Selective pulmonary pulsatile perfusion with oxygenated blood during cardiopulmonary bypass attenuates lung tissue inflammation but does not affect circulating cytokine levels†

Francesco Santini‡*, Francesco Onorati§, Mariassunta Telesca§, Tiziano Menon, Paola Mazzì, Giorgio Berton, Giuseppe Faggiana and Alessandro Mazzucco

Abstract

OBJECTIVE: Improved respiratory outcome has been shown after selective pulsatile pulmonary perfusion (sPPP) during cardiopulmonary bypass (CPB). No contemporary study has analysed the impact of sPPP on alveolar and systemic inflammatory response in humans.

METHODS: Sixty-four patients undergoing a coronary artery bypass graft (CABG) were randomized to sPPP or standard CPB (32 patients each). An alveolar-arterial oxygen gradient (A-aDO₂) was measured preoperatively (T0), at ICU arrival (T1), 3 h postoperatively (T2) and postextubation (T3). The bronchoalveolar lavage (BAL) was collected at T0, T1 and T2. White blood cells (WBCs), neutrophils, mononucleates and lymphocytes in BAL infiltrates were compared between the two groups. A cytokine assay for interleukin-1 (IL-1), IL-8, tumour necrosis factor alpha (TNF-α), monocyte chemotactic protein-1 (MCP-1), growth regulated oncogene-alpha (GRO-α) and interferon (IFN)-γ was collected from the BAL and peripheral blood at the same time-points. Repeated-measure analysis of variance and non-parametric statistics were used to assess the between-group and during time differences.

RESULTS: The two groups proved comparable for perioperative variables. A-aDO₂ proved better after sPPP (group P = 0.0001; group × time-P < 0.0001). BAL infiltrates after sPPP showed lower WBCs, neutrophils and lymphocytes (group-P = 0.0001, group × time-P = 0.0001 for all) together with higher mononucleates (group-P = 0.0001, group × time-P = 0.0001). Proinflammatory cytokines and chemokine MCP-1 were lower in BAL after sPPP (group-P = 0.0005, 0.034, 0.036 and 0.005, and group × time-P = 0.001, 0.009, 0.001 and 0.0001 for IL-1, IL-8, TNF-α and MCP-1, respectively), whereas the immune modulator IFN-γ significantly augmented after sPPP (time-P = 0.0001) but remained stable after the standard CPB (time-P = 0.101, group-P = 0.001, group × time-P = 0.0001). Indeed, serum cytokines were not different in the two groups during the study (P = NS at single time-points and as a function of time).

CONCLUSIONS: sPPP attenuates alveolar inflammation, as demonstrated by the lower neutrophilic/lymphocytic alveolar infiltration, and the secretion of anti-inflammatory rather than proinflammatory mediators.

Keywords: Cardiopulmonary bypass · Pulmonary perfusion · Pulsatile flow · Inflammation · Cytokines · Lung injury

INTRODUCTION

The development of lung tissue inflammatory response after cardiac surgery with cardiopulmonary bypass (CPB) is well documented [1]. Indeed, although the technical refinement of CPB has progressively improved the surgical outcomes of heart diseases, CPB-induced lung dysfunction may still represent a serious postoperative complication, ranging from well-tolerated minimal alterations of the respiratory functional parameters to severe clinical scenarios carrying high mortality rates, as in the adult respiratory distress syndrome (ARDS) [2].

Post-CPB lung damage derived from a cytokine/chemokine-mediated inflammation, predominantly triggered by an ischaemia–reperfusion (I/R) mechanism of injury, which results from the sequestration of activated leukocytes and platelets in the lung parenchyma, with consequential damage also mediated by oxygen-derived free radicals [2]. Based on the pioneer demonstration of a critical reduction in the nourishing bronchial blood flow during CPB, and to the possibility of a lessened post-CPB lung inflammation by the augmentation of the bronchial blood flow during CPB [3], the attention of the investigators moved towards the application of direct antegrade pulmonary perfusion during CPB [2, 4]. Different strategies of lung perfusion were therefore developed during the last decades, the majority of which showed potential functional or biochemical benefits, but overall
negligible clinical advantages [1–4]. Moreover, little is yet known about the cross-talk between local (alveolar) and systemic (serum) inflammatory cytokine secretion after pulmonary perfusion [5].

Our group has recently reported the results of the first clinical trial employing selective pulsatile pulmonary perfusion (sPPP) with oxygenated blood in humans, showing significant functional respiratory and pulmonary haemodynamic benefits [6]. In the light of these results, we sought to investigate the specific local (alveolar) and systemic (serum) inflammatory responses in humans undergoing sPPP. The results of this second investigational trial are herein reported.

