Role of Inhaled Nitric Oxide in vascular mechanics and right ventricular function following cardiac surgery.

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1. INTRODUCTION

Perioperative pulmonary hypertension (PH) and right ventricular dysfunction (RVD) during cardiac surgery are serious complications associated with delayed weaning from cardiopulmonary bypass (CPB) and significant postoperative morbidity and mortality (1). Their pathophysiology is multifactorial and includes factors such as left ventricular systolic or diastolic dysfunction, the systemic inflammatory response associated with CPB, reduced pulmonary endothelial NO production during CPB, and pulmonary factors like pulmonary ischemia-reperfusion injury, surfactant dysfunction, increased susceptibility to pulmonary collapse, impaired pulmonary mechanics, and gas exchange impairment (2, 3). Inhaled nitric oxide (iNO) is a selective pulmonary vasodilator that has been successfully used to treat postoperative PH and RVD. Specifically, by reducing pulmonary arterial tone and resistance, iNO improves right ventricular (RV) performance despite its mild negative inotropic effect. Furthermore, iNO has been shown to effectively reduce pulmonary vascular resistance (4, 5), mitigate CPB-related inflammation (6), and more recently, reduce acute kidney injury and provide protection against chronic kidney diseases (7). Although clear benefits in outcomes have not been established, due to the aforementioned reasons, experts consider the use of iNO useful for perioperative treatment of increased pulmonary vascular resistance during cardiac surgery (8).

Recently, we have studied the effects of lung collapse on RV function during CPB weaning and have described that a ventilation strategy aimed at minimizing collapse and maintaining homogeneously ventilated lungs, known as open lung approach (OLA), improves RV performance (9). Lung collapse is present in the majority of patients after cardiac surgery, often affecting more than 10% of the total lung tissue mass (10). Lungs remain collapsed during CPB, receiving only minimal blood flow through bronchial arteries, without protection against ischemia. Furthermore, the inflammatory response triggered by CPB circulation inactivates surfactant. This, along with atelectasis induced during anesthesia induction, results in a lung highly prone to collapse in the immediate perioperative period. Lung collapse has several negative consequences for both pulmonary function and pulmonary circulation. On one hand, it reduces functional lung size, affecting pulmonary mechanics, increasing transpulmonary pressure, and impacting gas exchange, reducing ventilation efficiency and increasing the demand for inspired oxygen. On the other hand, lung collapse reduces the cross-sectional area of the pulmonary vasculature, and unventilated or under-ventilated alveoli trigger and enhance hypoxic pulmonary vasoconstriction response, which is further amplified in the presence of concurrent respiratory or metabolic acidosis. All these factors lead to increased pulmonary vascular resistance, early PH, and RVD. Right ventricular dysfunction is related to difficulties in weaning from CPB, an increased demand for catecholamines and inotropic support extending into the postoperative period, where it can complicate early recovery by prolonging dependence on drugs and mechanical ventilation, ultimately affecting the individual patient's outcome.

The increase in pulmonary vascular resistance and PH after cardiac surgery and their response to different therapeutic strategies like iNO have been well documented. However, there is limited information on how such measures affect RV function and their true influence on perioperative evolution. In this study, we aim to evaluate modifiable pathophysiological treatments for postoperative PH and RVD.

One pharmacological, iNO, and one non-pharmacological, the OLA strategy that combines lung recruitment and stabilization with individually optimized positive end-expiratory pressure (PEEP), and the possible synergistic effects of both interventions on RV performance. Apart from specifically targeting the described pathophysiological mechanisms, the combination of an OLA strategy and iNO could be especially beneficial, as modifying the pulmonary state through OLA could theoretically enhance the effects of iNO by significantly increasing the gas exchange area and, thus, alveolar ventilation. We will also study a series of closely related physiological variables to better characterize the effects of both strategies and their combination. This can help establish a better indication for iNO in cardiac surgery patients and improve our understanding of mechanisms that are also present in patients with acute respiratory distress syndrome (ARDS), albeit on a different scale.

