



VECAR

Beneficial effects on maintaining mechanical ventilation during cardiopulmonary bypass for cardiac surgery on postoperative infections

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SPONSOR :	CHU Rennes (Rennes University Hospital)
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CLINICAL TRIAL PROTOCOL

VECAR

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STRATEGY	Cardiopulmonary bypass for cardiac surgery on postoperative infections : patients ventilated versus patients without mechanical ventilation during cardiopulmonary bypass
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COMPLETE TITLE	Beneficial effects on maintaining mechanical ventilation during cardiopulmonary bypass for cardiac surgery on postoperative infections
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INDICATION(S) (TARGET)	Cardiopulmonary bypass for cardiac surgery on postoperative infections
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COORDINATING INVESTIGATOR	Professeur Jean-Marc Tadié Service des maladies infectieuses et réanimation médicale CHU de Rennes – Hôpital de Pontchaillou 2 rue Henri le Guilloux 35033 Rennes Cedex 9
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PROTOCOL VERSION NO.	2.0
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DATE OF PROTOCOL	18/10/2017
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CPP	Approved on 30/08/2017 By the Committee for the Protection of Persons SOOM III
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SIGNATURES**INVESTIGATOR'S SIGNATURE**

I have read all pages of this clinical trial protocol for which the CHU of Rennes is the sponsor. I confirm that it contains all information necessary for the correct conduct of the study. I agree to conduct the study in compliance with the protocol and terms of conditions which are defined in it. I agree to conduct the study while complying with the conditions of:

- Principles of the "Declaration of Helsinki",
- The rules and recommendations of international good clinical practice ((ICH-E6) and French good clinical practice (rules of good clinical practice for biomedical research studies of medicinal products for human use - decisions of 24 November 2006),
- National legislation and regulations pertaining to clinical trials,
- Compliance with the Clinical Trials Directive of the EU [2001/20/EC].

I also agree that investigators and other qualified members of my team may have access to copies of this protocol and documents pertaining to the correct conduct of the study enabling them to work in compliance with conditions contained in these documents.

NAME : Professeur Jean-Marc TADIE

Signature :

Date : 18/10/2017

SPONSOR'S SIGNATURE

Sponsor : CHU de Rennes

NAME : GAUDRON Pascal

Signature :

Date : 18/10/2017

SYNOPSIS

TITLE	Beneficial effects on maintaining mechanical ventilation during cardiopulmonary bypass for cardiac surgery on postoperative infections
SPONSOR	CHU of Rennes
COORDINATING INVESTIGATOR	Professeur Jean-Marc Tadié Service des maladies infectieuses et réanimation médicale CHU de Rennes – Hôpital de Pontchaillou 2 rue Henri le Guilloux 35033 Rennes Cedex 9
PROTOCOL VERSION	2.0
RATIONALE / CONTEXT	<p>Cardiopulmonary bypass (CPB) during cardiac surgery induces a systemic inflammatory response associated with an immune dysregulation and a significant pulmonary dysfunction. First, the inflammatory response, usually attributed to surgical trauma, contact of blood with artificial surfaces, and ischemia reperfusion injury, is responsible for a postoperative immunodepression. For instance, an early impairment of lung cellular immune response after CPB, which could promote the development of postoperative pneumonia, has been found. Along these lines, a downregulation of human leukocyte antigen-DR antigen (HLA-DR) expression on monocytes and an increase in plasma interleukin 10 (IL-10) associated with the occurrence of nosocomial infections have been reported. Second, 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 (ARDS). This phenomenon is of multifactorial sources, but one of the main mechanisms is the occurrence of atelectasis during surgery. Atelectasis has been associated with lung injury and release of cytokines by shear forces on alveoli and small airways. However, it is not clear whether this injury is due to a recruitment/derecruitment phenomenon (i.e., atelectrauma) or whether it might by itself lead to the release of cytokine. Since CPB mechanically circulates and oxygenates blood bypassing the heart and lungs, usual procedure during CPB is to stop mechanical ventilation (MV) (apnea). Nevertheless, maintaining MV with positive expiratory pressure (PEEP) during CPB diminished the occurrence of atelectasis and the postoperative inflammatory response. Thus, we investigated the effects of maintaining MV during CPB for cardiac surgery on postoperative immunodepression and found that maintaining MV during CPB decreased postoperative immune dysfunction and could be an interesting strategy to diminish the occurrence of postoperative infection (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.</p>
ORIGINALITY AND INNOVATIVE ASPECTS	<p>While several researches projects studied the effects of maintaining MV on postoperative pulmonary function and/or inflammation, the innovative nature of this work is based on objective morbidity endpoint. Indeed, this study, based on our previous works, will explore the beneficial immunological changes induced by maintaining MV during CPB using a clinical endpoint: the reduction of postoperative infections. Indeed, we have demonstrated that maintaining MV decreased IL-10 levels and postoperative lymphopenia which is associated with postoperative infections. Moreover, because there is a lack of randomized controlled trials to guide optimal intra-operative ventilation, and because maintaining MV during CPB is not hampering the surgical procedure, without any additional cost, such a strategy reducing postoperative infection have to be studied. Lastly, since the menace of complete absence of effective antibiotics to treat hospital acquired infections exists, our study will participate to the development of innovative treatment and prophylactic approaches. Thus, expected public health benefits (or collective benefits) are:</p> <ul style="list-style-type: none"> - Clear benefits for public health since healthcare associated infections in hospitals impose significant economic consequences on the nation's healthcare system.

	<ul style="list-style-type: none"> - A decrease in the emergence of resistant strains in surgery, hospital and general population, since postoperative infections are responsible for antibiotic prescription which is the single most important cause of the emergence of drug resistance, both in the community and hospital settings.
PRIMARY OBJECTIVE	To measure the incidence of postoperative infections in 2 groups of patients: one group of patients ventilated and one group of patients without mechanical ventilation during cardiopulmonary bypass for cardiac surgery, and demonstrate that the incidence of postoperative infections is significantly lower in patients ventilated during CPB.
SECONDARY OBJECTIVES	<p>To demonstrate that, compared to patients without MV during CPB, maintaining MV will:</p> <ul style="list-style-type: none"> - Decrease postoperative immunodepression (human leukocyte antigen-DR antigen (HLA-DR) expression on monocytes, Indoleamine 2,3-Dioxygenase (IDO) activity, plasmatic levels of IL-10 and proportion of myeloid-derived suppressor cells (MDSCs)); - Decrease postoperative inflammation (plasmatic levels of IL-6 and quantity of extracellular vesicles (EV)); - Decrease the occurrence of lymphopenia after surgery (defined as an absolute lymphocyte count less than $1.2 \text{ cells}/\mu\text{L} \times 10.3$); - Decrease postoperative exposure to antimicrobials treatment; - Decrease postoperative mortality; - Decrease postoperative length of stay; - Increase the PaO₂/FiO₂ ratio after surgery; - Decrease postoperative duration of MV.
PRIMARY EVALUATION CRITERION	Primary end point will be the incidence of postoperative infections (ie nosocomial infections) within 28 days following cardiac surgery. The diagnosis of a postoperative infection will be based on clinical, biochemical, or morphological features and confirmed (if possible) by bacteriological data according to CDC definitions for nosocomial infections.
SECONDARY EVALUATION CRITERIA	<ul style="list-style-type: none"> - Postoperative expression of human leukocyte antigen-DR antigen (HLA-DR) on peripheral monocytes at day 0, day 1 and day 7, - Postoperative plasmatic levels of IL-10 at day 0, day 1 and day 7, - Postoperative indoleamine 2,3-Dioxygenase (IDO) activity at day 0, day 1 and day 7, - Postoperative proportion of myeloid-derived suppressor cells (MDSCs) at day 0, day 1 and day 7; - Postoperative plasmatic levels of IL-6 at day 0, day 1 and day 7, Postoperative quantity of extracellular vesicles (EV) at day 0 and day 1; - Postoperative lymphocytes count at day 0, day 1 and day 7; - Number of days of exposure to each antibiotic per 1000 inpatient days, defined as the number of days (>24 h) of continuous antibiotic treatment (from days 1 to 28) and duration of antibiotic treatment; - Mortality within 28 days following surgery ; - Hospital length of stay (max 28 days) defined as the number of days before first hospital discharge; - PaO₂/FiO₂ ratio at day 0 and day 1; - Duration of MV (max 28 days).
METHODOLOGY / STUDY SCHEDULE	A prospective, multicenter, single-blind, randomized, parallel-group trial.
CRITERIA FOR INCLUSION OF PATIENTS	<ul style="list-style-type: none"> - Age ≥ 18 years old; - Scheduled for any cardiac surgery (elective surgery) with cardio-pulmonary bypass, aortic clamp and cardioplegia, with median sternotomy and bi-pulmonary ventilation (cardiac valvular surgery (valve replacement or repair), coronary artery surgery, ascending aortic surgery and/or combined); - Written informed consent.
NON-INCLUSION CRITERIA OF PATIENTS	<ul style="list-style-type: none"> - Emergency surgery ; - Planned thoracotomy with one lung ventilation ; - Patients with known respiratory diseases (current respiratory infections, asthma,

