

# **Immune dysfunction after cardiac surgery with cardiopulmonary bypass: beneficial effects of maintaining mechanical ventilation**

Baptiste Gaudriot <sup>1\*</sup>, Fabrice Uhel <sup>2,3,4\*</sup>, Murielle Gregoire <sup>5</sup>, Arnaud Gacouin <sup>2,3</sup>, Sebastien Biedermann <sup>1</sup>, Antoine Roisne <sup>1</sup>, Erwan Flecher <sup>6</sup>, Yves Le Tulzo <sup>2,3,4</sup>, Karin Tarte <sup>4,5</sup>, Jean-Marc Tadie <sup>2,3,4</sup>

1. CHU Rennes, Anesthésie-Réanimation, F-35033 Rennes, France

2. CHU Rennes, Maladies Infectieuses et Réanimation Médicale, F-35033 Rennes, France.

3. Inserm, CIC-1414, Faculté de Médecine, Université Rennes I, F-35043 Rennes, France.

4. Biosit, Faculté de Médecine, Université Rennes 1, F-35043 Rennes, France

5. Inserm, U917, Faculté de Médecine, Université Rennes 1, F-35043 Rennes, France

6. CHU Rennes, Chirurgie Cardio-thoracique et Vasculaire, F-35033 Rennes, France.

\* Equally contributed to this work.

Requests for reprints should be addressed to Jean-Marc Tadié, MD, PhD, Service des Maladies Infectieuses et Réanimation Médicale, Hôpital Pontchaillou, 2 rue Henri Le Guilloux, 35033 Rennes Cedex 9, France. E-mail address: [jeanmarc.tadie@chu-rennes.fr](mailto:jeanmarc.tadie@chu-rennes.fr)

Conflicts of interest: none

Words count: 2480

Key words: Cardiopulmonary bypass, cardiac surgery, mechanical ventilation, immune dysfunction, myeloid derived suppressor cells

Funding: This work was supported by a Fondation de l'Avenir grant (Recherche médicale appliquée)

## **Abstract**

Introduction: Cardiac surgery with cardiopulmonary bypass (CPB) induces postoperative immunosuppression and impaired pulmonary function. Maintaining mechanical ventilation (MV) during CPB improves pulmonary function and diminishes postoperative systemic inflammation. However, there is no data about the influence of maintaining MV during CPB on postoperative immune dysfunction.

Material and methods: 50 patients were prospectively divided into two groups: without MV during bypass (n=25) and dead space MV with PEEP (n = 25). PaO<sub>2</sub>/FiO<sub>2</sub> ratio, CXCL10, CCL2, TNF- $\alpha$ , IL-10, HLA-DR, monocytic myeloid-derived suppressor cells (Mo-MDSC, CD14<sup>+</sup>HLA-DR<sup>lo/-</sup> monocytes) and blood cell count were collected before and after surgery.

Results: CPB induced a marked immunosuppression with a significant increase of plasmatic levels of TNF- $\alpha$  and IL-10, and a significant decrease in HLA-DR monocytic expression. The postoperative proportion of Mo-MDSC was subsequently significantly increased. Maintaining MV during CPB significantly improved PaO<sub>2</sub>/FiO<sub>2</sub> ratio and decreased postoperative plasmatic levels of TNF- $\alpha$  and IL-10 compared to patients without MV during CPB. Furthermore, non-ventilated patients had a lower lymphocyte count after surgery compared to patients with MV during CPB.

Conclusion: Our study suggests that maintaining MV during CPB for cardiac surgery decreases postoperative immune dysfunction and could be an interesting strategy to diminish the occurrence of postoperative nosocomial infection without hampering the surgical procedure. However, these findings have to be confirmed in a clinical trial using the incidence of nosocomial infection as an endpoint.

## Introduction

Cardiopulmonary bypass (CPB) during cardiac surgery induces a systemic inflammatory response associated with an immune dysregulation and a significant pulmonary dysfunction [1-5]. These two phenomena have been well characterized. Firstly, the inflammatory response, usually attributed to surgical trauma, contact of blood with artificial surfaces and ischemia-reperfusion injury, is responsible for a postoperative immunodepression. Mekontso et al. have shown that bactericidal activity of polymorphonuclear neutrophils against *Staphylococcus aureus* was decreased after cardiac surgery [3]. In a prospective study, Chalk et al. have also reported a dysfunction of pulmonary macrophages after CPB. In particular, they have described an early impairment of lung cellular immune response, which could promote the development of postoperative pneumonia [6]. Along these lines, a down-regulation of human leucocyte antigen-DR antigen (HLA-DR) expression on monocytes and an increase of plasma interleukine (IL)-10 associated with the occurrence of nosocomial infections have been reported [4,6,7]. Secondly, CPB induces a pulmonary dysfunction which ranges from a temporary and clinically insignificant reduction in arterial oxygenation to a life-threatening injury manifested as acute respiratory distress syndrome. This phenomenon is of multifactorial sources but one of the main mechanisms is the occurrence of atelectasis during surgery [8-15]. Atelectasis have been associated with lung injury and release of cytokines (TNF- $\alpha$ ) by shear forces on alveoli and small airways [12-15]. However, it is not clear whether this injury is due to a recruitment/de-recruitment phenomenon (ie atelectrauma) or whether it might by itself lead to the release of cytokines [15-17]. Nevertheless, addition of a positive end-expiratory pressure (PEEP) during CPB for cardiac surgery diminished the occurrence of atelectasis and the postoperative inflammatory response without hampering the surgical progress [10-12].

