nine patients prospectively randomised to continuous ventilation and no ventilation, during CABG on CPB, showed there was no statistically significant difference in most of inflammatory makers (IL-6, IL8, IL-10 & lactate) [32]. A recent metanalysis for patients undergoing cardiac surgery and received ventilation during CPB, included seventeen trials with 1162 patients, showed that ventilation during CPB significantly increased post-CPB $PaO2/FiO_2$ ratio, but there was no sufficient evidence to show that ventilation during CPB could influence long-term prognosis of these patients [33].

The discordant conclusion from all previous studies on the effect of low frequency ventilation during CPB on lung function, was what prompted us to design and conduct this study. This is the first RCT to investigate the effects of low frequency ventilation in patients undergoing CABG with CPB by measuring inflammation directly in lung biopsies and blood samples.

Strengths and limitations

Our trial to the best of our knowledge is the first to report the effects of LFV on pulmonary inflammation in the blood and directly in lung biopsy in patients undergoing CABG with CPB. Random allocation was concealed, retention was good, data collection was blinded, and analyses were carried out and reported in accordance with a prespecified analysis plan. Therefore, the trial was at low risk of bias [34]. Although the sample size was small, there was no suggestion of any benefit from the adoption of LFV.

The surgical team could not be blinded, and we cannot rule out the possibility that this led to variations in surgical technique by group. The use of PEEP in the intraoperative mechanical ventilation has been associated with a reduction of atelectasis in postoperative period as reported by studies using high PEEP level (10 cm H2O) [35-37]. Overall the role of PEEP in surgery has been extensively studied with positive impression [33, 38-40]. In cardiac surgery particularly as the chest cavity is open, the lungs are arbitrarily exposed to atmospheric pressure, rather than normal negative intrathoracic pressure. Hence the transpulmonary pressure (airway pressure minus intrathoracic pressure) becomes abnormally low at end-expiration leading to collapse of the lungs, if we do not apply PEEP of at least 3-5 cm H2O. Nevertheless, in our treatment group we applied the LFV without PEEP. This is probably a major limitation that we could have avoided by adding PEEP to our LFV group.

Conclusion

Contrary to our working hypothesis low frequency ventilation (LFV) during CPB has not been demonstrated to reduce pulmonary or systemic inflammation compared to both lungs left collapsed and may in fact increase the levels of specific inflammatory cytokines.

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Neither the BHF nor the NIHR had any role in the design, conduct, analysis or reporting of the trial.

Figure Legends

Figure 1. Consort diagram

Figure 2a, b. Forest plot illustrating the treatment effect for each inflammatory marker.
a: in the lung tissue biopsies
b: in the blood samples

Figure 3. Raw data on log scale (for y and x axis) [solid line=LFV, dash line=both lungs left collapsed] for p65, p38, ROS and HEME in blood

Reference List

1. Grover, F.L., *The Society of Thoracic Surgeons National Database: current status and future directions.* Ann Thorac Surg, 1999. **68**(2): p. 367-73; discussion 374-6.
2. Hall, R.I., M.S. Smith, and G. Rocker, *The systemic inflammatory response to cardiopulmonary bypass: pathophysiological, therapeutic, and pharmacological considerations.* Anesth Analg, 1997. **85**(4): p. 766-82.
3. Ascione, R., et al., *Economic outcome of off-pump coronary artery bypass surgery: a prospective randomized study.* Ann Thorac Surg, 1999. **68**(6): p. 2237-42.
4. Hill, G.E., *Cardiopulmonary bypass-induced inflammation: is it important?* J Cardiothorac Vasc Anesth, 1998. **12**(2 Suppl 1): p. 21-5.
5. Apostolakis, E.E., et al., *Strategies to prevent intraoperative lung injury during cardiopulmonary bypass.* J Cardiothorac Surg, 2010. **5**: p. 1.
6. Borgermann, J., et al., *Regulation of cytokine synthesis in cardiac surgery: Role of extracorporeal circuit and humoral mediators in vivo and in vitro.* Inflamm Res, 2007. **56**(3): p. 126-32.
7. Rothenburger, M., et al., *Prediction of clinical outcome after cardiac surgery: the role of cytokines, endotoxin, and anti-endotoxin core antibodies.* Shock, 2001. **16 Suppl 1**: p. 44-50.
8. Johnson, D., et al., *Neutrophil-mediated acute lung injury after extracorporeal perfusion.* J Thorac Cardiovasc Surg, 1994. **107**(5): p. 1193-202.
9. Dora, K.A., et al., *Isolated Human Pulmonary Artery Structure and Function Pre- and Post-Cardiopulmonary Bypass Surgery.* J Am Heart Assoc, 2016. **5**(2).
10. Wales, N., *Together for Health – A Delivery Plan for the Critically Ill* 2016.
11. Secondary Care Analysis, N.D., *Hospital Adult Critical Care Activity*, NHS, Editor. 2017: Health and Social Care Information Centre.
12. Tan, S.S., et al., *Direct cost analysis of intensive care unit stay in four European countries: applying a standardized costing methodology.* Value Health, 2012. **15**(1): p. 81-6.
13. guideline, N., *Costing statement:Implementing the NICE guideline on Transition between inpatient hospital settings and community or care home settings for adults with social care needs*. 2015.
14. Gao, D., et al., *Variation in mortality risk factors with time after coronary artery bypass graft operation.* Ann Thorac Surg, 2003. **75**(1): p. 74-81.
15. Ascione, R., et al., *Inflammatory response after coronary revascularization with or without cardiopulmonary bypass.* Ann Thorac Surg, 2000. **69**(4): p. 1198-204.
16. Ranieri, V.M., et al., *Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial.* JAMA, 1999. **282**(1): p. 54-61.
17. Seghaye, M., et al., *Interleukin-10 release related to cardiopulmonary bypass in infants undergoing cardiac operations.* J Thorac Cardiovasc Surg, 1996. **111**(3): p. 545-53.
18. Pinhu, L., et al., *Ventilator-associated lung injury.* Lancet, 2003. **361**(9354): p. 332-40.
19. Magnusson, L., et al., *Atelectasis is a major cause of hypoxemia and shunt after cardiopulmonary bypass: an experimental study.* Anesthesiology, 1997. **87**(5): p. 1153-63.
20. Goebel, U., et al., *Reduced pulmonary inflammatory response during cardiopulmonary bypass: effects of combined pulmonary perfusion and carbon monoxide inhalation.* Eur J Cardiothorac Surg, 2008. **34**(6): p. 1165-72.
21. Asimakopoulos, G., et al., *Lung injury and acute respiratory distress syndrome after cardiopulmonary bypass.* Ann Thorac Surg, 1999. **68**(3): p. 1107-15.