MATERIAL AND METHODS

Study design

Between November 2008 and April 2011, 65 consecutive patients (of 1080, 6%) undergoing an elective first-time isolated CABG were prospectively randomized by lottery, drawing preprepared sealed envelopes containing the group assignment at hospital admission, to receive sPPP during aortic cross-clamping (32 patients, sPPP group) or the standard CPB (33 patients, S group).

The inclusion/exclusion criteria have been already reported [6]: briefly, only a three-vessel low-risk CABG with an estimated logistic EuroSCORE ≤5% were enrolled, in order to minimize all the confounding factors potentially biasing the inflammatory response. Accordingly, any factor potentially affecting pulmonary function was considered as exclusion criteria [age >70 years, emergent/urgent/salvage procedures, left ventricular ejection fraction <45%, left ventricular end-diastolic pressure (LVEDP) >15 mmHg, associated redo, preoperative inta-aortic balloon pump (IABP), diabetes, obesity (body mass index >30 kg/m²), smoke (ex-smokers for <15 years were similarly excluded due to the risk of persistent lung dysfunction), chronic obstructive pulmonary disease or other lung diseases, pulmonary hypertension, renal disease (kidney disease outcome quality initiative class ≥2), previous irradiation, previous thoracic surgery, transfusions within 6 months, infections, liver dysfunction, ongoing steroids, alcohol/drug abuse, neurological dysfunction, immune diseases and cancer]. Furthermore, the following post hoc exclusion criteria mandating post-enrolment withdrawal were considered, in order to minimize the confounding effects of postoperative complications on lung/systemic inflammation: postoperative prolonged (>1 h) LVEDP >25% of the preoperative value or a cardiac index ≤1.4 l/min/m², postoperative IABP, perioperative acute myocardial infarction, transfusions (≥2 units of red packed cells, ≥600 ml of fresh frozen plasma or ≥1 unit of platelets), reoperation for bleeding, pneumothorax or pleural effusion requiring drainage [7]. The study protocol was approved by the institution’s Ethical Committee/Institutional Review Board, and informed consent was obtained from all patients.

Anaesthesia and surgery

All patients underwent preoperative Swan–Ganz catheter insertion for continuous haemodynamic monitoring. The anaesthetic management and surgical technique were standardized and have already been reported [6]. Briefly, the lungs were ventilated to normocapnia with volume-controlled ventilation at a frequency of 12 breaths/min, a tidal volume of 8 ml/kg, a FiO₂ of 0.5 and a positive-end expiratory pressure (PEEP) of 5 cm/H₂O. During CPB time, mechanical ventilation was discontinued. A standardized 30 s recruiting manoeuvre at the beginning of CPB weaning was always performed [6]. The lungs were then mechanically ventilated for at least 4 h after the termination of surgery, with a PEEP of 5 cm/H₂O, whereas FiO₂ and ventilation rates were adjusted to keep PaO₂ > 100 mmHg and PaCO₂ between 30 and 35 mmHg. Airway clearance was maintained by routine ‘close’ tracheal suctioning. Weaning from mechanical ventilation started after the 4th hour, provided haemodynamic stability (by invasive measurements), acid–base balance, chest drainage <80 ml/h and a body temperature ≥36°C. A negative preliminary neurological exam and patient cooperation were prerequisite to extubation.

Surgery and CPB management were standardized [6]. Briefly, surgery was always performed through a median sternotomy. The left internal mammary artery was always harvested, through an open pleural space, in a pedicled fashion and used as an in situ graft on the left anterior descending coronary artery. A non-pulsatile systemic CPB flow was established at 2.4 l/min/m², with moderate hypothermia ranging from 32 to 34°C. Myocardial protection was accomplished by intermittent antegrade and retrograde hyperkalemic cold blood (1:4 ratio) cardioplegia [7]. sPPP with the patient’s own oxygenated blood was achieved by the direct cannulation of the pulmonary trunk with a 14-Fr cannula (Edwards Fem-Flex, Edwards Lifesciences, Irvine, CA, USA) and set at a flow rate of 7 ml/kg/min and a pulsatile rate of 60 bpm by using a pulsatile pump (Jostra, Maquet Cardiopulmonary, Hirrlingen, Germany) integrated in the CPB machine, in order to reach a mean surplus haemodynamic energy of at least 10% [8]. Left ventricular venting was always accomplished. Pulmonary perfusion was initiated at the start of CPB and terminated at the beginning of the weaning period [7]. In contrast, the standard CPB without any pulmonary perfusion was carried out in the S group.

Preoperative and intraoperative data are reported in Table 1.