2. Hypothesis

Inhaled nitric oxide (iNO) administered at a dose of 20 to 40 ppm in the immediate postoperative period of cardiac surgery improves pulmonary vascular mechanics and right ventricular function and synergistically interacts with alveolar recruitment in patients presenting with postoperative atelectasis.

3. Aims

Primary aim

- To study the effects of inhaled iNO on pulmonary vascular mechanics, right ventricular function, and right ventricular-vascular coupling in patients in the immediate postoperative period of cardiac surgery.

Secondary aim

- To compare the effects of iNO with standard care and with a lung recruitment maneuver on pulmonary vascular mechanics, right ventricular function, and right ventricular-vascular coupling in patients in the immediate postoperative period of cardiac surgery.
- To study patterns of redistribution of pulmonary blood flow measured by Electrical Impedance Tomography (EIT) that predict a positive effect on vascular mechanics and right ventricular function

4. Patients

Inclusion Criteria

Patients undergoing cardiac surgery who meet the following criteria will be included in the study:

- Age > 18 years
- Under controlled mechanical ventilation in passive conditions
- Presence of postoperative lung collapse (confirmed by pulmonary echocardiography and Air test)
- Preoperative left ventricular ejection fraction (LVEF) ≥ 30%.
- Absence of hypovolemia: absence of "kissing" ventricles and/or collapsibility index of the superior vena cava < 20%.
- Stable spontaneous heart rhythm
- Postoperative hemodynamic stability:
- Mean arterial pressure (MAP) ≥ 60 mmHg
- Central venous pressure (CVP) ≥ 10 mmHg

- Heart rate (HR) ≤ 100 bpm without tachyarrhythmias
- Lactic acid ≤ 3 mmol/L
- Single vasopressor treatment
- Norepinephrine dose ≤ 0.2 µg/kg/min, without an increase ≥ 15% in the last 30 minutes.
- Obtained informed consent

Exclusion Criteria

- Cor pulmonale or preoperative pulmonary vascular pathology
- Absence of pre-existing chronic respiratory pathology.
- Pre-induction arterial oxygen saturation (SpO2) < 97% by pulse oximetry
- Chronic pulmonary hypertension (mPAP > 35 mmHg) related to advanced COPD r chronic thromboembolic disease.
- Tricuspid or pulmonary valve surgery.
- Intra or postoperative use of nitroprusside or nitroglycerin.
- Preoperative dependency on inotropic or vasoconstrictor drugs.
- Use of levosimendan. Patients receiving dobutamine may be included as long as the infusion is ≤ 2 µg/kg/min.
- Contraindication for transesophageal echocardiography (See Appendix I) (11)
- Absence of lung collapse
- Pacemaker dependency

5. Methods

5.1 Study Design

This is a prospective controlled and randomized physiological study that will be conducted at two hospitals. The intervention period will be limited to the first 2-3 postoperative hours. With a total of 54 patients, the study recruitment period is expected to last for 18 months.

Study Registration: EudraCT number 2022-003884-37

5.2 Follow-up and Study Variables

The main study variables will be right ventricular function parameters measured through transesophageal echocardiography (TEE), specifically those directly related to the estimation of right ventricular-vascular coupling (as described below). Additionally, variables derived from Electrical Impedance Tomography (EIT) will be recorded: regional distribution of ventilation and perfusion, and EIT pulsatility (12) (see below). Additional variables related to pulmonary vascular mechanics will be included in patients monitored with a pulmonary artery catheter.

5.2.1 Conventional Monitoring

Patients will receive routine postoperative monitoring, including invasive blood pressure measurement, surface electrocardiogram, SpO2, and central venous pressure (CVP).

Mechanical ventilation will be provided by a Servo-U ventilator (Getinge, Solna, Sweden). This ventilator allows for the recording of all respiratory parameters, pulmonary mechanics, and ventilatory waveforms (Pressure, flow, and volume - time).