22. Imura, H., et al., *Pulmonary injury after cardiopulmonary bypass: beneficial effects of low-frequency mechanical ventilation.* J Thorac Cardiovasc Surg, 2009. **137**(6): p. 1530-7.
23. O'Connor, S., et al., *Remifentanil and propofol undergo separation and layering when mixed in the same syringe for total intravenous anesthesia.* Paediatr Anaesth, 2016. **26**(7): p. 703-9.
24. Hayden, M.S., A.P. West, and S. Ghosh, *NF-kappaB and the immune response.* Oncogene, 2006. **25**(51): p. 6758-80.
25. Chen, C.C. and A.M. Manning, *Transcriptional regulation of endothelial cell adhesion molecules: a dominant role for NF-kappa B.* Agents Actions Suppl, 1995. **47**: p. 135-41.
26. Lee, J., et al., *Corticosteroid modulation of immunoglobulin expression and B-cell function in COPD.* FASEB J, 2016. **30**(5): p. 2014-26.
27. Oeckinghaus, A. and S. Ghosh, *The NF-kappaB family of transcription factors and its regulation.* Cold Spring Harb Perspect Biol, 2009. **1**(4): p. a000034.
28. Foster, S.L., D.C. Hargreaves, and R. Medzhitov, *Gene-specific control of inflammation by TLR-induced chromatin modifications.* Nature, 2007. **447**(7147): p. 972-8.
29. Loeckinger, A., et al., *Continuous positive airway pressure at 10 cm H(2)O during cardiopulmonary bypass improves postoperative gas exchange.* Anesth Analg, 2000. **91**(3): p. 522-7.
30. Reis Miranda, D., et al., *Open lung ventilation improves functional residual capacity after extubation in cardiac surgery.* Crit Care Med, 2005. **33**(10): p. 2253-8.
31. John, L.C. and I.M. Ervine, *A study assessing the potential benefit of continued ventilation during cardiopulmonary bypass.* Interact Cardiovasc Thorac Surg, 2008. **7**(1): p. 14-7.
32. Durukan, A.B., et al., *Ventilation during cardiopulmonary bypass did not attenuate inflammatory response or affect postoperative outcomes.* Cardiovasc J Afr, 2013. **24**(6): p. 224-30.
33. Chi, D., et al., *Ventilation during cardiopulmonary bypass for prevention of respiratory insufficiency: A meta-analysis of randomized controlled trials.* Medicine (Baltimore), 2017. **96**(12): p. e6454.
34. Higgins JPT, et al., *A revised tool for assessing risk of bias in randomized trials.* Chandler J, McKenzie J, Boutron I, Welch V (editors). Cochrane Methods. Cochrane Database of Systematic Reviews, 2016(10 (Suppl 1)).
35. Neumann, P., et al., *Positive end-expiratory pressure prevents atelectasis during general anaesthesia even in the presence of a high inspired oxygen concentration.* Acta Anaesthesiol Scand, 1999. **43**(3): p. 295-301.
36. Talab, H.F., et al., *Intraoperative ventilatory strategies for prevention of pulmonary atelectasis in obese patients undergoing laparoscopic bariatric surgery.* Anesth Analg, 2009. **109**(5): p. 1511-6.
37. Reinius, H., et al., *Prevention of atelectasis in morbidly obese patients during general anesthesia and paralysis: a computerized tomography study.* Anesthesiology, 2009. **111**(5): p. 979-87.
38. Hedenstierna, G. and H.U. Rothen, *Atelectasis formation during anesthesia: causes and measures to prevent it.* J Clin Monit Comput, 2000. **16**(5-6): p. 329-35.
39. Komal Ray, A.B., Elankumaran Paramasivam, *Pulmonary atelectasis in anaesthesia and critical care* Continuing Education in Anaesthesia Critical Care & Pain, 2013. **14**(5): p. 236–245.
40. Vargas, M., et al., *PEEP role in ICU and operating room: from pathophysiology to clinical practice.* ScientificWorldJournal, 2014. **2014**: p. 852356.