Endpoints

The primary endpoint of the study was to evaluate the role of sPPP on an alveolar inflammation. According to the demonstration of the primary role of alveolar neutrophils on a post-CPB lung injury, and the direct correlation of the neutrophil count in the bronchoalveolar fluid lavage (BAL) on the degree of post-CPB lung dysfunction [9], the neutrophil count in BAL samples was considered the primary outcome variable. Accordingly, three consecutive samples of BAL were collected from each patient: (i) preoperatively after intubation (T0), (ii) at ICU arrival (T1) and (iii) at the 4th hour of ICU stay (T2), according to a standard protocol [7]. BAL sampling was standardized [6, 9]: a flexible fiberoptic bronchoscope (Olympus BF-B3™; Olympus Co., Tokyo, Japan) was introduced through the endotracheal tube for BAL collection. The tip of the bronchoscope was wedged into a subsegment of the right inferior lobe of the lung, and 20 ml of sterile buffered saline solution (pH 7.4) was instilled through the bronroscope. The lavage fluid was aspirated by gentle suction. This procedure was repeated five times with instillation of 20 ml of buffered saline solution each time. The lavage fluid was centrifuged immediately at 1200 rpm for 10 min at 4°C. The cell-free supernatant was immediately stored at −80°C for the subsequent analysis of cytokines. The remainder cell-free supernatant was discarded, whereas 0.5 ml of sediment...
was sampled, resuspended in roswell park memorial institute medium + 10% fetal calf serum + glutamine medium and stored at 4°C for cell count, under sterile conditions with laminar flows. Subsequently, the cells were counted in a haemocytometer after cytocentrifugation and haematoxylin-eosin staining. The entire process for treatment and analysis of the BAL samples was identical in both groups. Apart from the absolute neutrophil count, which accounted for the primary endpoint, an absolute number of white blood cells (WBCs), monocytes/macrophages and lymphocytes were also collected at the same time-points and considered as secondary endpoints. Proinflammatory cytokine [interleukin-1 (IL-1), IL-8 and tumour necrosis factor alpha (TNF-α)], chemokine assay (GRO-α and MCP-1) and anti-inflammatory cytokine assay [interferon (IFN)-γ] were collected from BAL samples and central venous blood at the same three time-points and also considered as secondary endpoints. Blood was anticoagulated with ethylenediamine tetraacetic acid and centrifuged immediately, with plasma samples stored at −80°C until analysis. Cytokine and chemokine activities in the BAL and plasma were measured by using a commercial assay kit (Bio-Plex Human Cytokine Assays, Bio-Rad Laboratories, Inc., CA, USA). A single investigator, blinded towards group assignment, measured inflammatory cells and mediators.

Finally, because of the beneficial impact of sPPP on gas exchange [6] and the demonstration of a direct link between alveolar inflammation and respiratory function [9], we routinely measured the alveolar–arterial oxygen gradient (A-aDO2), which was considered as a secondary endpoint and calculated as follows:

\[ A - aDO2 = (PAO2 - PaO2) \]

where PAO2 is the partial pressure of alveolar oxygen and PaO2 the partial pressure of arterial oxygen. PAO2 was calculated as follows:

\[ PAO2 = FiO2[(BP - PH2O) - (PaCO2/RQ)] \]

where FiO2 is the fractional concentration of oxygen in the inspired air, BP the barometric pressure, PH2O the saturated vapour pressure, PaCO2 the arterial carbon dioxide tension pressure and RQ the respiratory exchange ratio. For simplification, the term (BP - PH2O) - (PaCO2/RQ) was considered to be constant (760 – 47) – (36/0.8) = 679 (mmHg). A-aDO2 was collected preoperatively after orotracheal intubation and just before surgical incision (T0), at ICU arrival (T1), 3 h postoperatively (T2) and postextubation (T3).

**Statistical analysis**

The enrolment of 64 patients (32 for each study arm) resulted in a 95% probability that the study detected a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is at least 60% of the mean of the primary outcome variable (i.e. the absolute neutrophil count of BAL sampling) of one treatment group, in terms of the percentile of the mean of the other treatment group (G Power 3.0.10, Kiel, Germany) [9]. Partial η2 of the model for the three-time absolute neutrophil count of BAL sampling was 0.934.