5.2.2 Echocardiography

At specified time points in the protocol, a specialist will perform transesophageal echocardiography (TEE) to assess right ventricular function and pulmonary circulation following the latest recommendations from the American and European Societies of Echocardiography (13). The following echocardiographic parameters will be studied:

Parameters of Right Ventricular Function

- Right ventricular end-diastolic and left ventricular end-diastolic diameters ratio
- Eccentricity index
- Right ventricular fractional shortening
- Tricuspid annular plane systolic excursion (TAPSE)
- S wave (Systolic Tissue Velocity)
- Myocardial Performance Index (MPI)
- Right ventricular ejection efficiency (RVEe) (14)
- TAPSE normalized to afterload (TAPSE/PAPs)
- Global longitudinal strain (speckle tracking)

Parameters of Afterload

- Estimated systolic pulmonary arterial pressure (PAPs)
- Estimated pulmonary vascular resistance using Doppler
- Notching pattern in the right ventricular outflow tract
- Right ventricular outflow tract acceleration time (RVOT-AT)
- Pulmonary pulse transit time (pPTT) (15)

From these right ventricular function variables, we have selected the primary variables (used for sample size estimation) that best inform about right ventricular-vascular coupling (16): RVEe; TAPSE/PAPs; pPTT, and contractility indicators: S' and TAPSE.

5.2.3 Electrical Impedance Tomography (EIT)

Patients will be monitored with Electrical Impedance Tomography (EIT) using the TMT impel Medical system (Sao Paulo, Brazil) (12):

Specifically for this study, the Enlight 18000 system will be used to monitor:

Regional distribution of lung ventilation and perfusion: Relative distribution of ventilation and perfusion in predefined regions of interest. The chest plane will be divided into ventral and dorsal regions, and additionally into four quadrants: Upper right and left lungs, and lower right and left lungs. The relative distribution (percentage) of ventilation (tidal volume) and perfusion (cardiac output) will be measured and compared in each selected region.

Changes in lung aeration (ΔEELZ delta end-expiratory lung volume): Direct measurement of static lung volume related to lung collapse and the effects of positive end-expiratory pressure (PEEP).

Pulmonary arterial pulsatility: After filtering the respiratory component using a high-pass filter, changes in impedance caused by pulmonary arterial pulsatility will be analyzed in each region of interest.

Distribution of pulmonary blood flow: Three perfusion studies by EIT will be conducted at each measurement point. To obtain the distribution of pulmonary perfusion, a small volume of 10 ml of 10% saline solution will be injected into the distal lumen of a central venous catheter in the jugular or subclavian position during a brief 20-second apnea. To induce apnea, ventilator settings will be changed to CPAP while maintaining the same PEEP level. The injected saline solution will be analyzed based on the first-pass kinetics, and the perfusion distribution result will be immediately displayed on the monitor. During EIT acquisition, the external temporary pacemaker will be turned off.

5.2.4 Pulmonary Artery Catheter

For patients monitored with a pulmonary artery catheter during the intraoperative period, the following parameters will be recorded: pulmonary artery pressure (PAP), SvO2, cardiac output (CO), right ventricular systolic work index (RVSWI), pulmonary vascular resistance index (PVRi), pulmonary pulsatility (PAp), effective pulmonary arterial elastance (effPAE), transpulmonary pressure gradient (TPG), and right ventricular pressure (RVP). Since a relatively low number of patients are expected to be monitored with a pulmonary artery catheter, these derived variables will have only descriptive value.