Since maintaining mechanical ventilation (MV) with PEEP during CPB diminishes postoperative inflammation, a relationship between pulmonary dysfunction and postoperative immunosuppression should be investigated. We thus conducted a prospective study to

investigate the effects of maintaining lung ventilation during CPB in patients undergoing cardiac surgery on postoperative immune dysfunction using IL-10 and HLA-DR as major endpoints. We also investigated the CD14<sup>+</sup>HLA-DR<sup>lo/-</sup> monocytes, which have been described as immunosuppressive monocytic myeloid derived suppressor cells (Mo-MDSC) in humans [18-20], before and after CPB.

## Materials and methods

### *Patient population*

All patients were > 18 years old and were scheduled for cardiac surgery using cardio-pulmonary bypass (valve replacement and/or coronary artery bypass grafting).

Exclusion criteria were as follows: systemic steroid therapy, chronic lung diseases (including chronic obstructive or restrictive pulmonary diseases), left ventricular dysfunction (left ventricular ejection fraction <50%) and the need for vasopressor or inotropic agents before surgery.

### *Anaesthesia, CPB, and surgical procedure*

General anaesthesia was induced with propofol, sufentanil 0.3µg/kg and was followed by cisatracurium 0.15 mg/kg for muscular relaxation. Anaesthesia, muscle relaxation and analgesia were respectively maintained with inhaled sevoflurane during off-pump surgery or propofol infusion during CPB, boluses of atracurium and sufentanil. Baseline ventilation: the patients received volume-controlled ventilation with a tidal volume of 8 mL/kg of predicted body weight (PBW) and a ventilatory frequency of 10–15 b.p.m. (PETCO<sub>2</sub> between 34 and 38 mmHg). Inspired oxygen fraction (FiO<sub>2</sub>) was 0.50, inhalation: exhalation ratio was 1:2, and the level of positive end-expiratory pressure (PEEP) was 5 cmH<sub>2</sub>O.

During CPB, two settings were used depending on the surgeon in charge: (1) absence of mechanical ventilation (and no positive end-expiratory pressure) by disconnecting the tracheal tube from the ventilator (MV- group), (2) dead space ventilation using tidal volume of 2.5 mL/kg (predicted body weight, PBW) with 5cmH<sub>2</sub>O PEEP (MV+ group). After CPB, ventilatory parameters were turned back to baseline settings and no recruitment manoeuvres were performed before oxygenation determination. PaO<sub>2</sub> /FiO<sub>2</sub> was obtained before surgery (after induction of anaesthesia) and 3 h after the end of CPB in the surgical intensive care unit (under baseline mechanical ventilation settings). We took advantage of the different ventilatory settings that are commonly used by the surgeon and the anaesthetist

in charge: consequently, the patients were not randomised in the different ventilatory settings for the purpose of this study. The study was approved by our Local Ethics Committee. Patients were informed of the observational nature of the study and gave their consent. The design of the study is summarized in Figure 1A.

### **Study procedures.**

Blood samples treated with heparin were collected for all patients immediately after induction of anesthesia (T1) and within an hour following the end of intervention (T2). The delay between blood sampling and the beginning of laboratory procedures was <1h. Plasma samples were stored at -80°C until use.

**Flow cytometry.** The level of HLA-DR expression on monocytes was measured on whole blood using fluorescein isothiocyanate-conjugated anti-human leukocyte antigen DR (HLA-DR-FITC, Beckman Coulter) with isotype- and fluorophore-matched control to confirm binding specificity, and phyco-erythrin-cyanin-7 anti-CD14 antibody (CD14-PC7; Beckman Coulter). Samples were run on a flow cytometer (Navios, Beckman Coulter) and data were analysed using Kaluza software (Beckman Coulter). Monocytes were characterized on the basis of their CD14 expression (See Figure 1B for gating strategy).

### **ELISA**

TNF- $\alpha$ , IL-10, C-X-C motif chemokine 10 (CXCL10) and chemokine ligand 2 (CCL2) were quantified by DuoSet ELISA (R&D Systems, Abingdon, UK) in peripheral blood plasma according to the manufacturer instructions.