Pre- and perioperative data were summarized as the mean and standard deviation (SD) or median and 25th–75th percentile if continuous and as counts and percent if categorical. Continuous variables were tested for normality with the Shapiro–Wilks test and compared between the two treatment groups with the Student’s t-test or the Mann–Whitney U-test accordingly. Categorical variables were compared with Fisher’s exact test. Ordinal variables were also compared with the Mann–Whitney U-test. Repeated-measure analysis of variance (ANOVA) with the Bonferroni correction for multiple measurements was used to compare serial data related to the inflammatory cell count, mediators and A-aDO2. A natural logarithmic transformation was applied to the data for all non-normally distributed variables, in order to normalize their distribution. Violations of sphericity were Greenhouse–Geisser corrected if F < 0.75 or Huynh–Feldt corrected if F > 0.75. Reported P-values include ‘group × time’, assessing the level of difference between groups; ‘time’, assessing the change over time of measured variables; ‘group × time × P’, assessing the group–time interaction. Comparisons were considered significant if P < 0.05, unless otherwise dictated by the Bonferroni correction. Statistical analysis was performed by the SPSS program for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Clinical outcome**

The two groups proved comparable for baseline and intraoperative variables (Table 1). All patients survived the operation, without intraoperative mortality. There were no perioperative acute myocardial infarctions. One patient belonging to the 5 group required postoperative transfusions and reoperation for bleeding (1.5%). The patient withdrew the protocol based on the above-mentioned rationale and exclusion criteria.

**Table 1: Preoperative and intraoperative variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>sPPP group (32 patients)</th>
<th>S group (32 patients)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>61.2 ± 5.9</td>
<td>62.3 ± 6.0</td>
<td>0.45</td>
</tr>
<tr>
<td>Female</td>
<td>11 (34.4%)</td>
<td>17 (53.1%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Hypertension</td>
<td>18 (56.3%)</td>
<td>14 (43.8%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>22 (68.8%)</td>
<td>21 (65.6%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Preoperative AMI</td>
<td>10 (31.3%)</td>
<td>7 (21.9%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Preoperative LVEF (%)</td>
<td>53 (50–60)</td>
<td>51 (48–60)</td>
<td>0.18</td>
</tr>
<tr>
<td>Preoperative Hb (g/dl)</td>
<td>13 (13–14)</td>
<td>12 (12–14)</td>
<td>0.83</td>
</tr>
<tr>
<td>Preoperative FEV1 (%)</td>
<td>83 (78–89)</td>
<td>85 (77–89)</td>
<td>0.57</td>
</tr>
<tr>
<td>Logistic EuroSCORE</td>
<td>2.3 ± 1.3</td>
<td>2.3 ± 0.9</td>
<td>0.98</td>
</tr>
<tr>
<td>N CABG (%)</td>
<td>3.0 (3.0–3.0)</td>
<td>3.0 (3.0–3.0)</td>
<td>0.66</td>
</tr>
<tr>
<td>ACC time (min)</td>
<td>50.6 ± 8.2</td>
<td>47.5 ± 9.2</td>
<td>0.15</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>72.5 ± 12.2</td>
<td>72.9 ± 13.1</td>
<td>0.91</td>
</tr>
<tr>
<td>Intraoperative transfusions (n units)</td>
<td>0.0 (0.0–2.0) 0.0 (0.0–1.0)</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Total perioperative transfusions (n units)</td>
<td>0.0 (0.0–1.0) 0.0 (0.0–1.0)</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Chest drainage (ml)</td>
<td>779 (621–895)</td>
<td>808 (589–904)</td>
<td>0.77</td>
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</table>

Bronchoalveolar lavage cellular count

Patients undergoing sPPP demonstrated at BAL analysis a significantly lower number of absolute WBC count when compared with the S group (group-P = 0.0001, group × time-P = 0.0001; Fig. 1A). The analysis of WBC subpopulations revealed that the overall higher infiltrates after the standard CPB resulted from a significantly higher sequestration in the alveolar spaces of both neutrophils (group-P = 0.0001, group × time-P = 0.0001; Fig. 1B) and lymphocytes (group-P = 0.009, group × time-P = 0.024; Fig. 1D). On the other hand, a significantly higher sequestration of monocytes/macrophages occurred after sPPP (group-P = 0.0001, group × time-P = 0.0001; Fig. 1C).

Bronchoalveolar lavage cytokine assay

When inflammatory mediators were considered, sPPP correlated with a higher anti-inflammatory and a lower proinflammatory lung activation (Fig. 2). More in detail, proinflammatory cytokines (IL-1, IL-8 and TNF-α) and proinflammatory chemokine MCP-1 levels proved all to be significantly lower in BAL samples after sPPP (group-P = 0.005, group-P = 0.006 and group-P = 0.005 and group × time-P = 0.001, 0.042, 0.003 and 0.0001 for IL-1, IL-8, TNF-α and MCP-1, respectively; Fig. 2A–D). Despite proinflammatory chemokine GRO-α showed a significant augmentation at T1 and T2 in both groups (time-P = 0.001 in either the sPPP group or the S group), it was the only proinflammatory mediator showing a comparable time-course regardless the employed technique of CPB (group-P = 0.46, group × time-P = 0.52; Fig. 2E).