	<p>chronic obstructive or restrictive pulmonary disease, obstructive apnea syndrome) ;</p> <ul style="list-style-type: none"> - Patients already intubated in the peri-operative period ; - Immunodepression defined by proven humoral or cellular deficiency, by continuous administration of steroids at any dose for more than one month prior to hospitalization, high-dose steroids (> 15 mg / kg / day of methylprednisolone or Equivalent), radiotherapy or chemotherapy in the previous year;; - Need for vasopressor or inotropic agents before surgery ; - Any acute infection in the last month before surgery ; - Hematological disorder, autoimmune disease, immunodeficiency, immunosuppressive therapy ; - Heart failure with an left ventricular ejection fraction<35% ; - Protected person (adults legally protected (under judicial protection, guardianship, or supervision), person deprived of their liberty.
STRATEGIES/PROCEDURES	<p>During CPB, two settings will be used depending on the randomization:</p> <ul style="list-style-type: none"> - absence of MV (and no PEEP) by disconnecting the tracheal tube from the ventilator (MV- group) ; - dead space ventilation using tidal volume of 2.5 mL/kg (predicted body weight) with 5-7 cmH2O PEEP (MV+ group). <p>Immunomonitoring parameters (HLA-DR expression, IL-10 and IL-6, lymphocytes count, IDO activity, MDSCs, EV) will be obtained after general anesthesia and before surgery (D0), at 24 hours (D1) and 7 days (D7) after surgery (except EV). HLA-DR expression, MDSCs, EV associated lung injury, IL-10, IL-6 and IDO activity will be measured in Rennes.</p> <p>Ratio of arterial oxygen tension (PaO2) to FIO2 will be obtained before surgery (D0, after induction of anesthesia) and 3 h after the end of CPB in the surgical intensive care unit (ICU; under baseline MV settings), and the day after surgery (D1).</p> <p>Occurrence of postoperative infections (an independent committee blinded to treatment group will ensure the classification of hospital-acquired infections), antibiotics treatment, length of stay, duration of MV, mortality will be recorded within the first 28 days following surgery.</p>
NUMBER OF PATIENTS	1400
DURATION OF STUDY	<p>Recruitment period : 24 months</p> <p>Duration of patient monitoring : 28 days</p> <p>Duration of analysis of data : 6 months</p> <p>Estimated total duration of study : 31 months</p>
EXPECTED FINDINGS AND IMPACT	<p>Expected patient benefits are:</p> <ul style="list-style-type: none"> - A significant decrease in the occurrence of postoperative infection. - A significant decrease in hospital length of stay. - A significant decrease in both mortality and morbidity. - A significant decrease in exposure to antibacterial agent which could be harmful (as clostridium difficile colitis). <p>Expected public health benefits are:</p> <ul style="list-style-type: none"> - Clear benefits for public health since healthcare associated infections in hospitals impose significant economic consequences on the nation's healthcare system. - A decrease in the emergence of resistant strains in surgery, hospital and general population since postoperative infections are responsible for antimicrobials prescription which is the single most important cause of the emergence of drug resistance, both in the community and hospital settings. - Lastly, the threat of complete absence of effective antibiotics to treat problematic hospital-acquired infections is fueling the development of prophylactic approaches.

LIST OF ABBREVIATIONS

AAR	Act Added by the Research
ADR	Adverse Reaction
AE	Adverse event
ANSM	Agence Nationale de Sécurité du Médicament et des produits de santé
AP-HP	Assistante Publique-Hôpitaux de Paris
ARC	Act of Routine Care
ARDS	Acute respiratory distress syndrome
CPB	Cardiopulmonary bypass
CDC	Center for Disease Control and Prevention
CHU	Centre Hospitalier Universitaire
CPP	Comité de Protection des Personnes
CRA	Clinical Research Associate
CRF	Case Report Form
EV	Extracellular Vesicles
FiO ₂	Fraction of inspired oxygen
GCP	Good Clinical Practices
HEGP	Hôpital Européen Georges-Pompidou
HLA-DR	Human leukocyte antigen-DR
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IDO	Indoleamine 2,3-Dioxygenase
IEC	Independent ethics committee
IL	Interleukin
INSERM	Institut national de la santé et de la recherche médicale
LVEF	Left ventricular ejection fraction
MDSCs	Myeloid-derived suppressor cells
MR	Methodology of Reference
MV	Mechanical ventilation
NO	Nitric Oxide
PaO ₂	Partial pressure of oxygen
PEEP	Positive end-expiratory pressure
PMN	Polymorphonuclear neutrophils
REVA	Réseau Européen de Recherche en Ventilation Artificielle
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAS	Statistical Analysis System
SUSAR	Suspected unexpected serious adverse reaction
TNF	Tumor necrosis factor

1 GENERAL INFORMATION

1.1 Title

Beneficial effects on maintaining mechanical ventilation during cardiopulmonary bypass for cardiac surgery on postoperative infections

1.2 Sponsor

1.2.1 Identification

CHU de Rennes
2, rue Henri le Guilloux
35033 Rennes cedex 9

1.2.2 Signature of protocol on behalf of the sponsor

Pascal GAUDRON, Director of Research
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1.2.3 Person responsible for study on the side of the sponsor

Pascal GAUDRON, Director of Research
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1.3 Coordination and monitoring of study

Direction of Research
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2, rue Henri le Guilloux
35033 Rennes cedex 9

1.4 Investigators

1.4.1 Coordinating investigator

Professeur Jean-Marc Tadié
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1.4.2 Associated investigators

See Appendix 1

1.5 Associated scientific partners

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1.6 Vigilance

Dr Catherine Mouchel, Centre d'Investigation Clinique, Inserm 1414, Unité de Pharmacologie Clinique, Hôpital de Pontchaillou, 2 rue Henri le Guilloux, 35033 Rennes Cedex 9, France. Tel.: 02.99.28.91.96 – Email: catherine.mouchel@chu-rennes.fr

1.7 Methodologist – biostatistician

Methodologist : Pr Bruno Laviolle, Centre d'Investigation Clinique, Inserm 1414, Unité de Méthodologie/biométrie, Hôpital de Pontchaillou, 2 rue Henri le Guilloux, 35033 Rennes cedex 9, France. Tel.: 02.99.28.96.68 – Email: bruno.laviolle@chu-rennes.fr

Biostatistician : Alain Renault, Centre d'Investigation Clinique, Inserm 1414, Unité de Méthodologie/biométrie, Hôpital de Pontchaillou, 2 rue Henri le Guilloux, 35033 Rennes cedex 9, France. Tel.: 02.99.28.91.93 – Email: alain.renault@chu-rennes.fr

1.8 Scientific Committee

The mission of the Scientific Committee is to settle scientific, methodological and ethical problems raised by the trial and to monitor its proper conduct. It will be constituted at the beginning of the study with 5 members, including the coordinating investigator, the methodologist, a representative from the sponsor, and two members among the associated teams. It will meet after each interim analysis to study the recommendations of the data and safety monitoring board and will take the final decision to stop the trial if needed.

The Scientific Committee will solve practical problems arising during the course of study and may decide to amend the protocol. If such amendments are done, the relevant Independent Ethics Committee and the Competent Authority will be notified. The Scientific Committee will send to investigators all the information necessary for the proper conduct of the study.