Consort Diagram

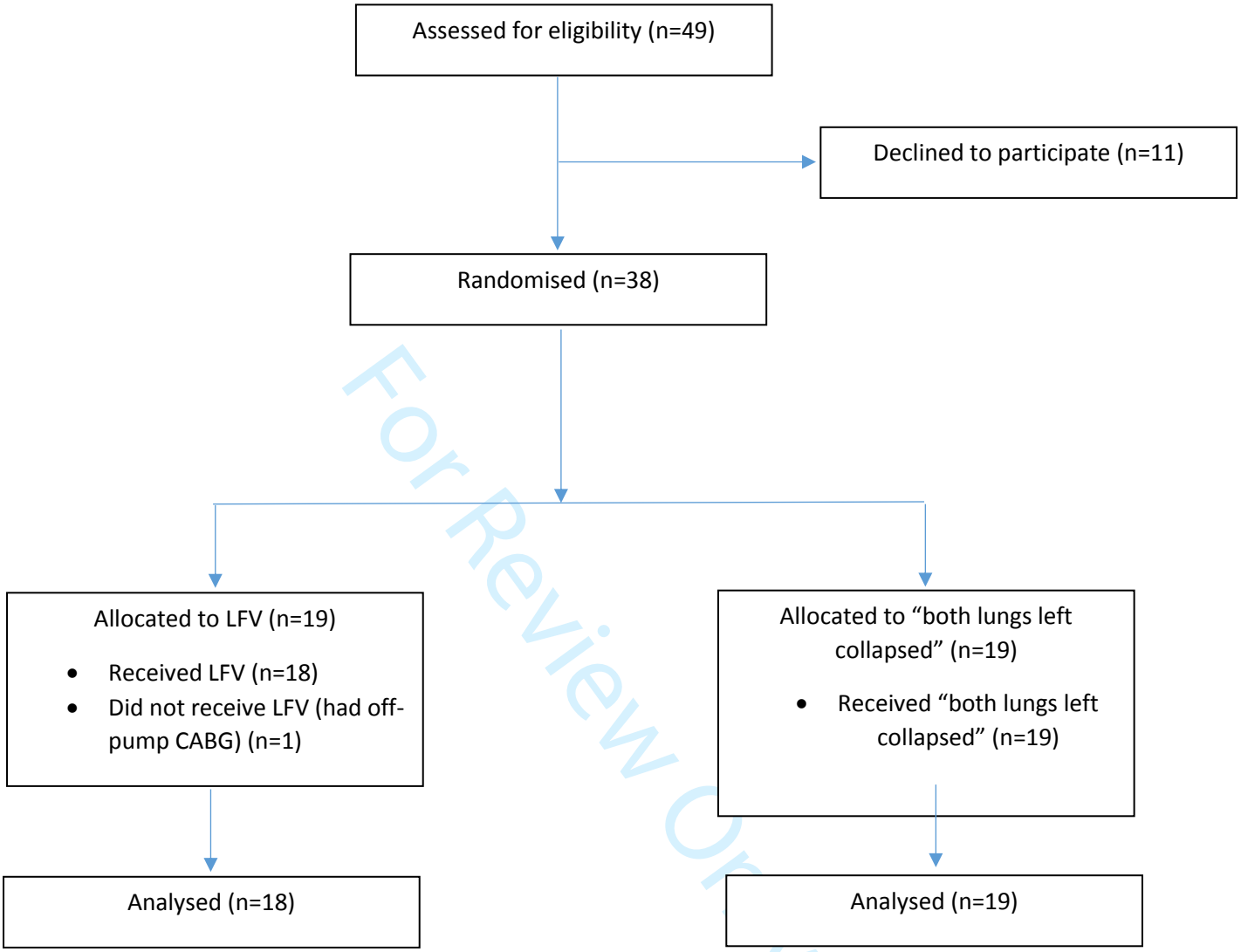