Finally, immune modulator IFN-γ leakage significantly augmented after sPPP (time-P = 0.0001), but remained stable after the standard CPB (time-P = 0.10), thus resulting in a significantly higher concentration in BAL samples of patients undergoing sPPP (group-P = 0.006, group × time-P = 0.004; Fig. 2F).

Serum cytokine assay

Serum proinflammatory cytokines and chemokines, as well as immune modulator IFN-γ, demonstrated an evident systemic response in both groups (time-P = 0.0001 for all biomarkers regardless of the group allocation, except for IL-8 showing a time-P = 0.001 after sPPP and a time-P = 0.0001 after the standard CPB; Fig. 3A–F). However, the serum levels of either proinflammatory IL-1, IL-8, TNF-α, MCP-1 and GRO-α or immune modulator IFN-γ did not differ significantly between the two groups (group-P = 0.47, 0.26, 0.32, 0.87, 0.35 and 0.43, respectively), also as a function of time (group × time-P = 0.36, 0.73, 0.12, 0.19, 0.41 and 0.67; Fig. 3A–F).

Alveolar–arterial oxygen gradient

$A-aDO_2$ deteriorated after surgery in both groups (time-P = 0.0001 for the sPPP and S groups, Table 2). However, the degree of such decline was significantly attenuated by sPPP (group-P = 0.0001) during the entire time-course under investigation (group × time-P = 0.0001, Table 2).
DISCUSSION

Post-CPB lung inflammation results in variable degrees of pulmonary dysfunction which ranges from a slight impairment of the oxygenation to the ARDS, whose estimated mortality approximates 15% with a non negligible incidence of 0.4% after routine cardiac surgery [2, 10]. A recent survey in 45 US Centres definitively proved that the development of ARDS in previous healthy adults required high costs of care and post-discharge resource consumption, with an overall associated poor long-term survival, function and quality of life [10]. In the last decades, researches deeply investigated the biochemical and cellular mechanisms underlying post-CPB lung injury and tested different refinements in the CPB technology capable to reduce the lung inflammation [1–6, 9, 10]. Furthermore, many pulmonary perfusion strategies were suggested during CPB to attenuate the I/R mechanisms of injury [3, 4, 6]. Among these, our group recently tested and validated the efficacy of sPPP with oxygenated blood to reduce the I/R lung damage, demonstrating a net advantage in terms of both perioperative pulmonary haemodynamics and respiratory function [6].

As in our preliminary experience, we confirmed in the present study a functional advantage after sPPP as shown by the reduced A-aDO2 values. The calculation of A-aDO2 is a well-recognized method for the assessment of perioperative changes in the lung function after cardiac surgery [9, 11]. Specifically, it has been demonstrated that CPB-induced lung ischaemia results in a progressive augmentation of the alveolar septal thickness, together with a parallel reduction in the alveolar surface area and that these ultrastructural changes improve with pulmonary perfusion [3]. Asimakopoulos et al. [11] also showed that a perioperative lung injury is characterized by an inflammatory-mediated interstitial and alveolar oedema, with epithelial damage and elastic fibre fragmentation, rapidly inducing progressive pulmonary fibrosis. Sievers et al. [4] confirmed the alveolar capillary leakage by the demonstration of high α2-macroglobulin levels in the BAL of patients receiving the standard CPB. All these data demonstrated an inflammatory-mediated alveolar damage resulting—from a clinical point of view—in a hampered oxygenation after surgery [3, 9, 11]. Accordingly, our results confirmed previous studies showing an improved oxygenation whenever antegrade pulmonary perfusion was maintained during CPB and cross-clamp time [4, 12].

Although multifactorial, post-CPB lung dysfunction is mainly caused by an I/R injury [13], involving leukocytes, cytokines, chemokines, free radicals and other inflammatory mediators. Specifically, it has been shown that the increased microvascular permeability have a bimodal pattern, peaking at 30 min and 4 h after reperfusion [13], with the first phase depending mainly on activated pulmonary mononucleates and the secretion of TNF-α,
IFN-γ and MCP-1 and the second phase depending more on products from activated neutrophils and TNF-α [13]. Interestingly enough, in our experience, we confirmed that the majority of proinflammatory mediators, as well as cellular infiltration, peaked at 4 h after surgery, in both groups.