1.9 Independent Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board will be constituted at the beginning of the study with 5 members who are not involved in the study, including an intensivist, an anesthesiologist, a cardiac surgeon, an infectiologist, a methodologist/statistician.

In order to prematurely stop the study in case of efficacy, two interim analyses are planned. These analyses will be performed on the primary endpoint but will also evaluate safety data in order to rapidly detect any unexpected serious adverse events.

The Data and Safety Monitoring Board will meet after each interim analysis and at the end of the study. It can also meet on request of the coordinating investigator or the methodologist if serious adverse events or results which can jeopardize the existence of the protocol occur.

The Data and Safety Monitoring Board will propose to stop the study if the interim statistical analyses reach significance or if it appears that study continuation would be contrary to ethics rules (occurrence of serious adverse events, publication of trial results providing the answer to the question...).

1.10 Independent validation committee

The validation committee will include 3 members, an infectiologist, a bacteriologist, and an intensivist. They will be in charge to validate the occurrence of nosocomial infections and the date of occurrence and will be blinded to the study arm (blinded evaluation of the primary endpoint). They will be provided with a standardized patient record containing all required document necessary harmonize patients' evaluations.

2 SCIENTIFIC RATIONALE AND GENERAL DESCRIPTION OF STUDY

2.1 Name and description of the disease

Cardiopulmonary bypass (CPB) is a technique that temporarily takes over the function of the heart and lungs during cardiac surgery. CPB during cardiac surgery induces a systemic inflammatory response associated with an immune dysregulation and a significant pulmonary dysfunction (1-9). These two phenomena have been well characterized. First, the inflammatory response, usually attributed to surgical trauma, contact of blood with artificial surfaces, and ischemia-reperfusion injury. Although often innocuous, such inflammation might contribute to postoperative complications such as lung injury, bleeding disorders and organs failure (1, 2, 4, 6, 10-12). Moreover, while the inflammation proceeds, some key players of the immune system become immunosuppressed exposing patients to postoperative infections (3, 13, 14). Secondly, CPB induced pulmonary dysfunction ranges from a temporary and clinically insignificant reduction in arterial oxygenation to life-threatening injury manifested as acute respiratory distress syndrome (7, 8, 15-17). This phenomenon is of multifactorial sources, but one of the main mechanisms is the occurrence of atelectasis during surgery, may be due to airway closure related to the absence of lung ventilation during CPB (9). Indeed, **since CPB mechanically circulates and oxygenates blood bypassing the heart and lungs, the usual procedure during CPB is to stop MV (apnea).**

Because maintaining MV during CPB diminishes postoperative inflammation, a relationship between pulmonary dysfunction and postoperative immunosuppression has been investigated with convincing results. However, these findings have to be confirmed in a clinical trial using the incidence of nosocomial infection as an endpoint.

A. *Cardiopulmonary bypass induced immunosuppression*

CPB is associated with a generalized inflammatory response with immune paresis which predisposes to the development of postoperative infections and sepsis. This systemic inflammatory response includes activation of platelets, polymorphonuclear neutrophils (PMNs), monocytes, macrophages (2, 3, 5, 18, 19). However, it is currently

accepted that CPB-induced inflammatory response is accompanied by immunosuppression, which can last several days or weeks after surgery, increasing the risk to develop postoperative infections. Several studies have demonstrated that circulating levels of interleukin 6 (IL-6), interleukin 10 (IL-10) and tumor necrosis factor alpha (TNF- α) increased dramatically (1). Furthermore, among changes of monocyte function observed after CPB, a down-modulation of monocyte molecules expressions, especially for HLA-DR (mHLA-DR), has been demonstrated (4, 11). In terms of functionality, monocytes with decreased HLA-DR have been shown to be unable to mount a pro-inflammatory response to any bacterial challenge or to properly present antigens to T cells. In terms of clinical information, decreased mHLA-DR has been shown to be predictive of both fatal outcome and septic complications after trauma, surgery, pancreatitis, burn and septic shock (6, 13, 20, 21). Of note, there are several lines of evidence suggesting that IL-6 and TNF- α play a pivotal role during acute inflammation, whereas IL-10 is an immunosuppressive cytokine that could be involved in HLA-DR downregulation (22).

Mekontso et al. have shown that bactericidal activity of PMNs 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 (13). Along these lines, observational studies have demonstrated that patients undergoing cardiac surgery with CPB have a high risk for nosocomial infections (23, 24). Moreover, a downregulation of HLA-DR expression on monocytes and an increase in plasma IL-10 levels were associated with the occurrence of nosocomial infections (5, 13, 21). In previous studies, the delayed-type hypersensitivity response, IL-6 concentrations and number of HLA-DR epitopes have been shown to correlate with clinical outcome for cardiac surgery, major abdominal surgery, and patients with trauma (5).

Infections are the most common non-cardiac complication after cardiac surgery. Infection in the setting of cardiac surgery increases morbidity, mortality, and cost. These infections can require prolonged treatment with antibiotics, additional surgery, or both. Several studies have found that 15-20% of patients will experience postoperative infections. In a recent prospective study, 15% of patients developed postoperative infections which dramatically affected survival and readmissions (23-26). The authors found that prolonged ventilation and transfusion were associated with adverse outcomes and emphasized the considerable opportunities for improving outcomes and preventing postoperative infection. However, no pharmacological strategies have reduced this immunosuppression (glucocorticoids, protease inhibitors, heparin, phosphodiesterase inhibitors, antioxidants...) (5, 18, 19, 25, 27-31). In a prospective, randomized, double-blind, placebo-controlled study, Tepaske et al. aimed to ascertain whether oral immune-enhancing nutritional supplement taken for at least 5 days before an operation in high-risk patients undergoing cardiac surgery improves preoperative host defense and subsequently reduces postoperative infections and organ dysfunction. They demonstrated that CPB decreased dramatically HLA-DR expression on monocytes in both group. However they found that concentration of IL-6 was significantly lower in the treatment group than in the control group (5). Although these results are interesting, no study has demonstrated that its finding could be generalized to other patients.

Cardiopulmonary bypass during cardiac surgery promotes inflammatory response and immune dysfunction that increase the occurrence of postoperative infection. Reducing postoperative inflammation will decrease postoperative immunosuppression and therefore will decrease patient morbidity.

B. Cardiopulmonary bypass induced pulmonary dysfunction

After cardiac surgery, pulmonary dysfunction is a frequently encountered complication which could increase postoperative ICU stay and result in a significantly increased mortality (7, 15, 32). Inflammatory response to CPB and ischemic damage of the lungs have been considered as major causes of respiratory failure after cardiac surgery, and there have been numerous reports of successful reduction of post-CPB lung injury by controlling inflammatory response or pulmonary ischemia (7, 16). However, recent studies have demonstrated that this phenomenon was of multifactorial sources, but one of the main mechanisms is the occurrence of **atelectasis** during surgery (8, 9, 33-37). Since CPB mechanically circulates and oxygenates blood bypassing the heart and lungs, usual procedure during CPB is to stop MV (apnea), which could induce atelectasis. **Atelectasis** has been associated with lung injury and release of cytokines (tumor necrosis factor α [TNF- α]) by shear forces on alveoli and small airways (9, 36, 38). However, it is not clear whether this injury is due to a recruitment/derecruitment phenomenon (i.e., atelectrauma) or whether it might by itself lead to the release of cytokines (38-40). This injury could be aggravated by MV. Although MV is an essential support during surgery or in patients admitted to the intensive care unit, clinical and experimental studies have shown that it could be harmful and could induce lung injury. Pulmonary and immune cells can convert mechanical stimuli into biological signals that will lead to inflammation (38). This sterile inflammation both locally and systemically will cause immunosuppression. Nevertheless, maintaining MV and 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** (1, 9, 35).

Continued ventilation during cardiopulmonary bypass results in lesser inflammatory responses and improves postoperative pulmonary function. Continuation of ventilation during CPB leads to an attenuated inflammatory and anti-inflammatory responses. This strategy might therefore improve patient outcome.