5.3 Specific Study Interventions

5.3.1 Inhaled Nitric Oxide (iNO)

The iNO administration device will be the So KinoxTM (Air Liquide). This device is one of the best and safest clinical devices for administering inhaled nitric oxide, routinely used in the intensive care unit. It ensures accurate dosing of iNO regardless of inspiratory flow and volume. It continuously and accurately monitors the concentration of nitric oxide in inspired gas and the fractions of inspired oxygen and nitrogen dioxide, a toxic byproduct of nitric oxide. The product technical information is available in the supplementary documentation. iNO will be initiated at a dose of 20 ppm immediately after randomization in the corresponding groups. After 30 minutes, a first assessment will be performed, and the iNO dose can be increased to 40 ppm as long as there is no increase in methemoglobin levels above 2.5% and in case there is no improvement in ventricular function or ventricular-vascular coupling, defined by the presence of any of the following:

- TAPSE ≤ 16 mm
- TAPSE/PAPs ≤ 0.26
- S' ≤ 9 cm·sec^-1
- RVOT-AT ≤ 100 ms

5.3.2 Recruitment Maneuver (RM) and PEEP Individualization

In the group assigned to pulmonary recruitment, the maneuver will be performed as follows:

Change the ventilation mode to pressure-controlled ventilation.
Respiratory rate of 15 cmH2O, FiO2 0.4, Inspiratory pressure of 15 cmH2O, Increase PEEP from 5 to 20 cmH2O every 5 breaths (20 seconds each step at a respiratory rate of 15). The last step increases inspiratory pressure from 15 to 20 cmH2O, maintained for 10 breaths (40 seconds). Immediately after completing the maneuver, the ventilation mode will return to volume-controlled ventilation, and a rapid decremental PEEP titration will be performed. PEEP will be reduced stepwise from 16 cmH2O in 2 cmH2O steps (30 seconds at each step) while keeping baseline ventilator settings. The closing pressure will be identified based on maximum compliance (17) and/or EIT (18). After a new short recruitment maneuver, ventilation will continue with baseline settings but with PEEP set to the detected closing pressure level.

Safety criteria: The RM will only be performed if stability conditions described in the inclusion criteria are met. The maneuver will be interrupted if any of the following situations occur during the maneuver:
- Desaturation: SpO2 ≤ 89%
- Mean arterial pressure < 55 mmHg or a decrease ≥ 30% from baseline
- Arrhythmias (tachyarrhythmias >120 bpm or bradyarrhythmias < 50 bpm)
- If the patient requires a pacemaker, heart rate of 70-90 bpm based on hemodynamic response.

5.3.3 Transesophageal Echocardiography (TEE)

An expert cardiologist will perform TEE at three time points during the investigation in all patients following standard procedures. A Philips CX50 portable device will be used.

5.5 Study Protocol

5.5.1 Patient Admission

Routine and specific study monitoring will be initiated. According to the clinical protocol, baseline ventilation will consist of volume-controlled ventilation with VT 6-8 ml/kg; PEEP 5 cmH2O; RR 15 bpm; I:E 1:2; FiO2 1. The first routine blood gas measurement will be obtained with these parameters. Subsequently, FiO2 will be reduced to 0.4.

5.5.2 Diagnosis of Atelectasis

The presence of atelectasis will be determined using lung ultrasound:

Lung Ultrasound:
Lung ultrasound will be used to confirm atelectasis. Ecographic collapse (consolidation pattern) must be present for the patient to be included in the study. Lung ultrasound will be performed at the posterior axillary line in the subdiaphragmatic region. Echographic collapse will be defined as the presence of a consolidation pattern and absence of "lung sliding" or B-lines at the posterior axillary line in the subdiaphragmatic region (19). Alternatively, atelectasis can be confirmed during transesophageal echocardiography (TEE): Presence of a consolidation pattern with dynamic air bronchogram associated with B-lines in the left lower lobe during medial-lower esophageal position of the probe (20). If no ultrasound-visible collapse is present, the patient will not be randomized and will be labeled as a screened patient. Additionally, an air test will be performed to confirm the functional impact of collapse through the air test.

5.7 Study Groups

After confirming atelectasis using the air test and lung ultrasound, patients will be randomly assigned using block randomization to one of the following three groups:

1) Standard treatment (CONT): Patients will be managed according to routine clinical protocol, standardizing interventions in both participating centers.