### **Data collection**

The following data were collected: Age, Body Mass Index (BMI), central venous pressure (CVP) and mean pulmonary arterial pressure (mPAP) before and after CPB, PaO<sub>2</sub> /FiO<sub>2</sub> ratio before and after CPB, type of heart surgery (valvular replacement, coronary artery bypass grafting (CABG) or mixed), surgery duration, ICU and hospital length of stay. The occurrence of postoperative infections (nosocomial infections) within 14 days was also recorded and defined as previously described [21]. Lastly, blood cell count with differential (including lymphocyte, neutrophil (PMN), and monocyte counts) was collected before and after surgery.

### **Statistical analysis**

Quantitative variables were expressed as median and interquartile range (IQR). Differences observed between groups were analyzed using the nonparametric Mann-Whitney *U* test. In each group, differences between pre- (T1) and post-CBP times (T2) were analyzed using the Wilcoxon matched-pairs signed rank test. Correlations between two continuous variables were investigated using the nonparametric Spearman rank correlation test. All statistical analyses were performed using GraphPad Prism Version 5 (GraphPad Prism Software Inc) at a significance level of  $p < 0.05$ .

## Results

### *Patients characteristics*

50 patients completed the study. The baseline characteristics are given in Table 1. No difference was found between the two groups except for body mass index (BMI).

### *Effects of maintaining MV during CPB on pulmonary function*

The effects of mechanical ventilation on outcome as well as clinical parameters are summarized in Table 2. Of note, maintaining MV during CPB did not influence procedure duration.

Maintaining MV during CPB significantly improved postoperative PaO<sub>2</sub> /FiO<sub>2</sub> ratio (+6% in ventilated patients vs. -15% in non-ventilated patients, p= 0.005, Figure 2A). No difference was found between the two groups regarding hemodynamic parameters (CVP and mPAP) before and after CPB (Table 1). Lastly, MV duration, length of stay in ICU and hospital stay were not different between the two groups.

Plasmatic levels of CXCL10 and CCL2 were significantly increased after cardiac surgery (Figure 2B). No difference was found between patients with and without MV during CPB (Figure 2C).

### *CPB during cardiac surgery decreased HLA-DR expression, increased CD14<sup>+</sup>HLA-DR<sup>low/neg</sup> monocytes count and induced IL-10 and TNF- $\alpha$ production.*

A significant decrease in HLA-DR expression occurred after CPB (MFI 7.3 [IQR, 5.4 – 9.3] before vs 2.8 [2.3 – 3.4] after; p<0.001, Figure 3A). CPB was subsequently associated with a significant increase in CD14<sup>+</sup>HLA-DR<sup>low/neg</sup> monocytes (10.3% of CD14<sup>+</sup> monocytes [IQR, 3.9 – 17.4] before vs. 29.5% [IQR, 20.9 – 40.9] after; p<0.0001). TNF- $\alpha$  and IL-10 levels were higher after surgery (respectively, 45.24 pg/mL [IQR, 43.14 – 48.47] before vs. 48.47 pg/mL [43.14 – 56.14] after; p=0.0183, and 66.77 pg/mL [IQR, 62.78 – 72.95] before vs. 114.7 pg/mL [IQR, 86.55 – 153.9] after; p<0.0001, Figure 3B). White blood cell counts were also

modified after surgery, compared to WBC before CPB: we found a significant increase in PMN count ( $4.42 /\text{mm}^3$  [IQR, 3.31 – 5.22] before vs.  $8.58 /\text{mm}^3$  (IQR, 6.47 – 12.69] after;  $p<0.0001$ ) and a significant decrease in lymphocyte count ( $1.69 /\text{mm}^3$  [1.34 – 2.205] before vs.  $1.3 /\text{mm}^3$  [IQR, 0.9 – 1.625] after;  $p<0.0001$ ), while monocyte count remained unchanged ( $0.63 /\text{mm}^3$  (IQR, 0.53 – 0.795) before vs.  $0.63 /\text{mm}^3$  IQR, 0.39 – 0.9] after; ns, Figure 3C). Lastly, there was a significant inverse correlation between postoperative levels of IL-10 and lymphocytes count (Figure 3D).