C. Previous studies supporting our hypothesis

We have already conducted three different studies that strongly support our hypothesis. Main results are summarized as follow:

1. Partitioning of exhaled nitric oxide (NO) in ventilated patients undergoing cardiac surgery

(Appendix 4): In this observational study, our objectives were to assess the changes in exhaled nitric oxide (NO) after CPB, taking into account the anatomical origin of its production using a method allowing the partitioning of exhaled NO between its bronchial and alveolar origins. We hypothesized that exhaled NO change often evidenced after CPB could be due to the absence of ventilation during CPB resulting in airway closure (transient atelectasis), and that this change could be prevented by MV with positive end-expiratory pressure during CPB. We found that a frank decrease in exhaled NO originating from the bronchial tree after CPB without MV. This significant decrease seemed related to airway closure since dead space MV with PEEP prevented it. At least two hypotheses could have been raised, firstly persistent airway closure (~atelectasis) may explained the decrease in exhaled NO by reducing epithelial surface and consequently maximum flux from bronchi (41-43); secondly, inflammatory processes related to CPB may led to a generalized decrease in NO production due to the post-insult immune paralysis (44).

Thus, we 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.

2. Immune dysfunction after cardiac surgery with cardiopulmonary bypass: beneficial effects of maintaining mechanical ventilation (Appendix 5)

Because maintaining MV (MV) with PEEP during CPB diminishes postoperative inflammation, we decided to investigate the relationship between pulmonary dysfunction and postoperative immunosuppression. 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 (Appendix 5). We also investigated the CD14+HLA-DR^{lo/-} monocytes, which have been described as immunosuppressive monocytic myeloid-derived suppressor cells (Mo-MDSCs) in humans (45), before and after CPB.

Main results were as follow:

1. We found that patients ventilated during CPB had lower postoperative levels of immunosuppressive IL-10 and proinflammatory TNF- α , and a higher postoperative lymphocytes count. These beneficial effects on postoperative immunosuppression could be related to an enhancement in pulmonary function because maintaining MV during CPB abolished the postoperative decrease in PaO₂/FIO₂ ratio.
2. We also found that total lymphocyte count was significantly decreased after cardiac surgery in non-ventilated patients compared with patients ventilated during CPB. This finding was of major interest since 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 (46, 47). 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 because lymphopenia predicted an increased risk of secondary infection (48). However, no study has been conducted in patients undergoing cardiac surgery.

Since we found that total lymphocyte count was significantly decreased after cardiac surgery in non-ventilated patients compared with patients ventilated during CPB.

Mechanisms involved in postoperative lymphopenia had to be studied.

3. Cardiopulmonary bypass induced lymphopenia and decreased lymphocyte proliferation ability: IL-10 as a potential therapeutic target to reduce postoperative infection (Appendix 6)

Perioperative lymphopenia has been linked with an increased risk of postoperative infectious complications in orthopedic surgery or abdominal surgery (47, 49). There is no data in cardiac surgery. We performed an observational study in patients undergoing cardiac surgery with CPB and found:

1. In a cohort of patients (n=986), persistent lymphopenia within 5 days following cardiac surgery with CPB was associated with the occurrence of postoperative infection (ie nosocomial infections).
2. Cardiac surgery induced lymphocytes apoptosis after CPB.
3. Lymphocytes from patients were less likely to proliferate after CPB.
4. Indoleamine 2,3-dioxygenase (IDO) and Interleukin 10 (IL-10) were involved in CPB-induced decrease lymphocyte proliferation ability.

D. Selected biomarkers for immunomonitoring and pulmonary function

Interleukin 6 (IL-6): IL-6 is an inflammatory cytokine with wide-ranging biological effects. Cytokines are released in response to tissue injury or an inflammatory stimulus. They act locally and systemically to generate a variety of physiologic responses, principal among them is the acute phase response (50). Since maintaining MV should decrease postoperative inflammation, plasmatic IL-6 levels should be lower in the ventilated group than in patients without MV during CPB.

Interleukin 10 (IL-10): IL-10 is an immunosuppressive cytokine that could be involved in HLA-DR downregulation (22), and an increase in TNF- α has been shown to be counterbalanced by early expression of anti-inflammatory IL-10 (51). Thus, strategies that could decrease postoperative IL-10 levels could be of interest to decrease postoperative infectious complications. Since maintaining MV should decrease postoperative immunosuppression, plasmatic IL-10 levels should be lower in the ventilated group than in patients without MV during CPB.

Lymphocytes: 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 (46, 47). 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 because lymphopenia predicted an increased risk of secondary infection (48).

Indoleamine 2,3-dioxygenase (IDO): IDO plays a pivotal role in immune tolerance (52). IDO catalyzes the first and limiting step of tryptophan catabolism, resulting in suppression of T cell proliferation in vitro and in vivo. In addition, the tryptophan-depleting activity of IDO affects the growth of several pathogens, so that IDO activation could have beneficial, as well as detrimental, consequences on host defenses against infections (53).

mHLA-DR: The loss of HLA-DR expression on monocytes indicates their functional deactivation and is supposed to be a determining factor of the immunodeficiency. Several studies have found that decreased mHLA-DR has been shown to be predictive of both fatal outcome and septic complications after trauma, surgery, pancreatitis, burn and septic shock (20, 21).

Myeloid-derived suppressor cells (MDSCs): MDSCs described in cancer and inflammatory processes are involved in sepsis-induced immune suppression and have been found to contribute to T-cell dysfunction in septic patients (54). We have demonstrated that postoperative proportion of MDSCs was subsequently significantly increased after cardiac surgery with CPB (1)

Extracellular vesicles: EV are emerging as important “surrogate markers” of many diseases states as well as of physiologically less accessible tissues such as endothelial cells, liver, lungs or bone marrow. In the post cardiac surgery context a recent and promising literature described the vast implication of EVs in the inflammation, endothelial failure and immunosuppression development (55). These recent progresses in understanding the post cardiac surgery pathophysiology remain to be validated in clean clinical settings.

PaO₂/FiO₂ ratio: The PaO₂/FiO₂ ratio is a commonly used indicator of lung function in critically ill patients, to define and characterize the severity of the acute respiratory distress syndrome (ARDS) and this ratio is still a central element of the new ARDS definition (Berlin definition). In addition, clinicians utilize this ratio to track change in lung conditions, to set positive end expiratory pressure (PEEP), to assess the response to different ventilatory strategies (56).

E. Postoperative infections diagnosis

Patients were examined daily by the clinical team for the presence of infection. Definitions of The diagnosis of a postoperative infection will be based on clinical, biochemical, or morphological features and confirmed (if possible) by bacteriological data according to CDC definitions for nosocomial infections (**Appendix 7**). Of note, an independent committee blinded to treatment group will ensure the classification of hospital-acquired infections.

Our different works suggest 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. This is the aim of this present study

2.2 Summary of results of non-clinical trials and clinical trials available and relevant regarding the research involving the human person study concerned

A. Summary of results of non-clinical trials and clinical trials available regarding CPB induced immunosuppression

Several studies have found that CPB induced immunosuppression (1-6, 9-13, 19, 25, 28, 29, 32, 57).

However, to our best knowledge, only one study have demonstrated that pharmacological approach could decreased CPB induced immunosuppression and decreased postoperative infection. In a prospective, randomized, double-blind, placebo-controlled study, Tepaske et al. aimed to ascertain whether this oral immune-enhancing nutritional supplement taken for at least 5 days before an operation in high-risk patients undergoing cardiac surgery improves preoperative host defense and subsequently reduces postoperative infections and organ dysfunction. They demonstrated that CPB decreased dramatically HLA-DR expression on monocytes in both group. However they found that concentration of interleukin 6 was significantly lower in the treatment group than in the control group. Authors concluded that intake of an oral immune enhancing nutritional supplement for 5 days preoperatively improves preoperative host defense, as expressed by improved delayed-type hypersensitivity response, increased number of HLA-DR epitopes, decreased concentrations of interleukin 6, reduced number of postoperative infections, and better preserved renal function. However, in the control group, patients received significantly higher levels of blood cell transfusions and presented a higher postoperative fluid balance, two major factors of postoperative immune dysfunction (5).

B. Summary of results of non-clinical trials and clinical trials available regarding CPB induced pulmonary dysfunction

Several ventilatory strategies have been suggested as beneficial when applied during CPB (15, 16, 34, 35). However, there is still a questionable impact on the long term clinical outcome in treated patients.