2) iNO 20-40 ppm (iNO): iNO at 20 ppm will be initiated immediately after randomization and data collection. The dose will be re-evaluated after the first 30 minutes and may be increased to a maximum of 40 ppm depending on the observed response in ventricular function.

3) iNO 20-40 ppm + Pulmonary Recruitment (iNO-RM)

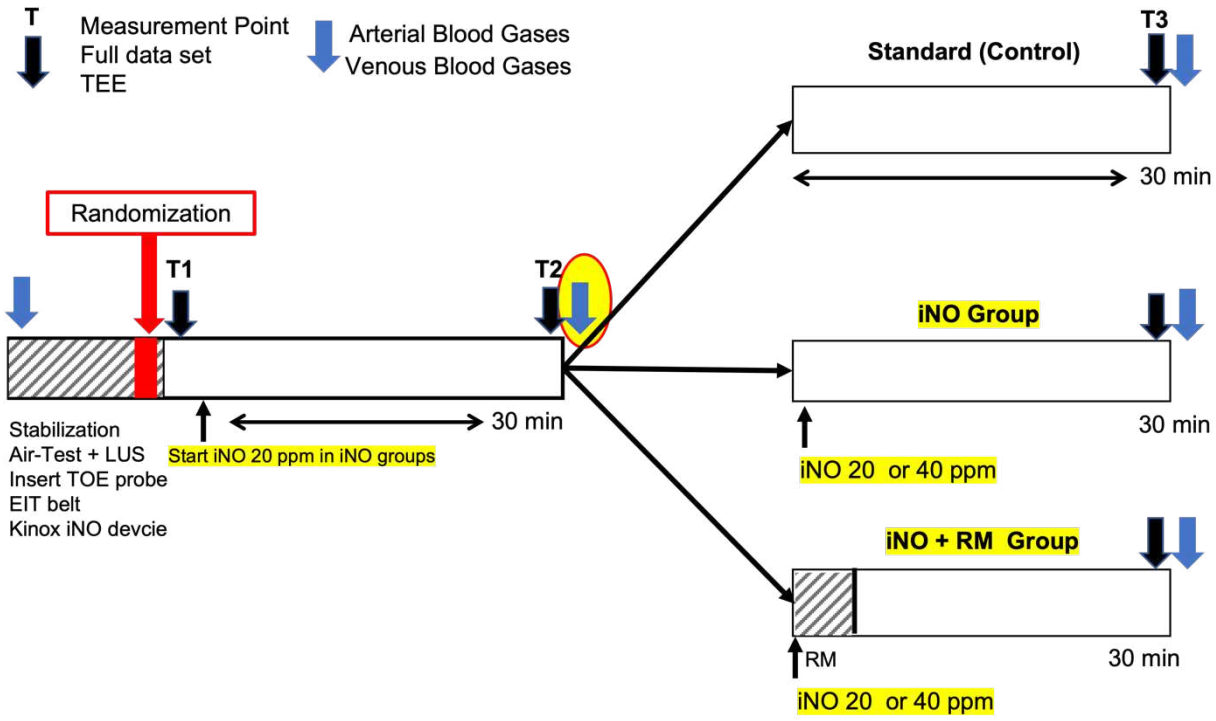

Figure 1: Study protocol

5.7.1 Admission Evaluation

Upon admission, patient monitoring and stability will be checked. After routine arterial and venous blood gas extraction, the air test and lung ultrasound will be performed to determine the presence of atelectasis. If the presence of collapse is confirmed, informed consent will be obtained from the family if it has not been given previously. If affirmative, randomization will be conducted, and impedance belts will be placed.

5.7.2 Protocol Measurement Time Points:

T1: Admission

Baseline measurements will be taken upon arrival in the intensive care unit, once the patient has been monitored, postoperative atelectasis has been confirmed (using lung ultrasound or air test), and the patient is in a stable condition after initial laboratory tests.

T2: Baseline

30 minutes after the first measurement under reference ventilation conditions. Depending on the observed response in right ventricular function, the iNO dose will be adjusted. If no improvement is observed, the iNO dose will be increased to 40 ppm.