#### *Effects of maintaining MV during CPB on immune parameters*

Patients ventilated during CPB showed lower levels of TNF- $\alpha$  and IL-10 compared to non-ventilated patients ( $44.2 \text{ pg/mL}$  [IQR, 42.1 – 48.5] and  $98.7 \text{ pg/mL}$  [IQR, 86.5 – 129.0] vs.  $53.5 \text{ pg/mL}$  [IQR, 48.4 – 64.4] and  $129.1 \text{ pg/mL}$  [IQR, 89.0 – 198.7],  $p<0.05$  and  $p<0.05$  respectively, Figure 4A). Furthermore, non-ventilated patients had a lower lymphocyte count after surgery compared to patients ventilated during CPB ( $1.05 /\text{mm}^3$  [IQR, 0.88 – 1.465] vs.  $1.48 /\text{mm}^3$  [IQR, 1.128 – 1.74] respectively,  $p=0.04$ , Figure 4B). Lymphocyte count remained significantly lower 48 hours after surgery ( $0.73 /\text{mm}^3$  [IQR, 0.59 – 1.05] vs.  $1.36 /\text{mm}^3$  [IQR, 0.96 – 1.47],  $p<0.05$ , Figure 4C).

No significant difference was found in HLA-DR down-regulation between the two groups (MFI (T2-T1)  $-4.3$  [IQR,  $-6.7$  -  $-2.4$ ] in non-ventilated patients vs.  $-3.8$  [IQR,  $-5.0$  -  $-2.8$ ] in ventilated patients; ns, Figure 4D). Consequently, CD14<sup>+</sup>HLA-DR<sup>low/neg</sup> monocytes count was not different between the two groups (32.23% of CD14<sup>+</sup> monocytes [IQR, 24.23 – 40.40] in non-ventilated patients vs. 24.87% [IQR, 15.81 – 41.73] in ventilated patients;  $p=0.2$ , Figure 4E).

## Discussion

Our results suggested that maintaining MV during cardiac surgery with CPB could be of interest to decrease postoperative immunosuppression. We found that patients ventilated during CPB had lower postoperative levels of immunosuppressive IL-10 and proinflammatory TNF- $\alpha$ , and a higher postoperative lymphocytes count. However, the proportion of CD14<sup>+</sup>HLA-DR<sup>lo/-</sup> monocytes was not statistically different between both groups. These beneficial effects on postoperative immunosuppression could be related to an enhancement in pulmonary function since maintaining MV during CPB abolished the postoperative decrease of PaO<sub>2</sub> /FiO<sub>2</sub> ratio.

Firstly, cardiac surgery with CPB induced postoperative immunosuppression. This effect has been well characterized and previous studies have demonstrated that HLA-DR expression was significantly decreased, and IL-10 was increased after cardiac surgery [4,5,18]. The loss of HLA-DR expression on monocyte, which indicates their functional deactivation, had previously been described after cardiac surgery and sepsis [5], and the persistence of this alteration is associated with severity, nosocomial infection, and death [7,22]. IL-10 is an immunosuppressive cytokine which could be involved in HLA-DR down regulation [23] and an increase in TNF- $\alpha$  has been shown to be counterbalanced by early expression of anti-inflammatory IL-10 [24]. Thus, strategies that could decrease postoperative IL-10 levels could be of interest to decrease postoperative infectious complications. In agreement, we found that total lymphocyte count was significantly decreased after cardiac surgery in non-ventilated patients compared to patients ventilated during CPB. Lymphopenia has been associated with immune dysfunction during septic shock and it has been shown that low absolute lymphocyte counts were predictive of postoperative sepsis [25-26]. In a recent retrospective study, Drewry et al. have demonstrated that persistent lymphopenia after sepsis predicted mortality, and could be a valuable biomarker of immunosuppression since lymphopenia predicted an increased risk of secondary infection [27]. Lastly, we found an increased number of CD14<sup>+</sup>HLA-DR<sup>lo/-</sup> monocytes after cardiac surgery. This phenotype is

consistent with a monocytic-MDSC phenotype. To our knowledge, no study has previously reported this finding. MDSC comprise a heterogeneous population of immature myeloid cells at different stages of differentiation [19,20]. Although many subtypes of MDSC have been described in mice and humans, they can be classified into two major subsets: a monocytic MDSC population (CD14<sup>+</sup>HLA-DR<sup>low</sup>) and a granulocytic MDSC population ([CD3, CD19, CD56, CD14]<sup>-</sup>CD15<sup>+</sup>HLA-DR<sup>+</sup>). Those cells have been shown to be increased in critically ill patients and associated with a poor prognosis [28]. This finding raises the question of targeting those cells to reduce immunosuppression after cardiac surgery. However, as the proportion of CD14<sup>+</sup>-HLA-DR<sup>low</sup> monocytes was not different between both groups, mechanical ventilation doesn't seem to have any impact on HLA-DR expression.

Since we found significantly lower TNF- $\alpha$  and IL-10 plasma levels, as well as an increased lymphocyte count in ventilated patients compared to non-ventilated patients during CPB, we assume that maintaining MV during CPB may decrease both pro- and compensatory anti-inflammatory responses by preventing lung injury and/or atelectasis.