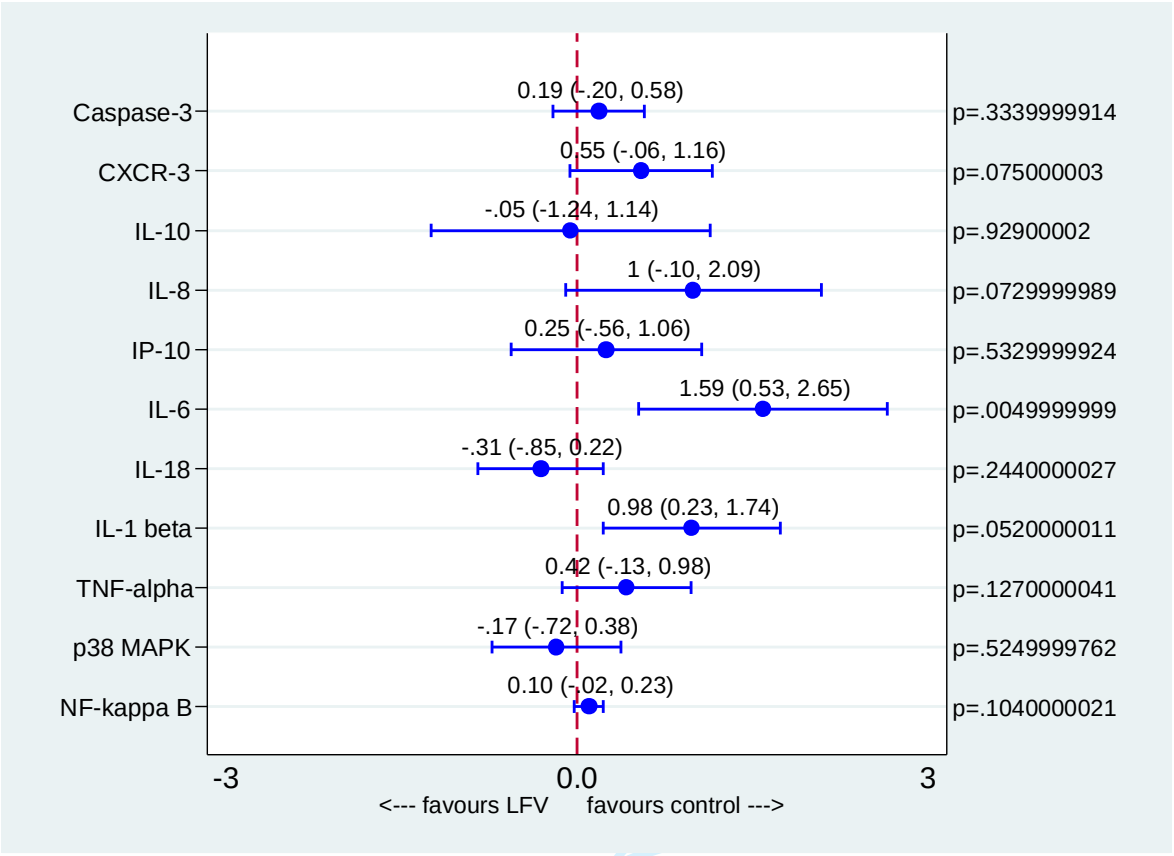
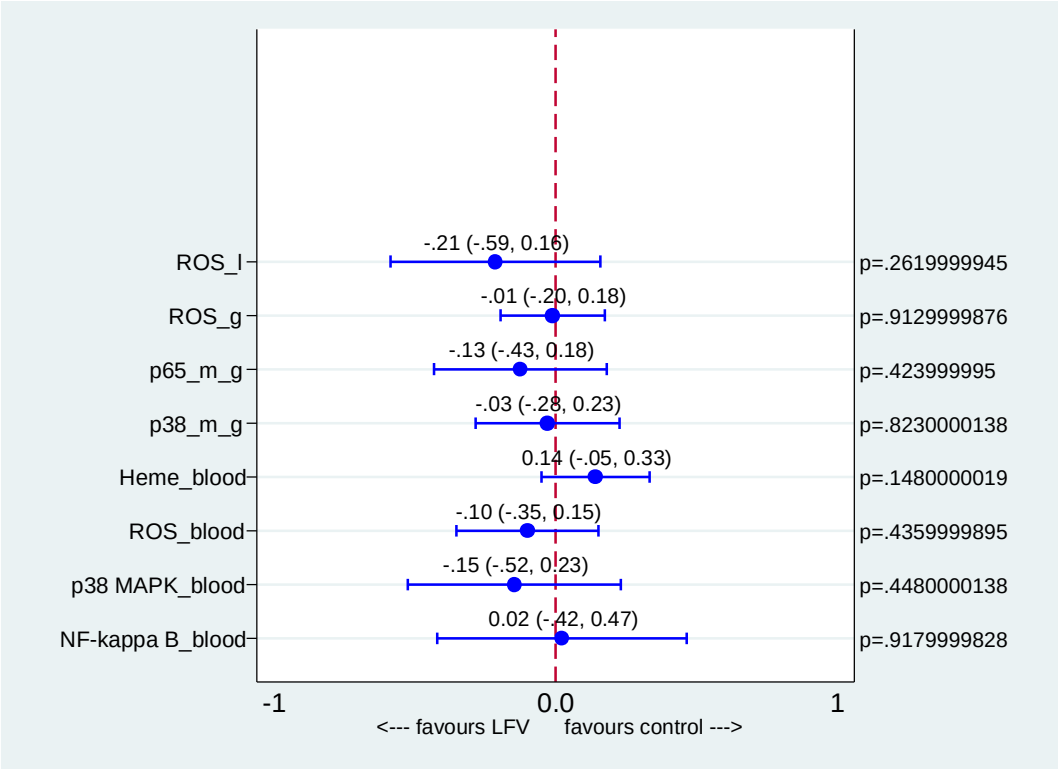