T3: Final

30 minutes after applying the specific protocol for each group.

5.8 Statistical Analysis

Patients will be assigned to intervention groups through block randomization to ensure equitable distribution among the three intervention groups.

5.8.1 Sample Size

At the participating hospital (Hospital Universitario de la Princesa), cardiac surgery provides a continuous daily flow of patients (one or two per day) who could potentially become candidates for the study if they meet inclusion criteria and not exclusion criteria. Assuming a 15% improvement in right ventricular function measured by echocardiographic parameters in response to iNO or pulmonary recruitment (9) and a confidence level of 95%, the estimated sample size will be 54 patients, 18 in each group (27 per center). However, due to the lack of

sufficient studies analyzing right ventricular response to iNO, RM, or both, the final sample size may be adjusted based on preliminary results from initial pilot patients and study progress.

5.8.2 Statistical Methods

Variables will undergo descriptive analysis of central tendency measures including mean, median, dispersion, standard deviation, and interquartile range, respectively. Homoscedasticity and normality assumptions will be confirmed using Levene's and Shapiro-Wilk tests, respectively.

Variables with normal distribution and similar variance will be compared using the t-test, and variables with non-normal distribution using the non-parametric Wilcoxon test. Pearson's chi-squared test will be used for categorical variables to compare groups at the three measurement points in the study, and Fisher's exact test when expected frequencies are < 5.

For the comparison of more than two means with homoscedastic variances and normal distribution, ANOVA with post hoc testing will be conducted. For variables with heteroscedastic variances and/or non-normal distribution, the Kruskal-Wallis post hoc test will be applied. To analyze temporal evolution, a repeated measures ANOVA will be used with the intervention type as the main factor. In case of non-normality, the non-parametric Friedman test will be applied. Statistical significance will be set at $p \leq 0.05$. Statistical analysis will be performed using Stata v.15.

6. Safety and Adverse Events

To ensure safety during the 2-hour duration of the research protocol, the following precautions will be taken:

The inclusion of unstable patients or those who have had significant intraoperative complications leading to instability will be avoided.

6.1 Use of Inhaled Nitric Oxide (iNO)

1. The use of high and prolonged doses of iNO can be associated with adverse effects such as the formation of methemoglobin and NO2. Methemoglobin levels above 8-10% can lead to symptoms related to functional anemia, as methemoglobin cannot bind oxygen. NO2 is a common air pollutant that can be toxic at elevated levels due to the formation of reactive oxidative stress species.

Given the doses used and the duration of use, it is unlikely that elevated levels of both will occur. However, methemoglobin levels will be monitored through two blood gas measurements, one at 30 minutes and another at the end of iNO administration (1 hour). The SoKinox system continuously monitors NO2 levels in the inspired gas and has safety alarms.

iNO may precipitate or worsen left heart failure in patients with preexisting ventricular dysfunction due to increased left preload. This protocol specifically excludes patients with poor preexisting ventricular function or instability. Cardiac function will be monitored through echocardiography performed at 30 minutes (T2) and immediately after (T3) iNO administration. If there are clear signs of deteriorating left ventricular function (more than 20% drop in LVEF) during the examination compared to the initial function measured upon admission (T1), administration will be stopped.

The following criteria (any of them) will be established for dose reduction or discontinuation of iNO:

- NO_2 levels > 1 ppm
- Methemoglobin fraction > 2.5%
- Variations in inspired oxygen concentration ± 5% compared to ventilator settings
- Development of left ventricular failure data (drop in LVEF > 20% compared to initial level (T3) in echocardiographic controls.

6.2 Pulmonary Recruitment Maneuver

The recruitment maneuver can cause transient hemodynamic deterioration and, less frequently, barotrauma. Safety criteria have been established to interrupt the maneuver (see 5.3.2):

- Desaturation: $SpO_2 \leq 89\%$
- Mean arterial pressure < 55 mmHg or decrease ≥ 30% from baseline
- Arrhythmias (tachyarrhythmias >120 bpm or bradyarrhythmias < 50 bpm)
- If the patient requires a pacemaker, a heart rate of 70-90 bpm based on hemodynamic response.