Indeed, we found that maintaining MV during CPB improved postoperative index of oxygenation. This result was expected and several studies have already demonstrated the beneficial effects of MV with PEEP during cardiac surgery with CPB [10-12]. As previously reported, postoperative plasmatic levels of CXCL10 and CCL2 were significantly increased [29]. CXCL10 is involved in acute lung injury and ARDS and induces neutrophils migration into the lung [30]. In particular, a recent study has shown that CXCL10 and its receptor CXCR3 were critical factors for the exacerbation of the pathology of ARDS [31]. CCL2 has been shown to be involved in neutrophils recruitment during ARDS. In a mouse model of ARDS, neutralization of this chemokine significantly decreased severity of lung injury [32]. Our ventilation strategy did not have a significant influence on post-CPB increased permeability edema since postoperative levels of CCL2 and CXCL10 were similar in ventilated and non-ventilated patients with a similar count of PMN. Thus, we believe that maintaining ventilation during CPB decreases rather the occurrence of post-CPB atelectasis which has been regarded as a main cause of post-CPB lung injury [13-14]. Furthermore, we

found that maintaining MV during CPB significantly decreased postoperative levels of TNF- $\alpha$ . TNF- $\alpha$  is released by a large number of pulmonary cells including alveolar macrophages and epithelial cells including pulmonary cells (alveolar macrophages and pulmonary epithelial cells...) and has been considered as a marker of lung injury [15,33,]. Occurrence of atelectasis when MV is discontinued during CPB [13,14,34] could generate alveolar injury and inflammation in the surrounding lung tissue [16] which could be worsened by MV after CPB (ie atelectrauma). Alternatively, hypoxia associated with atelectasis may be injurious and has been associated with an increased wet-to-dry ratio in an animal study [15,17,].

### Limitations

This study has several limitations. First, it is an observational study and the lack of randomization may have induced some bias. We found a difference between two groups with a significantly higher BMI in the ventilated group, and a nearly statistically significant difference in baseline PaO<sub>2</sub> /FiO<sub>2</sub> ratio. Despite BMI having been described as a risk factor for early onset of severe pulmonary dysfunction after surgery [35], we still found that PaO<sub>2</sub> /FiO<sub>2</sub> ratio was improved in ventilated patients. Secondly, in contrast to murine MDSC, human MDSC are not so clearly defined because of the lack of specific markers. Although CD14<sup>+</sup>HLA-DR<sup>low/neg</sup> phenotype is consistent with a Mo-MDSC phenotype, *in vitro* studies would be required to confirm the suppressive properties of those cells after CPB [20].

Acknowledgment: we thank Eric Barr for his careful review of the manuscript

## Figure legends

### Figure 1: Design of the study and gating strategy for HLA-DR monocytic expression measurement.

(A) Design of the study.

(B) Following an initial FS/SS discrimination of the monocytic subset, CD14<sup>+</sup> monocytes were gated, and HLA-DR mean fluorescence intensity (MFI) was measured before (T1) and after CBP (T2). Green areas represent mean channel fluorescence for FITC anti-HLA-DR. Unfilled histograms are isotype control results from the same sample. As monocytic MDSC (Mo-MDSC) in humans were previously described as CD14<sup>+</sup>HLA-DR<sup>lo/-</sup> monocytes, we also quantified the proportion of this subset among CD14<sup>+</sup> monocytes.

CBP, Cardio-pulmonary bypass; CCL2, Chemokine ligand-2; CXCL-10, CXC motif chemokine-10; IL-10, Interleukin-10; FS, Forward scatter; ICU, Intensive care unit; mHLA-DR, Monocytic expression of human leukocyte antigen-DR; MV, Mechanical ventilation; PaO<sub>2</sub>/FiO<sub>2</sub>, Ratio of arterial oxygen tension over inspired oxygen fraction; PEEP, Positive end-expiratory pressure; RR, respiratory rate; SS, Side scatter; TNF- $\alpha$ , Tumor necrosis factor alpha.

### Figure 2: Effects of maintaining mechanical ventilation during CPB on pulmonary function

(A) Variation of PaO<sub>2</sub>/FiO<sub>2</sub> ratio between pre- (T1) and post-CBP time (T2) in patients with (MV+) and without (MV-) mechanical ventilation during CPB.

(B) Plasma levels of CCL2 and CXCL10 before (T1) and after (T2) CPB.

(C) Plasma levels of CCL2 and CXCL10 according to maintenance (MV+) or not (MV-) of mechanical ventilation during CPB.

Boxes represent median, 25<sup>th</sup> and 75<sup>th</sup> percentiles. Whiskers represent 5<sup>th</sup> and 95<sup>th</sup> percentiles. Bars on dot plots represent medians. P values were obtained using Mann-Whitney *U* test for comparisons between MV+ and MV- groups, and Wilcoxon match-pairs signed rank test for comparisons between T1 and T2 periods within each group.