Figure 2 (a-b)

a: forest plot illustrating the treatment effect for each inflammatory marker measured in the lung tissue biopsies



b: forest plot illustrating the treatment effect for each inflammatory marker measured in the blood samples

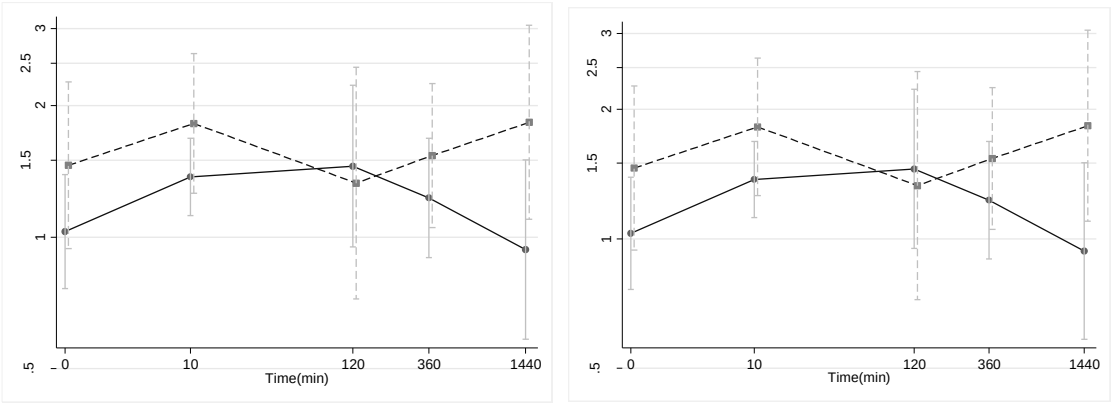


LFV=Low frequency ventilation

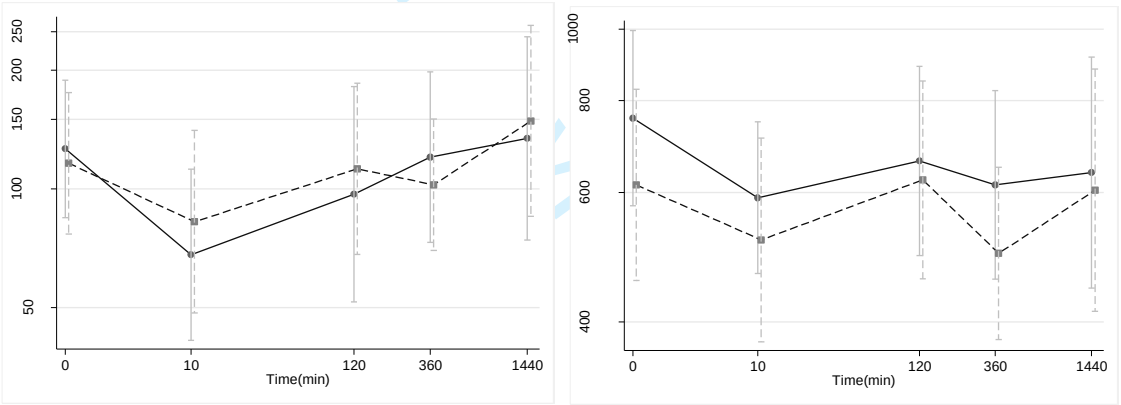
Control= both Lungs left collapsed

Figure 3

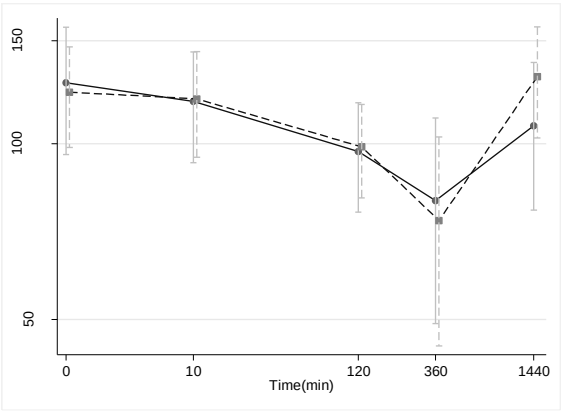
a) p65 in leukocytes and monocyets/granulocytes