The main results could be summarized as follow:

1. Continued MV with PEEP during CPB improved postoperative PaO₂/FiO₂ ratio (1, 9).
2. Continued MV with PEEP during CPB may decrease duration of MV after surgery (17).
3. Continued MV with PEEP during CPB attenuated the postoperative systemic immune response (7, 17).
4. Continued MV with PEEP during CPB attenuated the postoperative immunodepression (1).

Of note, there is no recruiting or ongoing clinical trial studying CPB induced immunosuppression and mechanical ventilation registered to *clinicaltrials.gov*.

2.3 Summary of benefits, if applicable, and of foreseeable and known risks for the person who is a Patient in the research study

2.3.1 Benefits

2.3.1.1 Individual benefits

Expected patient benefits are:

- A significant decrease in the occurrence of postoperative infection.
- A significant decrease in hospital length of stay.
- A significant decrease in both postoperative mortality and morbidity.
- A significant decrease in exposure to antibacterial agent which could be harmful (as clostridium difficile colitis).

2.3.1.2 Collective benefits

Since the menace of complete absence of effective antibiotics to treat problematic hospital acquired infections exists, our study will participate to the development of innovative treatment and prophylactic approaches. Thus, expected public health benefits (or collective benefits) are:

- Clear benefits for public health since healthcare associated infections in hospitals impose significant economic consequences on the nation's healthcare system.
- A decrease in the emergence of resistant strains in surgery, hospital and general population since postoperative infections are responsible for antimicrobials prescription which is the single most important cause of the emergence of drug resistance, both in the community and hospital settings.

2.3.2 Risks

No specific risks (individual or collective) are likely to occur:

- Maintaining MV during CPB did not hamper surgical procedure ;
- Several studies have found benefits to maintain MV, mainly in biological parameters; however no study has demonstrated its impact on postoperative infections.

2.3.2.1 Individual risks

Physical risks and constraints

Main constraints are blood withdrawn from patients. However, volume withdrawn will not induce any physical side effect.

Psychological risks and constraints

The patients will not present any specific psychological risks or constraints associated with this study. The psychological risks to the patients included in this trial are the same as for any patient requiring cardiac surgery.

Socio-economic risks and constraints

The patients will not present any specific socio-economic risks or constraints associated with this study. The psychological risks to the patients included in this trial are the same as for any patient requiring cardiac surgery.

2.3.2.2 Collective risks

None.

2.3.3 Benefit / risk ratio

Given the expected benefits and low specific risks, the benefit/risk ratio is highly favourable.

2.4 Statement indicating that the study will be conducted in compliance with the protocol as well as with good clinical practices and legislative and regulatory conditions in force

The sponsor and the investigator also agree that this study will be conducted:

- in compliance with the protocol,
- in compliance with local and international good clinical practice currently in force,
- in compliance with legislative and regulatory conditions currently in force in participating countries.

2.5 References to the scientific literature and to relevant data used as a reference for the study

See appendix 2

3 STUDY OBJECTIVES

3.1 Primary objective

To measure the incidence of postoperative infections in 2 groups of patients: one group of patients ventilated and one group of patients without mechanical ventilation during cardiopulmonary bypass for cardiac surgery, and demonstrate that the incidence of postoperative infections is significantly lower in patients ventilated during CPB.

3.2 Secondary objectives

To demonstrate that, compared to patients without MV during CPB, maintaining MV will:

- Decrease postoperative immunodepression (human leukocyte antigen-DR antigen (HLA-DR) expression on monocytes, Indoleamine 2,3-Dioxygenase (IDO) activity, plasmatic levels of IL-10 and proportion of myeloid-derived suppressor cells (MDSCs));
- Decrease postoperative inflammation (plasmatic levels of IL-6 and quantity of extracellular vesicles (EV));
- Decrease the occurrence of lymphopenia after surgery (defined as an absolute lymphocyte count less than $1.2 \text{ cells}/\mu\text{L} \times 10.3$);
- Decrease postoperative exposure to antimicrobials treatment;
- Decrease postoperative mortality;
- Decrease postoperative length of stay;
- Increase the PaO₂/FiO₂ ratio after surgery;
- Decrease postoperative duration of MV.

4 STUDY DESIGN

4.1 Study evaluation criteria

4.1.1 Primary evaluation criteria

Primary end point will be the incidence of postoperative infections (ie nosocomial infections) within 28 days following cardiac surgery. The diagnosis of a postoperative infection will be based on clinical, biochemical, or morphological features and confirmed (if possible) by bacteriological data according to CDC definitions for nosocomial infections (appendix 7).

4.1.2 Secondary evaluation criteria

Immunomonitoring:

- Postoperative expression of HLA-DR on peripheral monocytes at day 0, day 1 and day 7,
- Postoperative levels of IL-10 at day 0, day 1 and day 7,
- Postoperative indoleamine 2,3-Dioxygenase (IDO) activity at day 0, 1 and 7
- Postoperative proportion of MDSCs at day 0, day 1 and day 7;
- Postoperative levels of IL-6 at day 0, day 1 and day 7,
- Postoperative quantity of EV at day 0 and day 1;
- Postoperative lymphocytes count at day 0, 1 and 7;
- Number of days of exposure to each antibiotic per 1000 inpatient days, defined as the number of days (>24 h) of continuous antibiotic treatment (from days 1 to 28); duration of antibiotic treatment;
- Mortality within 28 days following surgery ;
- Hospital length of stay (max 28 days) defined as the number of days before first hospital discharge;
- PaO₂/FiO₂ ratio at day 0 and day 1;
- Duration of MV (max 28 days).

4.2 Description of study methodology

4.2.1 Experimental design

A prospective, multicenter, single-blind, randomized, parallel-group trial.

4.2.2 Conduct of study

4.2.2.1. Screening phase

During the day of the consultation with a cardiac surgeon, the patients fulfilling the inclusion criteria and after verification of the exclusion criteria, will be proposed to participate to the study. Informed consent will be obtained during the consultation. If necessary, after this information session the patient can reconsider his/her decision within a period of time.

4.2.2.2. Inclusion phase

The patients will be randomized the day of surgery before induction of anesthesia.

Data collected at inclusion: The following data were collected: age, sex male, body mass index (BMI), EuroScore, type of surgery (isolated coronary artery bypass graft (CABG), single valve, surgery on multiple valve, CABG plus valve), coexisting medical conditions (Hypertension, diabetes mellitus, previous cerebrovascular event (stroke/transient ischemic attack), peripheral vascular disease, preoperative creatinine, chronic renal dysfunction), antibiotic prophylaxis.

Description of surgery:

- General anesthesia will be induced with propofol and sufentanil (or remifentanil) and will be followed by cisatracurium for muscular relaxation. Anesthesia, muscle relaxation, and analgesia will be respectively maintained with inhaled sevoflurane during off-pump surgery or propofol infusion during CPB and boluses of atracurium and sufentanil (or remifentanil).
- For baseline ventilation, the patients will receive volume-controlled ventilation with a tidal volume of 6-8 mL/kg of predicted body weight and a ventilatory frequency of 10 to 15 per minute (end-tidal carbon dioxide between 34 and 38 mmHg). Inspired oxygen fraction (FIO₂) will be adjusted to ensure a peripheral oxygen saturation of > 92%, inhalation-to-exhalation ratio will be 1:2, and the level of PEEP will be 5-7 cmH₂O.
- During CPB, two settings will be used depending on the study arm:
 - o In the control group (MV- group): absence of MV (and no PEEP) by disconnecting the tracheal tube from the ventilator;
 - o In the MV + group: dead space ventilation using tidal volume of 2.5 mL/kg/pbw (predicted body weight) with 5-7 cmH₂O PEEP.
- After CPB, a recruitment maneuver will be performed. Recruitment maneuver consisted of applying a continuous positive airway pressure of 30 cm of water for 30 seconds, and then ventilatory parameters will be turned back to

baseline settings. All postoperative patients will be admitted to the intensive care unit (ICU) and will be ventilated with volume-controlled ventilation, VT=6-8 ml/Kg/pbw, FiO2 of 60% and PEEP of 5cmH2O.