CCL2, Chemokine ligand 2; CXCL-10, CXC motif chemokine 10; MV, Mechanical ventilation; PaO<sub>2</sub>/FiO<sub>2</sub>, Ratio of arterial oxygen tension over inspired oxygen fraction; T1, before cardiopulmonary bypass; T2, After cardiopulmonary bypass.

### Figure 3: Immunological effect of CPB during cardiac surgery

(A) HLA-DR expression on CD14<sup>+</sup> monocytes determined by flow cytometry, and proportion of CD14<sup>+</sup>HLA-DR<sup>lo</sup> monocytes among CD14<sup>+</sup> monocytes, before (T1) and after (T2) CPB.

(B) Plasma levels of TNF- $\alpha$  and IL-10 before (T1) and after (T2) CPB.

(C) Number of circulating neutrophils (PMN), lymphocytes and monocytes before (T1) and after (T2) CPB.

(D) Correlation between circulating lymphocyte count and plasma level of IL-10 after CPB (T2).

P values were obtained using Wilcoxon match-pairs signed rank test for comparisons between T1 and T2 periods. Correlations were investigated using the nonparametric Spearman rank correlation test.

IL-10, Interleukin-10; mHLA-DR, Monocytic expression of human leukocyte antigen-DR; MFI, Mean fluorescence intensity; MV, Mechanical ventilation; PaO<sub>2</sub>/FiO<sub>2</sub>, Ratio of arterial oxygen tension over inspired oxygen fraction; T1, before cardiopulmonary bypass; T2, After cardiopulmonary bypass; TNF- $\alpha$ , Tumor necrosis factor alpha.

### Figure 4: Immunological effect of maintaining mechanical ventilation (MV) during CPB

(A) Plasma levels of TNF- $\alpha$  and IL-10, (B) Number of circulating neutrophils (PMN), lymphocytes and monocytes, (C) Number of circulating lymphocytes at day 2, (D) expression

of HLA-DR on CD14<sup>+</sup> monocytes and (E) percentage of CD14<sup>+</sup>HLA-DR<sup>lo/-</sup> monocytes according to maintenance (MV+) or not (MV-) of mechanical ventilation during CPB.

Bars on dot plots represent medians. P values were obtained using Mann-Whitney *U* test for comparisons between MV+ and MV- groups, and Wilcoxon match-pairs signed rank test for comparisons between T1 and T2 periods within each group.

IL-10, Interleukin-10; mHLA-DR, Monocytic expression of human leukocyte antigen-DR; MFI, Mean fluorescence intensity; MV, Mechanical ventilation; PMN, Neutrophils; T1, before cardiopulmonary bypass; T2, after cardiopulmonary bypass; TNF- $\alpha$ , Tumor necrosis factor alpha.

|                             | MV-<br>N=25  | MV+<br>N=25  | p     |
|-----------------------------|--------------|--------------|-------|
| Age (years)                 | 75[65-79]    | 73[69-80]    | 0.79  |
| Surgery                     |              |              |       |
| - Valvular replacement      | 17           | 8            |       |
| - CABG                      | 5            | 9            |       |
| - Mixed (valvular and CABG) | 3            | 8            |       |
| BMI (kg/m <sup>2</sup> )    | 26[23-28]    | 30[27-33]    | 0.007 |
| Euroscore II                | 2.2[1.4-2.9] | 2.3[1.4-3.3] | 0.6   |
| CVP before CPB (mmHg)       | 7[6-10]      | 10[7-12]     | 0.15  |
| mPAP before CPB (mmHg)      | 23[20-24]    | 19[11-25]    | 0.28  |
| P/F ratio before CPB        | 326[225-381] | 240[173-320] | 0.07  |

**Table1. Baseline characteristics of patients before CBP**

|                          | MV-<br>N=25  | MV+<br>N=25  | p    |
|--------------------------|--------------|--------------|------|
| CVP after CPB (mmHg)     | 8[6-11]      | 11[8-12]     | 0.16 |
| mPAP after CPB (mmHg)    | 19[18-27]    | 21[16-24]    | 0.74 |
| P/F ratio after CPB      | 276[199-360] | 242[211-310] | 0.34 |
| CPBP duration (min)      | 61[52-76]    | 54[48-68]    | 0.32 |
| Surgery duration (min)   | 170[148-210] | 150[139-182] | 0.3  |
| ICU stay (days)          | 3[3-6]       | 3[3-5]       | 0.59 |
| Hospital stay (days)     | 10[4-16]     | 10[4-13]     | 0.52 |
| Postoperative Infections | 5 (20%)      | 2 (8%)       | 0.5  |