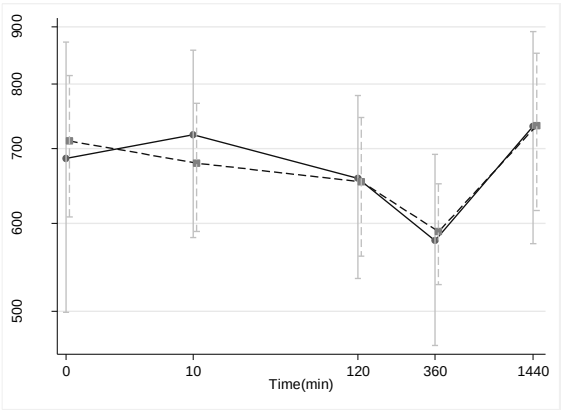


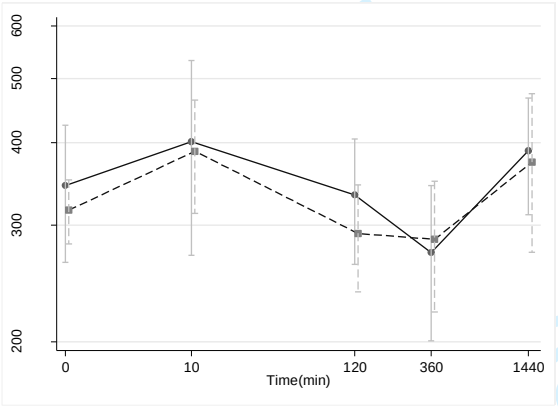
b) p38 in leukocytes and monocyets/granulocytes



c) ROS in leukocytes, granulocytes and monocytes







d) Heme

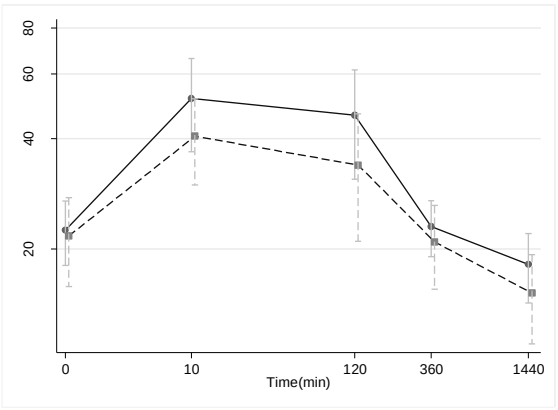


Table 1 - Patient population characteristics and operative details

Variable*	LFV (N=18)	Lungs left collapsed (N=19)
Age (years) – mean (SD)	65.39 (12.09)	62.86 (10.08)
Height (m) - mean (SD)	1.70 (0.09)	1.69 (0.06)
Weight (kg) - mean (SD)	85.72 (15.49)	80.84 (15.29)
BMI (kg/m2) - mean (SD)	29.69 (4.14)	28.15 (4.82)
NYHA class – no. of patients (%)		
0	0	0
1	4 (22%)	9 (47%)
2	12 (67%)	9 (47%)
3	2 (11%)	1 (5%)
4	0	0
Left Ventricular Function – no. of patients (%)		
Poor (<30%)	0	0
Moderate (30-50%)	4 (22%)	4 (21%)
Good (>50%)	14 (78%)	15 (79%)
Smoker/ex-smoker – no. of patients (%)	14 (78%)	12 (63%)
Asthma – no. of patients (%)	4 (22%)	0
COPD – no. of patients (%)	0	1 (5%)
Operation details		
Bypass, minutes - median (IQR)	87.5 (68-97)	69 (54-79)
Cross-clamp, minutes - median (IQR)	44.5 (37-50)	35 (30-43)
Intubation, hours - median (IQR)	8.7 (7.1-10.3)	7 (6.4-10)
Time to discharge, days - median (IQR)	6 (6-7)	6 (5-7)

*The median and interquantile range are reported for the variables whose distribution is skewed.

Table 2 - Adverse events

Adverse event	LFV n (%) N=18		Lungs left collapsed (%) N=19	
	In hospital	At follow up	In hospital	At follow up
Respiratory				
Re-intubation/Ventilation	0	0	0	0
Mask CPAP	1 (5.6%)	0	2 (10.5%)	0
Tracheostomy	0	0	0	0
Prolonged ventilation (24 h)	0	0	0	0
ARDS	0	0	0	0
Pneumothorax / Effusion	0	0	0	0
Cardiovascular				
MI	0	0	0	0
Cardiac arrest	0	0	0	0
Arrhythmias	9 (50%)	0	7 (36.8%)	0
Haemodynamic support	16 (89%)	0	9 (47.4%)	0
Neurological				
Permanent Stroke	0	0	0	0
TIA	0	0	0	0
Renal				
Hemofiltration / dialysis	0	0	0	0
Other				
GI complications	0	0	0	0
Thromboembolic complications	0	0	0	0
Bleeding complications	0	0	0	0
Wound complications	0	0	0	1 (5.3%)
Infective complications	4 (22.2%)	3 (17%)	4 (21.1%)	3 (15.8%)
Reoperation	0	0	0	0

Table A1 (a-b)

a) Comparison between LFV and both lungs left collapsed on lung tissue inflammatory markers