4.2.2.3. Follow-up

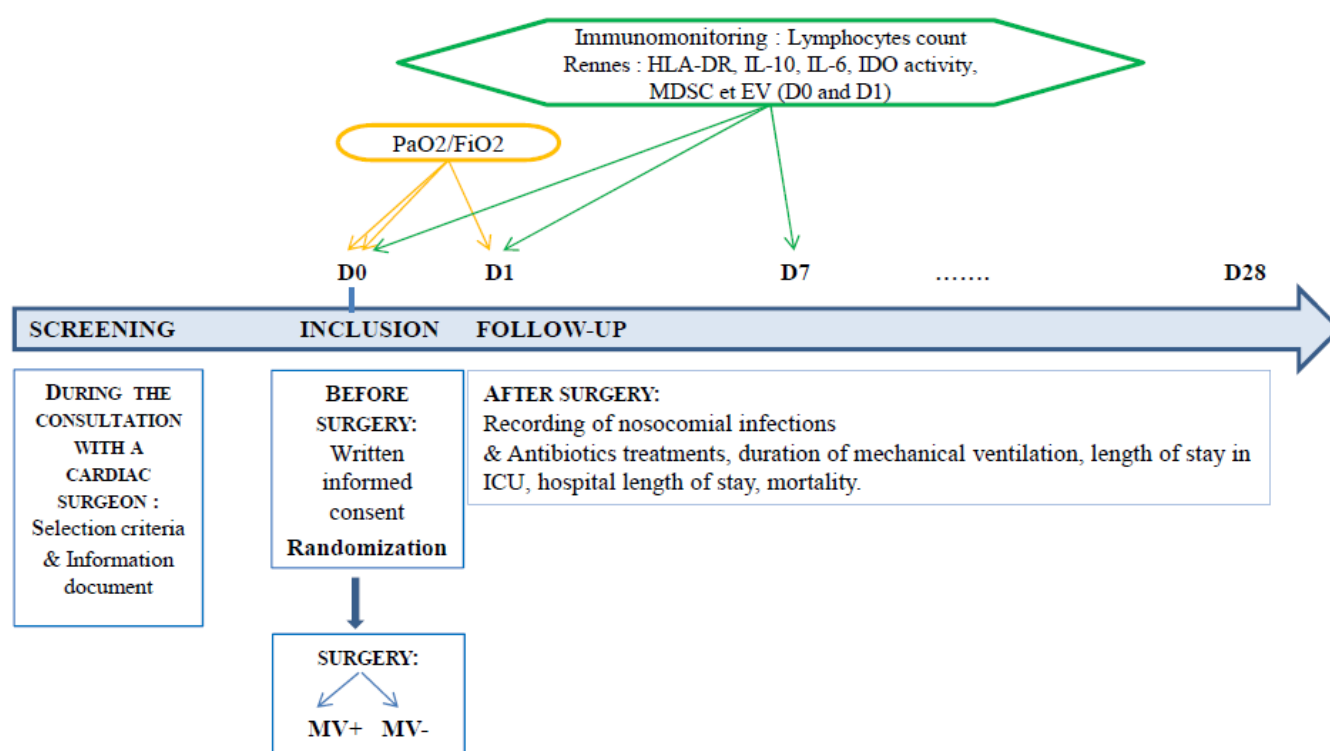
Data collected after surgery: duration of procedure, duration of CPB, duration of aortic cross-clamping, antibiotics treatments, duration of MV, length of stay in ICU, hospital length of stay, mortality.

Immunomonitoring parameters (HLA-DR expression, IL-10 and IL-6, lymphocytes count, Indoleamine 2,3-Dioxygenase activity, MDSCs and EV) will be obtained after general anesthesia and before surgery (D0, at 24 hours (D1) and 7 days (D7) (except EV) after surgery.

HLA-DR expression, IL-10, IL-6 and IDO activity will be measured in Rennes.

Ratio of arterial oxygen tension (PaO2) to FIO2 will be obtained before surgery (D0) after induction of anesthesia and 3 h after the end of CPB in the surgical intensive care unit (ICU; under baseline MV settings), and the day after surgery (D1).

Occurrence of postoperative infections within 28 days following cardiac surgery will be monitored. An independent committee blinded to treatment group will ensure the classification of hospital-acquired infections. Antibiotics treatment, length of stay, duration of MV, mortality will be recorded within the first 28 days following surgery.



	Screening Phase	Inclusion Phase (D0) Surgery	D1	D7	D28
Information document	X				
Clinical Examination (check the selection criteria)	X				
Written informed consent	X	Or X (later)			
Randomization		X			
Patient characteristics and clinical data		X			
Data surgery		X			
HLA-DR* and MDSCs*		X	X	X	
IL-10*		X	X	X	
IL-6*		X	X	X	
IDO activity*		X	X	X	

EV*		X	X		
Lymphocytes count		X	X	X	
Pa02/FiO2		X <i>After induction of anesthesia <u>and</u> 3 h after the surgery</i>	X		
Nosocomial infections recording		X			
Antibiotics treatment		X			
Length of stay		X			
Duration of MV		X			
Mortality		X			
Adverse events recording		X			

* HLA-DR expression, MDSCs, EV, IL-10, IL-6 and IDO activity will be measured in Rennes.

4.3 Description of the measures taken to reduce and prevent bias

4.3.1 Randomization

Randomization will be stratified per centre.

Randomization will be performed in the centres via a webinterface (Ennov Clinical, Ennov Group, Paris, France). A document to help use this tool will be provided to the investigators.

4.3.2 Methods of blinding

This project is a randomized single-blinded (patient) trial.

4.4 Expected duration of participation of persons and description of chronology and of duration of all study periods including monitoring, if applicable

Recruitment period: **24 months**

Duration of patient monitoring: **28 days**

Duration of analysis of data: **6 months**

Estimated total duration of study: **31 months**

Starting with inclusion of the first patient, the sponsor has to inform without delay the local health agency and the ethics committee of the actual date of start-up of the study. The actual date of start-up = date of signature of consent form by the first person who is a Patient in the study.

The date of end of the study will be transmitted by the sponsor to the ethics committee and to the local health agency within 90 days or earlier according to local regulations. The date of end of the study corresponds to the last visit of the last person who is a Patient in the study.

4.5 Description of rules for permanent or temporary discontinuation

4.5.1 Discontinuation of participation of a person in study

Patients can withdraw their consent and ask to withdraw from the study at any time and for whatever reason. If a Patient discontinues the study before its completion, the investigator must document the reasons as completely as possible.

In fact, the investigator can temporarily or permanently discontinue the study treatment for any reason which is in the best interest of the Patient.

If a Patient is lost to follow-up, the investigator will make every effort to resume contact with that person.

4.5.2 Discontinuation of part or of the entire study

Unexpected events or new information pertaining to the study strategy, in light of which the study objectives or clinical programme probably would not be achieved, can lead the sponsor to terminate the study.

In case of early permanent discontinuation of the study, the information will be sent by the sponsor within 15 days to the relevant country health agency and to the ethics committee or earlier according to local regulations.

5 SCREENING AND INCLUSION OF PERSONS FROM THE STUDY

5.1 Inclusion criteria

- Age \geq 18 years old;
- Scheduled for any cardiac surgery (elective surgery) with cardio-pulmonary bypass, aortic clamp and cardioplegia, with median sternotomy and bi-pulmonary ventilation (cardiac valvular surgery (valve replacement or repair), coronary artery surgery, ascending aortic surgery and/or combined);
- Written informed consent.

5.2 Non-inclusion criteria

- Emergency surgery ;
- Planned thoracotomy with one lung ventilation ;
- Patients with known respiratory diseases (current respiratory infections, asthma, chronic obstructive or restrictive pulmonary disease, obstructive apnea syndrome) ;
- Patients already intubated in the peri-operative period ;
- Immunodepression defined by proven humoral or cellular deficiency, by continuous administration of steroids at any dose for more than one month prior to hospitalization, high-dose steroids (> 15 mg / kg / day of methylprednisolone or Equivalent), radiotherapy or chemotherapy in the previous year ;
- Need for vasopressor or inotropic agents before surgery ;
- Any acute infection in the last month before surgery ;
- Hematological disorder, autoimmune disease, immunodeficiency, immunosuppressive therapy ;
- Heart failure with an left ventricular ejection fraction $< 35\%$;
- Protected person (adults legally protected (under judicial protection, guardianship, or supervision), person deprived of their liberty.

5.3 Methods of recruitment

Informed consent will be obtained the day of consultation for cardiac surgery or before randomization the day of surgery.