A lung I/R injury is associated with a significant burst of proinflammatory cytokines and chemokines, initiated by macrophages but maintained by neutrophils [1, 13, 14]. Kotani et al. [9] demonstrated a significant increase in BAL IL-6, IL-8 and TNF-α after CPB, with IL-8 concentration paralleling the PaO₂/FiO₂ value. Similarly, we found a burst of proinflammatory cytokines and chemokines which paralleled an A-aDO₂ decline in both groups, with an early alveolar mononucleates and a progressive and late neutrophil infiltration. Furthermore and most importantly, the higher proinflammatory cytokine/chemokine levels in S-group patients paralleled with a higher neutrophil infiltration and a worse A-aDO₂.

IFN-γ is a well-known immune modulator cytokine promoting the shift from quiescent monocytes/macrophages to activated macrophages [7]. Macrophages significantly augment in the last phases of an acute inflammatory process in order to terminate it by inhibiting the proliferation of smooth muscle cells.

### Table 2: Perioperative course of A-aDO₂

<table>
<thead>
<tr>
<th></th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>P-value*</th>
<th>P-value**</th>
<th>P-value***</th>
</tr>
</thead>
<tbody>
<tr>
<td>S group</td>
<td>24.2 ± 2.7</td>
<td>245.4 ± 27.5</td>
<td>292.5 ± 34.1</td>
<td>96.5 ± 19.7</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>sPPP</td>
<td>22.6 ± 1.8</td>
<td>175.9 ± 17.8</td>
<td>178.5 ± 12.5</td>
<td>59.6 ± 6.9</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

A-aDO₂: alveolar-arterial oxygen delivery.
*Time-P (change within group).
**Group-P (level of difference between groups).
***Group × time-P (difference between groups as a function of time).
endothelial cell division and platelet-derived growth factor production, platelet aggregation, matrix synthesis and myofibroblast collagen synthesis, all of which result in irreversible tissue remodelling [7].

Our study confirmed a direct link between IFN-γ levels and macrophage's infiltration (as for BAL sampling) and showed an attenuated inflammatory reaction after sPPP. According to the demonstration that the balance between proinflammatory and anti-inflammatory mediators determines the magnitude and course of post-CPB end-organ inflammatory damage [14], sPPP appeared to attenuate the lung proinflammatory state by combining a lower production of IL-1, IL-8, MCP-1 and TNF-α, with an increased synthesis of immune modulator mediators (as IFN-γ).

When BAL cells are considered, it has been shown that the neutrophils that migrate to the distal airway become activated under the macrophage-derived proinflammatory mediators and release elastase that damages alveolar architecture [9, 13]. Elastase, in turn, stimulates the release of IL-8 from neutrophils in the distal airways, which attracts additional neutrophils, thus completing a vicious cycle [9]. Furthermore, pulmonary neutrophil sequestration initially occurs in the ischaemic period but it progresses significantly following lung reperfusion [9]. Indeed, an experimental study demonstrated that pharmacological strategies aimed at decreasing neutrophil sequestration into the lungs, prevented the lung endothelium injury and improved both post-I/R lung function and arterial oxygenation [15]. Thus, the lower the neutrophils, the lower the alveolar damage [9]. In our study, sPPP patients showed a lower concentration of neutrophils in BAL samples at all postoperatively tested time frames which correlated with a better preservation of A-aDO₂, underscoring the previous observations that at lower neutrophil infiltration corresponds a lower activation of deleterious mechanisms of injury in the lung parenchyma.

Recent studies have questioned an exclusive role of BAL neutrophils on the development of an I/R injury [9]. Reperfusion in a rat model of lung I/R in the absence of neutrophils did not significantly attenuate the degree of lung injury [16]. Similarly, in a canine lung transplant model, capillary oedema formed during organ preservation when the alveolar capillary white cell count was normal, but then decreased after reperfusion when the number of neutrophils increased 3-fold, thus indicating that oedema may not be exclusively neutrophil-dependent [17]. Moreover, a recent elegant experimental model has demonstrated that cellular types in BAL sampling change with time during an I/R injury: a significant rise in BAL macrophages and lymphocytes progressively occur during the 'early ischaemic' time, whereas BAL neutrophils significantly increase only during the 'subsequent reperfusion' period, and in a percentage proportional to the ischaemic time [18]. Furthermore, it is well known from lung-transplant studies that lymphocytes promote the acute/subacute rejections, whereas neutrophils trigger later-occurring rejections [19]. Finally, a positive correlation has been demonstrated between IL-1 levels and the number of macrophages and lymphocytes in 'early' BAL from 'ischaemic' lungs, as well as a positive correlation between IL-1 levels and the number of neutrophils in 'late' BAL from 'ischaemic/reper fused' lungs [18]. All these experimental and clinical studies overall confirm a predominant role of neutrophils in the development of lung damage, either when reperfusion occurs after ischaemia or whenever a lung proinflammatory state is prolonged during time; however, an early inflammatory state seems to be predominantly triggered by both lymphocytes and macrophages [9, 15, 18].