Inflammatory Marker	LFV (N=18)		Lungs left collapsed (N=18)	
	Before CPB	Before chest	Before CPB	Before chest
NF-κB p65	-1.92 (0.27)	-1.86 (0.32)	-1.87 (0.18)	-1.91 (0.22)
p38 MAPK	-1.12 (1.09)	-1.19 (1.05)	-1.26 (1.05)	-1.08 (0.75)
TNFα	-7.36 (1.12)	-6.91(0.73)	-7.34(1.12)	-7.33(0.86)
IL-1β	-5.72(1.05)	-3.15(1.13)	-5.64(1.16)	-4.10(1.17)
IL-18	-8.63(0.81)	-8.67(0.56)	-8.67(0.71)	-8.42(1.09)
IL-6	-0.99(1.31)	-6.12(1.10)	-2.56(1.75)	-6.03(0.87)
IP-10	-7.86(1.27)	-7.54(1.24)	-7.84(1.01)	-7.66(1.33)
IL-8	-5.45(1.77)	-1.30(1.39)	-5.64(0.78)	-2.30(1.72)
IL-10	-6.03(1.05)	-5.03(2.30)	-6.32(1.09)	-5.26(1.63)
CXCR3	-4.22(1.77)	-4.28(1.07)	-4.59(1.95)	-4.98(1.20)
Caspase 3	-2.46(0.38)	-2.60(0.47)	-2.56(0.60)	-2.50(0.64)

b) Comparison between LFV and both lungs left collapsed on blood inflammatory markers with time fitted in the model

Inflammatory Marker Mean(SD)*	**LFV**					**Lungs left collapsed**				
	Post-induction	**10 min post CPB**	**2 h**	**6 h**	**24 h**	**Post-induction**	**10 min post CPB**	**2 h**	**6 h**	**24 h**
Leukocytes										
NF-κB p65	0.16 (0.54)	0.40 (0.63)	-0.03 (0.80)	0.08 (0.99)	-0.30 (1.26)	0.44 (0.44)	0.10 (0.67)	0.11 (1.22)	0.21 (1.56)	-0.05 (0.94)
p38 MAPK	4.84 (0.80)	4.22 (1)	4.57 (1.26)	4.79 (1)	4.90 (1.19)	4.75 (0.83)	4.41 (1.07)	4.72 (1)	4.63 (0.77)	5.00 (1.11)
ROS**	4.43 (1.48)	4.46 (1.33)	4.41 (0.76)	3.80 (1.60)	4.35 (1.13)	4.71 (0.51)	4.69 (0.45)	4.51 (0.46)	3.79 (1.24)	4.75 (0.54)
Monocytes/Granulocytes***										
NF-κB p65	0.03 (0.60)	0.32 (0.41)	0.37 (0.85)	0.21 (0.63)	-0.07 (0.94)	0.38 (0.88)	0.60 (0.73)	0.28 (1.22)	0.43 (0.76)	0.60 (1.02)
p38 MAPK	6.63 (0.55)	6.38 (0.48)	6.50 (0.59)	6.42 (0.59)	6.46 (0.72)	6.42 (0.60)	6.25 (0.64)	6.44 (0.62)	6.21 (0.54)	6.40 (0.76)
Monocytes										
ROS**	5.54 (1.11)	5.52 (1.58)	5.47 (1.45)	5.38 (0.91)	5.78 (0.89)	5.73 (0.24)	5.87 (0.45)	5.58 (0.49)	5.55 (0.51)	5.82 (0.46)
Granulocytes										
ROS**	6.25 (1.07)	6.27 (1.38)	6.21 (1.27)	6.17 (0.93)	6.42 (0.94)	6.52 (0.36)	6.48 (0.33)	6.44 (0.31)	6.36 (0.21)	6.54 (0.38)

Heme (µM)	3.05 (0.38)	3.79 (0.57)	3.65 (0.64)	3.08 (0.34)	2.81 (0.43)	2.95 (0.51)	3.58 (0.50)	3.35 (0.56)	2.93 (0.48)	2.60 (0.49)

*Data are transformed because their distribution is not normal, the mean of the transformed data is tabulated.
**Negative data for ROS was set to missing. Most time points and cell type had only one value set to missing. ROS in Leukocyte at baseline and 2 hours had three missing values, ROS in Leukocyte at 6 hours had 6 missing values.
***Monocytes overlapped with granulocytes for NF-κB p65 and p38 MAPK

Table A2

Summary of the main effects for primary and secondary endpoints in inflammatory markers (LFV vs Lungs left collapsed)

	Event	**LFV vs Lungs left collapsed**		**p value**
		Treatment effect	**95% CI**	
In Biopsy	**NF-κB p65**	0.102	-0.022 0.226	0.104
	p38 MAPK	-0.173	-0.723 0.376	0.525
	TNFα	0.425	-0.128 0.977	0.127
	IL-1β	0.985	0.228 1.742	0.012
	IL-18	-0.310	-0.845 0.224	0.244
	IL-6	1.59	0.528 2.654	0.005
	IP-10	0.251	-0.567 1.06	0.533

	IL-8	0.995	-0.098 2.088	0.073
	IL-10	-0.053	-1.244 1.138	0.929
	CXCR3	0.551	-0.058 1.159	0.075
	Caspase 3	0.186	-0.204 0.576	0.334
In Blood	**NF-κB p65** leukocytes	0.023	-0.420 0.467	0.918
	NF-κB p65 monocytes_granulocyte	-0.125	-0 .433 0.182	0.424
	p38 MAPK leukocytes	-0.146	-0 .524 0.232	0.448
	p38 MAPK monocytes_granulocyte	-0.029	-0.284 0.226	0.823
	ROS monocytes	-0.100	-0.353 0.152	0.436
	ROS granulocyte	-0.010	-0.196 0.176	0.913
	ROS leukocytes	-0.213	-0.585 0.159	0.262
	Heme	0.142	-0.050 0.334	0.148

Results of the statistical inference summary showing the main effect for primary and secondary end points in standard care vs. LFV. Cytokines increased following surgery. A positive value indicates that standard care was better than LFV whilst a negative value indicates an improved response to LFV.