6 TREATMENTS ADMINISTERED IN THE STUDY

6.1 Authorised treatments

Besides immunosuppressive drugs listed below, every treatment are authorized during the study period.

6.2 Unauthorised treatments

Only immunosuppressive drugs within 7 days following cardiac surgery.

6.3 Emergency treatment

All emergency treatment required during anesthesia are allowed in the study.

7 EVALUATION OF SAFETY

7.1 Definitions

- Adverse event (AE): any untoward medical occurrence in a person who is a Patient in a research involving the human person study, whether or not this occurrence is related to the study.
- Serious Adverse Event (SAE): Severity is defined as one of the following occurrences :
 - . Death
 - . A life-threatening condition
 - . persistent or significant disability or incapacity
 - . Hospitalisation
 - . Prolongation of hospitalisation
 - . Malformation/congenital anomaly
 - . Potentially serious event (an adverse clinical event or a laboratory test result with a serious feature or considered as such by the investigator)
- Adverse Reaction (ADR): any untoward and unintended reaction linked to the study.
- Serious adverse reaction (SAR): a serious adverse event with a causal relationship to the study.
- Unexpected adverse reaction: an adverse reaction whose type, severity, intensity or outcome does not agree with informations contained in the protocol.

- Causal relationship: relation between the AE and the study. An Adverse Event related to the the study will become an AR.

7.2 Investigator's role

7.2.1 Notification of serious adverse events

Every serious adverse event related to the study strategy or not, expected or unexpected, expect those mentioned on the paragraph 7.2.3, must be reported within 24 hours by the investigator to the sponsor on a "Serious adverse event" form on which will be indicated the date of occurrence, criterion of severity, intensity, relationship with the strategy evaluated (or the study), and the outcome.

It must be sent by fax to the number indicated on the form. The person in charge of Pharmacovigilance is Catherine Mouchel (phone: 02-99-28-91-96, e-mail: catherine.mouchel@chu-rennes.fr).

The period in which serious adverse events should be reported begins from the day of the written informed consent to the last visit of the patient.

The narrative must be completed and sent to the sponsor as soon as new relevant information is obtained. Copies of the patient's medical record must be attached as well as results of laboratory tests.

Whenever a serious adverse event persists at the end of the study, the investigator must follow the patient until the event is considered resolved.

7.2.2 Notification of non-serious adverse events

All other Adverse Events, except those mentionned on the paragraph 7.1.3, will be reported on the "adverse event" page of the case report form specifying the date of occurrence, the description, severity, duration, method of resolution, causal relationship and the decisions made.

7.2.3 Protocol specific features

The following events: postoperative infections will be recorded as primary evaluation criterion in the case report form and not as adverse events. Indeed, for this trial where efficacy endpoint can also be SUSARs, the integrity of the clinical trial may be compromised if the blind is systematically broken. Under these circumstances, the sponsor will treat postoperative infections as disease related and not subject to systematic unblinding and expedited reporting (Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ('CT-3') (2011/C 172/01) paragraph 7.11.1). As planned in the study, they will be analysed at the time of interim analyses (two interim analyses after inclusion of 1/3 and 2/3 of the patients) which will permit to show potential difference between the two groups during the study.

7.3 Sponsor's role

7.3.1 Analysis of serious adverse events

The sponsor must evaluate the following:

- the causal relationship of serious adverse events (all adverse events for which the investigator or the sponsor considers that a causal relationship with the strategy can be reasonably considered, are considered as suspected adverse reactions. If the sponsor's evaluation of the event differs from the investigator's, both opinions are mentioned in the statement sent to the competent authority if this statement is necessary),
- and their expected or unexpected feature, using the reference document in force.

7.3.2 Scoring of causal relationship

Conforming to the recommendations of ICH on the management of adverse events in clinical trials – ICH E2B (R3), version of May 12th, 2005 – an evaluation of the imputability is required for all declared SAE's.

7.3.3 Declaration of unexpected serious adverse events

The sponsor reports all suspected unexpected serious adverse reactions (SUSAR) to ANSM (Agence Nationale de Sécurité du Médicament et des produits de santé), and to investigators within the regulatory time periods for reporting: if seriousness is death or life-threatening, SUSARs must be notified immediately; for other type of seriousness, SUSARs must be notified within 15 days (follow-up will be sent within 8 days).

7.3.4 Transmission of annual safety reports

On the anniversary date of the first consent, the sponsor writes a safety report containing:

- the list of serious adverse events which may be related to the strategy studied, including expected and unexpected serious effects,
- a succinct and critical analysis of safety of patients who are Patients in the study.

It is sent to local regulatory agency and to the ethics committee within 60 days following the anniversary of the first consent.

7.3.5 Declaration of other safety data

Every safety finding that can alter the benefit/risk ratio of the strategy or changes in conduct of the trial must immediately be notified to the sponsor. The report form will be the subject of an immediate report to the competent authorities (ANSM) and the ethic committee.

7.4 Expected adverse effects

No adverse effects are expected since several clinical studies, using the same ventilator strategies did not describe AE. No specific risks (individual or collective) are likely to occur:

- Maintaining MV during CPB did not hamper surgical procedure ;
- Several studies have found benefits to maintain MV, mainly in biological parameters; however no study has demonstrated its impact on postoperative infections.

Main constraints are blood withdrawn from patients. However, volume withdrawn will not induce any physical side effect.

8 STATISTICS

Statistical analysis will be performed on all randomized and evaluated patients (intention to treat analysis). It will be performed with SAS software (SAS Institute, Cary, NC, USA) in the Methodology/Biometrics department of the Inserm 1414 Clinical Investigation Centre of Rennes (certified ISO 9001 for the methodology, management and analysis of clinical studies).

8.1 Description of planned statistical methods, including schedule of planned interim analysis

8.1.1 Descriptive analysis

A first overall descriptive analysis and analysis by group will be performed. This consists of separate estimates, numbers and percentages for qualitative variables, means, standard error, medians and interquartile intervals for quantitative variables. The normal feature of the distribution of quantitative variables will be checked (Shapiro test).

8.1.2 Comparison of groups at inclusion

Student's t test or a Mann-Whitney test if necessary will be used to compare quantitative variables, and a Chi² or Fisher's exact test if necessary will be used to compare qualitative variables between two groups at inclusion.

8.1.3 Analysis of the primary criteria

The primary endpoint (rate of nosocomial infection at day-28 after CPB) will be compared between the two groups with the Chi² test.

Two interim analyses after inclusion of 1/3 and 2/3 of the patients, and one final analysis are planned. Stopping rules will use the alpha spending function with the O'Brien-Fleming boundary. The cumulative values of alpha for each analysis are: 0.00021 at the first analysis, 0.01202 at the second analysis and 0.04626 at the final analysis (nTerim, V1.1, Statistical solutions Ltd, Cork, Ireland). The trial will be stopped early if the significance of the Chi² test is below these alpha values.

8.1.4 Analysis of other criteria

For the analysis of the other endpoints, the same strategy as for baseline comparisons will be used.

In addition, censored endpoints (survival) will be compared using the log-rank test.

Continuous endpoints repeatedly measured during the study will be compared using a repeated measure two-way (time, group) analysis of variance.

For all these analyses, adjustments can be made in case of heterogeneity at inclusion.

8.1.5 Analysis of adverse events

Possible adverse events are coded according to the MedDRA classification and are the patient of a descriptive analysis.

8.2 Planned number of persons to be enrolled in the study, and planned number of persons in each centre with its statistical justification

Our hypothesis is that 15% of the patients without MV during CPB (MV- group) will have post-operative infection at day 28. A total sample size of 1392 patients (696 per group) is required to achieve 80% power to detect a decrease in the rate of postoperative infection in patients with MV during CPB (MV+ group) to 10%, using a two-sided test with a type I error of 5% (nTerim, V4.0, Statistical solutions Ltd, Cork, Ireland), **1400 will be included to take into account potential non evaluable patients (death during surgery, lost to follow-up...).**

8.3 Planned degree of statistical significance

Except for the interim analyses described in chapter 8.1.3, a p value <0.05 will be considered as significant for all analyses.

8.4 Method of management of missing, unused or invalid data

Missing data will not be replaced. Mixed models can be used in analysis of repeated data.