Similarly, our study showed an overall significant augmentation of WBC count after surgery. As far as cell titration is concerned however, our findings seem to suggest a different inflammatory cascade after the two utilized techniques, with a predominant early lymphocyte and late neutrophil activation following the standard CPB (with a higher degree of lung damage due to the higher and prolonged neutrophil infiltration) versus a predominant macrophage-induced lung inflammation after sPPP.

Furthermore, according to de Perrot et al. [20], who demonstrated that lymphocytes also play a significant role during reperfusion in a rat model, our findings in the sPPP group (lower lymphocytes and neutrophils, lower proinflammatory and higher immune modulator mediators) appear to suggest a potential limitation of the mechanisms of injury involved in the 'reperfusion' phase. From this perspective, we could also interpret the similar augmentation of GRO-α levels found in the BAL of both groups, given the demonstration that GRO-α is an 'early' biomarker, whose synthesis is predominantly triggered by ischaemia (i.e. the initial trigger) rather than by reperfusion (i.e. the perpetuating mechanism) [21].

If the lower functional impairment after sPPP can be explained by the lower neutrophil infiltration [6, 9, 13], the higher macrophage count observed in the same group deserves further studies. The recent demonstration of an 'immune modulator' effect of lung macrophages during inflammation, together with their potential shifting from a proinflammatory state to an anti-inflammatory one in the self-limiting phases of various inflammatory-mediated lung diseases [22, 23], might help to explain our findings. Nevertheless, the shifting of alveolar macrophages from proinflammatory to anti-inflammatory phenotypes in the context of a CPB-induced lung I/R injury has never been demonstrated. However, the demonstration of a macrophage anti-inflammatory phenotype has been recently detected in the serum of patients undergoing CABG, thus suggesting its potential protective role [24].

Finally, the present study did not demonstrate any difference between serum cytokines of patients undergoing either sPPP or the standard CPB. These data are in line with previous research investigating the lung injury: in an elegant human study on 18 CABG patients, Massoudy et al. [2] demonstrated increased proinflammatory cytokines with the retention of platelet–monocyte coaggregates during the pulmonary passage, thus proving a specific lung parenchyma inflammatory response during CABG. Similarly, Kotani et al. [9] showed how IL-8, neutrophils and elastase levels from BAL— but not the corresponding serum levels—correlate with post-CPB PaO₂/FiO₂ and the degree of intrapulmonary shunt. Furthermore, Tsuchida et al. demonstrated that alveolar macrophages, but not circulating monocytes, enhance their surface expression of adhesion molecules during the reperfusion phase after aortic declamping, as well as that alveolar macrophages (but not serum monocytes) synthesized higher levels of IL-8 and TNF-α when cultured in vitro [5]. These data together with our findings confirm that any investigation of lung injury after cardiac surgery deserves lung parenchyma sampling, rather than circulating serum cytokine and leukocyte evaluation, the latter being totally unreliable markers of a specific lung inflammation.

Although, in our experience, sPPP did not show any advantage in terms of overall survival, possibly in view of the low-risk profile of the chosen patient population, an improved outcome, still to be tested, in higher risk patients, such as those with
moderate-to-severe pulmonary comorbidities, might justify the invasiveness of this tool. Moreover, the use of sPPP in patients with impaired pulmonary function deserves future comparison with alternative strategies, such as off-pump surgery, already suggested but never definitely proved to overcome the negative effects of CPB on perioperative lung function [25].

Limitations of the study

The main limitation of the study is related to the employed technique of sPPP. Indeed, it can be questioned to which extent perfusion and pulsatility determined the observed results. Several studies, however, have already showed the benefit of continuous pulmonary perfusion on lung function, and pulsatility was chosen as a further adjunct to provide additional benefit. Careful extrapolation of our results to different techniques of pulmonary perfusion is mandatory. Certainly, future studies analysing the optimal pulmonary flow, the best oxygen content of blood used for sPPP, and/or the role of different strategies of mechanical ventilation during CPB to maximize the ventilation/perfusion ratio (e.g. low volumes with high rates, low rates with high volumes, presence/absence of positive end-expiratory pressures etc.) should be encouraged.

On the other hand, the standardization of the protocol and its execution in a single institution guarantees the uniformity of the perioperative management of the patient population throughout the experimentation, thus limiting the potential for human bias. Moreover, on an intention-to-treat basis, we adopted strict enrolment criteria in order to minimize potential confounding factors and misleading results.