Table A3 (a,b)

a) Pulmonary gas exchange parameters (LFV vs Lungs left collapsed)

Pulmonary gas exchange Median (IQR)	LFV							Lungs left collapsed						
	Post-induction	10 min post CPB	2 h	4h	Post extubation	12 h	24 h	Post-induction	10 min post CPB	2 h	4h	Post extubation	12 h	24 h
PaO_2 (mmhg)	215.25 (144.7-344.3)	168 (145.5-264)	133.9 (103.5-203)	119.25 (105-130.5)	110.25 (84.7-117.8)	102.35 (94.5-122.3)	99 (86.3-114.8)	229.55 (164.3-293.3)	211.5 (117-328.5)	129 (117-184.5)	132 (106-5-157.5)	114.8 (94.5-148.5)	122.3 (105.8-146.3)	97 (90.8-111.8)
A-a Gradient	148.19 (33.18 - 204.96)	176.74 (121.39 - 233.55)	114.89 (108.5 - 150.53)	116.26 (91.63 - 126.95)	113.63 (77.56 - 133.00)	97.56 (80.36 - 116.88)	104.04 (81.97 - 132.83)	111.59 (85.05 - 168.65)	178.21 (40.63 - 336.49)	113.78 (95.1 - 146.83)	90.63 (62.75 - 144.45)	105.95 (58.43 - 122.40)	62.68 (35.86 - 99.70)	67.98 (46.47 - 114.63)

b) Lung function parameters (LFV vs Lungs left collapsed)

Lung Function test Mean(SD)*	LFV		Lungs left collapsed	
	Pre-op	Follow up	Pre-op	Follow up
FEV1 (% predicted) litres - mean (SD)	90.94 (21.58)	82.06 (19.86)	97.22 (18.83)	91 (20.20)
FVC (% predicted) litres - mean (SD)	93.24 (20.73)	83.00 (18.37)	97.11 (18.51)	91.25 (16.55)
MEF75(% predicted) litres/sec - mean (SD)	84.13 (42.32)	85.31 (39.01)	87.87 (30.83)	87.00 (30.37)
MEF25(% predicted) litres/sec - mean (SD)	68.27 (36.25)	60.56 (25.77)	70.47 (25.82)	67.33 (23.94)
TLC(% predicted) litres - mean (SD)	97.40 (14.53)	88.94 (11.76)	103.27 (14.11)	90.08 (13.57)
RV (% predicted) litres - mean (SD)	112.73 (25.60)	98.75 (24.47)	116.33 (34.67)	95.17 (21.44)
TL_{CO}(% predicted) (mmol/Kpa/min) - mean (SD)	89.79 (18.13)	84.75 (18.05)	84.14 (12.98)	76.92 (18.13)
K_{CO} (% predicted) (mmol/Kpa/min) - mean (SD)	103.71 (17.29)	108.56 (17.53)	96.00 (12.17)	97.75 (15.36)
FEV1(measured) litres - mean (SD)	2.51 (0.91)	2.36 (0.81)	2.75 (0.81)	2.58 (0.89)
FVC(measured) litres - mean (SD)	3.27 (1.07)	3.04 (0.99)	3.47 (0.95)	3.30 (0.99)
MEF75 (measured) litres/sec - mean (SD)	5.40 (3.32)	5.87 (2.93)	5.93 (2.41)	6.09 (2.45)
MEF25 (measured) litres/sec - mean (SD)	1.40 (2.02)	1.17 (1.57)	0.97 (0.51)	0.97 (0.55)
TLC(measured) litres - mean (SD)	5.87 (1.19)	5.59 (1.07)	6.15 (1.07)	5.58 (1.16)
RV (measured) litres - mean (SD)	2.57 (0.61)	2.29 (0.7)	2.61 (0.87)	2.17 (0.51)
TL_{CO}(average) (mmol/Kpa/min) - mean (SD)	7.63 (1.93)	7.29 (1.8)	7.53 (2.56)	6.69 (2.40)
K_{CO}(average) (mmol/Kpa/min) - mean (SD)	1.39 (0.26)	1.46 (0.25)	1.29 (0.19)	1.31 (0.27)
Percentage O_2 saturation - mean (SD)	96.33 (1.05)	96.88 (1.58)	97.07 (1)	98 (0.85)

*If data are transformed because of the distribution is non-normal, then the mean of the transformed data will be tabulated.