**Table 2. Effects of mechanical ventilation on outcome and clinical parameters.**

## References

1. Hadley JS, Wang JE, Michaels LC, Dempsey CM, Foster SJ, Thiernemann C, Hinds CJ. Alterations in inflammatory capacity and TLR expression on monocytes and neutrophils after cardiopulmonary bypass. *Shock*. 2007;27:466-73.
2. Rankin JS, Oguntolu O, Binford RS, Trochtenberg DS, Muhlbaier LH, Stratton CW. Management of immune dysfunction after adult cardiac surgery. *J Thorac Cardiovasc Surg*. 2011;142:575-80.
3. Mekontso-Dessap A, Honoré S, Kirsch M, Plonquet A, Fernandez E, Touqui L, Farcet JP, Soussy CJ, Loisanse D, Delclaux C. Blood neutrophil bactericidal activity against methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* during cardiac surgery. *Shock*. 2005;24:109-13.
4. Tepaske R, Velthuis H, Oudemans-van Straaten HM, Heisterkamp SH, van Deventer SJ, Ince C, Eysman L, Kesecioglu J. Effect of preoperative oral immune-enhancing nutritional supplement on patients at high risk of infection after cardiac surgery: a randomised placebo-controlled trial. *Lancet*.;358:696-701.
5. Wilhelm W, Grundmann U, Rensing H, Werth M, Langemeyer J, Stracke C, Dhingra D, Bauer M. Monocyte deactivation in severe human sepsis or following cardiopulmonary bypass. *Shock*. 2002;17:354-60.
6. Chalk K, Meisel C, Spies C, Volk T, Thuenemann K, Linneweber J, Wernecke KD, Sander M. Dysfunction of alveolar macrophages after cardiac surgery and postoperative pneumonia?--An observational study. *Crit Care*. 2013;17:R285.
7. Muehlstedt SG, Lyte M, Rodriguez JL. Increased IL-10 production and HLA-DR suppression in the lungs of injured patients precede the development of nosocomial pneumonia. *Shock*. 2002;17:443-50.
8. Ng CS, Arifi AA, Wan S, Ho AM, Wan IY, Wong EM, Yim AP. Ventilation during cardiopulmonary bypass: impact on cytokine response and cardiopulmonary function. *Ann Thorac Surg*. 2008;85:154-62.
9. Zupancich E, Paparella D, Turani F, Munch C, Rossi A, Massaccesi S, Ranieri VM. Mechanical ventilation affects inflammatory mediators in patients undergoing cardiopulmonary bypass for cardiac surgery: a randomized clinical trial. *J Thorac Cardiovasc Surg*. 2005;130:378-83.
10. John LC, Ervine IM. A study assessing the potential benefit of continued ventilation during cardiopulmonary bypass. *Interact Cardiovasc Thorac Surg*. 2008;7:14-7.
11. Loeckinger A, Kleinsasser A, Lindner KH, Margreiter J, Keller C, Hoermann C. Continuous positive airway pressure at 10cm H<sub>2</sub>O during cardiopulmonary bypass improves postoperative gas exchange. *Anesth. Analg*. 2000 91, 522-527.
12. Tadié JM, Zegdi R, Trinquart L, Kurdi O, Journois D, Latremouille C, Diehl JL, Louis B, Fagon JY, Fabiani JN, Delclaux C. Partitioning of exhaled NO in ventilated patients undergoing cardiac surgery. *Respir Physiol Neurobiol*. 2010 ;171:151-6.