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REFERENCES


APPENDIX. CONFERENCE DISCUSSION

Dr C. Choong (Melbourne, Australia): Post cardiopulmonary bypass lung dysfunction is a recognized complication and the aetiology is clearly multifactorial. Ischaemic reperfusion injury is one of the factors involved and is an important area in cardiothoracic surgery and may explain and contribute to some of the respiratory complications seen in the postoperative period following cardiopulmonary bypass. This is a very well-designed clinical study, with care taken to carefully select appropriate patients to avoid confounding factors, and with good collection of data peroperatively. I think it is important to point out that, in general, most surgeons performing cardiac surgery would not routinely practice selective pulmonary perfusion during the period of aortic cross clamping and cardiopulmonary bypass support.

I have two questions. Firstly, the selective pulmonary perfusion increased coronary collateral blood flow during the performance of coronary artery anastomosis; did it add to the complexity of the surgery? Secondly, the incidence of serious respiratory complications in straightforward low-risk cardiac surgical patients is indeed low. What are your thoughts as to who should be selected for consideration of selective pulmonary perfusion during cardiopulmonary bypass? I think it is important to keep in mind that the use of selective pulmonary perfusion requires additional cannulation of the main pulmonary artery and the additional placement of a left ventricular vent.

Dr Santini: Concerning the coronary flow during the procedure, we didn’t have this problem because we routinely used a left ventricular vent which we positioned through the right superior pulmonary vein. So the procedure per se was not complicated by the pulmonary perfusion. However, at the very beginning of the study we also elected to perfuse with just 7 ml/kg/min to avoid the potential for technical problems during the procedure.

Concerning the indication for this tool, which definitely requires more invasiveness in the organization of the surgical strategy, I don’t know what the answer is as yet since we chose to start with a very low-risk profile group of patients to prove the safety and efficacy of the technique. Indeed, we proved that the impact of pulmonary perfusion on the respiratory function and the haemodynamic parameters really deserves attention. We plan to choose more complicated and high-risk patients in the future to be able to find out retrospectively which characteristics may indicate in which patients to use this tool.

Dr A. Wahba (Trondheim, Norway): There are several studies that have shown beneficial effects of pulmonary perfusion. Now, you chose to add pulmonary perfusion and pulsatility. What is, in your opinion, the most important part and why did you choose this model?

Dr Santini: I think there is a lot of confusion still on the difference between continuous flow and pulsatile flow. I think that a real pulsatile flow has the potential to give more haemodynamic energy to the perfusion. And the idea was to provide the best available model to perfuse the lung. We calculated the energy, the extra energy, according to the available formulas that we all know. And we set up the minimal flow with the potential to fulfil the criteria for a pulsatile flow on the one hand, without interfering with the technical detail of the operation on the other.

Dr D. Paparella (Bari, Italy): We performed a study a few years ago in which we could demonstrate that by using a lung protective ventilation strategy with high PEEP and low volumes and low pressures, we could have reduced inflammatory response following CPB measured in the lung and also in the systemic circulation. We concluded that the lungs after cardiopulmonary bypass are probably one of the main sources of inflammatory response. You could show very nice reduction of inflammatory response in the lungs but not in the systemic circulation. Could it be because actually with this method we add some excess extracorporeal circulation to the one that we usually do?

Dr Santini: Concerning the ventilation, we did not ventilate these patients during the procedure, although we provided for all, both groups, a recruitment manoeuvre at the end just to minimize the amount of atelectasis. Definitely ventilation is a variable to be considered, because lung oxygen supply has a bronchial artery nature but also a pulmonary artery and a ventilatory support one. So I think that lung ventilation during this procedure may indeed have a potential role in protecting the tissue.

We thought it was very interesting that the amount of cytokine levels was significantly different between the groups in the bronchoalveolar lavage, but not in the systemic circulation. Our interpretation of this data is that possibly many other tissues, the myocardium first of all, but also others, contribute to the systemic inflammatory cascade and so what pertains to the lung might be overlooked should you check the systemic circulation.

In view of these considerations also looking at several studies from the past, I wonder whether checking on the inflammatory reaction of the lung by collecting samples from the systemic circulation is really a reliable method.

Dr W. Harringer (Braunschweig, Germany): In this cohort you are ultimately aiming at the really sick patients with preoperative lung injury. One way to get around it would be to avoid the extracorporeal circulation at all. Have you gathered any anecdotal or additional data in your study to see how these values compare to off-pump surgery patients?

Dr Santini: In our institution the number of patients operated on off-pump is currently minimal so we didn’t have the chance to check on that group. I think, however, that the importance of this study is not only as a potential tool to reduce mortality but also as a means to reduce postoperative morbidity. This will certainly have an impact on ICU stay and costs.