8.5 Choice of persons to include in the analysis

This trial is an intention to treat study, that is all randomized and evaluated patients will be analysed.

9 RIGHT OF ACCESS TO DATA AND SOURCE DOCUMENTS

9.1 Access to data

In compliance with GCP:

- the sponsor is in charge of obtaining the agreement of all parties involved in the study to ensure direct access to all sites of study conduct, source data, source documents and reports with the aim of quality control and of an audit by the sponsor,
- investigators will make available to persons in charge of monitoring, of quality control or audit of the study documents and individual data which are strictly necessary for such checking, in compliance with legislative and regulatory conditions in force (articles L.1121-3 and R.5121-13 of the French public health code).

9.2 Source documents

Source data defined as all documents or original items which make it possible to demonstrate the existence or accuracy of data or of a finding recorded in the clinical study will be kept for 15 years by the investigator or by the hospital if it involves a hospital medical dossier.

Source documents consist of a medical dossier, originals of laboratory test results, imaging examination reports, etc.

9.3 Confidentiality of data

In compliance with conditions concerning confidentiality of data to which persons in charge of quality control of the biomedical study have access (article L.1121-3 of the French public health code), in compliance with conditions pertaining to confidentiality of information in particular concerning the type of investigational medicinal products, the tests, persons who are Patients in the study and results obtained (article R. 5121-13 of the French public health code), persons having direct access will take all necessary precautions with the aim of ensuring confidentiality of information pertaining to the investigational medicinal products, tests, the persons who are patients in the study and in particular concerning their identity as well as that of results obtained.

These persons, in the same capacity as the investigators themselves, are patient to professional secrecy (according to conditions defined by articles 226-13 and 226-14 of the French penal code).

During the study or at its end, data collected on persons who are patients in the study and forwarded to the sponsor by the investigators (or all other specialised participants) will be made anonymous (deletion of Patients' names).

They must not in any event clearly reveal the names of the persons concerned or their address.

Only the first letter of the surname and of the first name of the patient are recorded, together with the code number specific for the study indicating the order of inclusion of patients.

The sponsor will make certain that each person who is a patient in the study has provided his or her written agreement for access to his/her personal individual data and strictly necessary for quality control of the study.

10 QUALITY CONTROL AND ASSURANCE

A Clinical Research Associate (CRA) designated by the sponsor will ensure proper conduct of the study and the quality of data collected, in agreement with the Standard Operating Procedures applied in the CHU of Rennes and in compliance with Good Clinical Practice as well as legislative and regulatory conditions in force.

The investigator and members of his team agree to make themselves available at Quality Control visits carried out at regular intervals by the Clinical Research Associate. At these visits the following items will be reviewed:

- informed consent,
- compliance with study protocol and procedures defined in it,
- quality of data collected in the case report forms: accuracy, missing data, consistency of data with "source" documents (medical dossiers, appointment diaries, originals of laboratory test results, etc.).

In addition, the investigators agree to be audited by the sponsor as well as inspected by the competent authorities. All data, all documents and reports can be subject to audits and regulatory inspections without being opposed by medical secrecy.

11 ETHICAL CONSIDERATIONS

11.1 Regulatory and institutional review

The protocol, information form and certificate of consent of the study will be submitted to the relevant independent ethics committee (IEC) for review.

Notification of a favourable opinion from the IEC will be transmitted to the sponsor of the study and to the competent authority. An application for authorisation will be sent by the sponsor to local regulatory authority prior to start of study.

11.2 Substantial changes

In case of a substantial change made to the study protocol by the investigator, it will be approved by the sponsor. The latter must obtain prior to start of study a favourable opinion from the ethics committee and authorisation from the relevant health authority in the setting of their respective competence. Additional consent from persons participating in the study will be collected if necessary.

11.3 Information for patients and written informed consent form

Patients will be completely and truly informed in terms which are understandable to them of the objectives and constraints of the study, the possible risks incurred, measures of supervision and safety necessary, of their right to refuse to participate in the study or of the possibility of retracting their agreement at any time.

All this information is contained in an information and consent form given to the patient. Free, informed and written consent from the patient will be collected by the investigator, or a doctor representing him prior to final inclusion in the study. A copy of the information and consent form signed by both parties will be given to the patient; the investigator will keep the original of it.

11.4 Compensation of patients

None.

11.5 Registration in the national file of persons who are patients in research involving the human person

None.

12 DATA PROCESSING AND RETENTION OF DOCUMENTS AND DATA

12.1 Case report forms and data entry

All information required by the study protocol must be recorded in the case report forms and an explanation must be provided for any missing data. Data must be collected progressively as obtained and recorded explicitly in the case report forms.

An electronic case report form (e-CRF) will be made available and data entry will be completed in centres through a WEB interface (Clinsight software, Ennov, Paris (75), France). It solely requires an internet connection and a browser. An aid document for use of this tool will be provided to investigators. The interface between the CRA and the investigator thus will be promoted, making possible the collection and control of data at a distance. Tests of control of consistency of data will be incorporated in electronic format. An audit function is incorporated in the e-CRF thus making it possible to follow any change in study data. This function also makes it possible to clearly identify the person who made a change as well as the date. A justification possibly can be incorporated in comment.

If requested, a paper copy will be printed at the end of the study, authenticated (dated and signed) by the investigator and a copy will be sent to the sponsor and archived.

Data analysis will be carried out by statisticians of the Biostatistics Unit Department of Pharmacology of Rennes.

12.2 CNIL

This study falls within the scope of the "Methodology of Reference" (MR-001) established by the Commission Nationale Informatique et Liberté (CNIL). The CHU of Rennes, sponsor of the study, has signed an agreement of compliance with this "Methodology of Reference".

12.3 Archiving

The following documents will be kept in the respective departments until the end of the period of practical utility.

These documents are:

- The study protocol and its Appendixes, and possible amendments,
- The original signed informed information and consent forms,
- Individual data (authenticated copies of raw data),
- Monitoring documents,
- Statistical analyses,

- The study final report.

At the end of the period of practical utility, the documents will be archived by the sponsor for at least 15 years after the end of the study or its early discontinuation, in compliance with institutional practices.

Archived documents cannot be moved or destroyed without agreement of the sponsor. All data, all documents and reports can be subject to an audit or inspection.

13 INSURANCE

The sponsor will take out, for the duration of the study, insurance covering the sponsors civil responsibility as well as that of all doctors involved in conduct of the study. They will also insure the total compensation of all harmful consequences of the study for persons who are Patients in it and their heirs, except when evidence is provided that the harm is not causally related to a fault of the sponsor or that of any participant, without it being possible to oppose the intervention of a third party or the voluntary withdrawal of a person who had initially consented to be participated in the study.

14 STUDY FEASIBILITY

Two smaller scale studies (pilot studies) have been already conducted to test the plan and method of this research study with convincing results (Appendixes 4-5-6).

Eligibility criteria and study design will not hinder enrollment expectations. With 7 centers (almost 10 000 CPB/years) enrollment goals will be achieved.

There are no known open competing studies registered to *clinicaltrials.gov* (September 2016).

Study plan does not conflict with currently accepted practice.

Study has been found to be of great scientific interest among potential co-investigators in surgery, anesthesiology and intensivist.

This study has been endorsed by the REVA network (Appendix 8).

15 RULES PERTAINING TO PUBLICATION

Scientific presentations and reports corresponding to the study will be written under the responsibility of the coordinating investigator of the study with the agreement of the responsible investigators.

First author: Jean-Marc Tadié

Last author: Erwan Flecher

The co-authors of the report and of publications will be the investigators and clinicians involved (cardiac surgeon and anaesthesiologist), on a pro rata basis of their contribution in the study, as well as the biostatistician and associated researchers.

Rules on publication will follow international recommendations (N Engl J Med, 1997; 336:309-315).

The study will be recorded on a freely accessible website (Clinical Trials) prior to inclusion of the first patient in the study.

16 LIST OF APPENDIX

Appendix 1: List of investigators

Appendix 2: References to the scientific literature

Appendix 3: Lettre d'information et formulaire de consentement

Appendix 4: Nitric Oxide cardiac surgery

Appendix 5: Mechanical ventilation immunological effects

Appendix 6: CPB induced lymphopenia

Appendix 7: CDC Infections criteria

Appendix 8: Endorsement REVA