13. Magnusson L, Zemgulis V, Wicky S, Tyden H, Thelin S, Hedenstierna G. Atelectasis is a major cause of hypoxemia and shunt after cardiopulmonary bypass. An experimental study. *Anesthesiology*. 1996;87:1153-63.
14. Verheij J, van Lingen A, Raijmakers PG, Spijkstra JJ, Girbes AR, Jansen EK, et al. Pulmonary abnormalities after cardiac surgery are better explained by atelectasis than increased permeability oedema. *Acta Anaesthesiol Scand*. 2005;49:1302-10
15. Chu EK, Whitehead T, Slutsky AS. Effects of cyclic opening and closing at low- and high-volume ventilation on bronchoalveolar lavage cytokines. *Crit Care Med*. 2004;32:168-74.
16. Retamal J, Bergamini B, Carvalho AR, Bozza FA, Borzone G, Borges J, Larsson A, Hedenstierna G, Bugedo G, Bruhn A. Non-lobar atelectasis generates inflammation and structural alveolar injury in the surrounding healthy tissue during mechanical ventilation. *Crit Care*. 2014 Sep 9;18(5):505.
17. Fukuse T, Hirata T, Nakamura T, Kawashima M, Hitomi S, Wada H. Influence of deflated and anaerobic conditions during cold storage on rat lungs. *Am J Respir Crit Care Med*. 1999;160(2):621-7.
18. Sbrana S, Bevilacqua S, Buffa M, Spiller D, Parri MS, Gianetti J, De Filippis R, Clerico A. Post-reperfusion changes of monocyte function in coronary blood after extracorporeal circulation. *Cytometry B Clin Cytom*. 2005;65:14-21.
19. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol*. 2009;9(3):162-74.
20. Greten TF, Manns MP, Korangy F. Myeloid derived suppressor cells in human diseases. *Int Immunopharmacol*. 2011 Jul;11(7):802-7.
21. Tadié JM, Trinquart L, Jannié-Nartey C, Guerot E, Louis B, Fagon JY, Diehl JL, Delclaux C. Prediction of nosocomial infection acquisition in ventilated patients by nasal nitric oxide: proof-of-concept study. *Shock*. 2010;34:217-21.
22. Le Tulzo Y, Pangault C, Amiot L, Guilloux V, Tribut O, Arvieux C, Camus C, Fauchet R, Thomas R, Drénou B. Monocyte human leukocyte antigen-DR transcriptional down regulation by cortisol during septic shock. *Am J Respir Crit Care Med*. 2004;169:1144-51.
23. Fumeaux T, Pugin J. Role of interleukin-10 in the intracellular sequestration of human leukocyte antigen-DR in monocytes during septic shock. *Am J Respir Crit Care Med*. 2002 ;166:1475-82.
24. Walley KR, Lukacs NW, Standiford TJ, Strieter RM, Kunkel SL. Balance of inflammatory cytokines related to severity and mortality of murine sepsis. *Infect Immun* 1996; 64: 4733–4738.
25. Le Tulzo Y, Pangault C, Gacouin A, Guilloux V, Tribut O, Amiot L, Tattevin P, Thomas R, Fauchet R, Drénou B. Early circulating lymphocyte apoptosis in human septic shock is associated with poor outcome. *Shock*. 2002;18:487-94
26. Lewis RT, Klein H: Risk factors in postoperative sepsis: significance of preoperative lymphocytopenia. *J Surg Res* 26(4):365Y371, 1979.
27. Drewry AM, Samra N, Skrupky LP, Fuller BM, Compton SM, Hotchkiss RS. Persistent lymphopenia after diagnosis of sepsis predicts mortality. *Shock*. 2014;42:383-91.

28. Gey A, Tadie JM, Caumont-Prim A, Hauw-Berlemont C, Cynober L, Fagon JY, Terme M, Diehl JL, Delclaux C, Tartour E. Granulocytic Myeloid-Derived Suppressor Cells inversely correlate with plasma arginine and overall survival in critically ill patients. *Clin Exp Immunol*. 2014 Dec 4. doi: 10.1111/cei.12567. [Epub ahead of print].
29. Beer L, Szerafin T, Mitterbauer A, Debreceni T, Maros T, Dworschak M, Roth GA, Ankersmit HJ. Low tidal volume ventilation during cardiopulmonary bypass reduces postoperative chemokine serum concentrations. *Thorac Cardiovasc Surg*. 2014;62:677-82.
30. Michalec L, Choudhury BK, Postlethwait E, Wild JS, Alam R, Lett-Brown M, Sur S. CCL7 and CXCL10 orchestrate oxidative stress-induced neutrophilic lung inflammation. *J Immunol* 168: 846–852, 2002.
31. Ichikawa A, Kuba K, Morita M, Chida S, Tezuka H, Hara H, Sasaki T, Ohteki T, Ranieri VM, Dos Santos CC, Kawaoka Y, Akira S, Luster AD, Lu B, Penninger JM, Uhlig S, Slutsky AS, Imai Y. CXCL10-CXCR3 enhances the development of neutrophil-mediated fulminant lung injury of viral and nonviral origin. *Am J Respir Crit Care Med* 187: 65–77, 2013.
32. Williams AE, Chambers RC. The mercurial nature of neutrophils: still an enigma in ARDS? *Am J Physiol Lung Cell Mol Physiol*. 2014 Feb;306(3):L217-30.
33. Tremblay LN, Miatto D, Hamid Q, Govindarajan A, Slutsky AS. Injurious ventilation induces widespread pulmonary epithelial expression of tumor necrosis factor- $\alpha$  and interleukin-6 messenger RNA. *Crit Care Med*. 2002;30:1693-700.
34. Shim JK, Chun DH, Choi YS, Lee JY, Hong SW, Kwak YL. Effects of early vital capacity maneuver on respiratory variables during multivessel off-pump coronary artery bypass graft surgery. *Crit Care Med*. 2009;37(2):539-44.
35. Rady MY, Ryan T, Starr NJ. Early onset of acute pulmonary dysfunction after cardiovascular surgery: risk factors and clinical outcome. *Crit Care Med*. 1997;25:1831-9.