Barotrauma is a much less frequent and highly unlikely complication in patients with healthy lungs (as included in this study) and with the proposed pressures and duration (very brief and at moderate pressures) in this study.

A section for recording adverse effects will be added to the Case Report Form (CRF) of the study (in Excel format).

7. Ethical Considerations

The intervention period is very short, and most procedures are applied routinely in the postoperative period. Special precautions will be taken to interfere as little as possible with normal postoperative times and management (an average of 4 to 6 hours until patients are fully awake and extubated). However, since patients are randomized to receive different interventions, a study insurance will be organized.

During the study period, patients will remain sedated, primarily due to the continuous effects of anesthesia and, secondly, because routine administration of short-acting hypnotics and opioids is given during these early postoperative hours to ensure gradual and incident-free awakening. In any circumstance of patient discomfort or inappropriate early awakening with agitation, pain, or fighting against the ventilator, additional sedation/analgesia will be provided as per standard clinical practice. This will ensure patient comfort and prevent additional stress.

All research procedures will strictly adhere to the principles of good clinical practice and ethical principles established in the Declaration of Helsinki and the Oviedo Convention on Human Rights and Biomedicine.

7.1 Direct Access to Data/Source Documents

The information generated and obtained through the implementation of this study is considered confidential and should be treated as such at all times. Study subjects will be identified by a coded subject number. All data generated in the study will be subject to the General Data Protection Regulation (GDPR) and the Spanish Organic Law 3/2018 of December 5 on the Protection of Personal Data.

Both the responsible trial investigators and a representative of the sponsor or health authorities will have access to the information recorded throughout the study. Patient identities will not be revealed in any publication of study results.

7.2 Control and Quality Assurance

During the study's development, the sponsor or its representatives will conduct periodic monitoring visits to ensure that the protocol and Good Clinical Practice (GCP) standards are followed. The investigator and the center will allow sponsor monitors or their representatives, as well as competent health authorities, direct access to the original documents for verification. The study center may undergo reviews by the Ethics Committee or quality assurance audits conducted by the sponsor or its affiliates, or inspections by competent health authorities. It is

important that investigators and relevant personnel are available during monitoring visits and possible audits or inspections and allocate sufficient time for the process.

7.3 Ethics

General and specific guidelines for investigators:

Investigators will strictly adhere to the provisions of this protocol, fully completing Case Report Forms (CRFs), which will be submitted in a timely manner to the sponsor for data analysis. The trial will be conducted in accordance with the recommendations for clinical trials and drug evaluation in humans outlined in the Declaration of Helsinki (revised in various locations) and the current Spanish Legislation on Clinical Trials.

7.4 Informed Consent (IC)

The IC document must comply with ICH GCP, local administrative regulations, and legal requirements. The IC document used in this study and any changes made to it during the study must be pre-approved by the Ethics Committee (CEIm) and the sponsor before its use. The investigator must ensure that all study patients, or their legal representatives, are fully informed about the nature and objectives of the study and the potential risks associated with participation. The investigator or their designee will obtain written informed consent from each patient or their legal representative before performing any specific study activity, and a copy will be provided to the patient or their legal representative for retention. The investigator will retain the original copies of all signed consent documents.

For this study, informed consent will be obtained from each patient before the surgical intervention.z

7.5 Data Handling and Record Archiving

Documentation Archive:

There will be a documentation archive for all data, which will be kept intact in both paper and electronic formats for 25 years following the completion of the study. This archive should contain the following elements:

1. CEIm approval of the protocol and informed consent form.
2. Copies of the signed informed consent form and approved protocol, with any amendments if applicable.
3. Any correspondence related to the study with the sponsor during its course.
4. Any correspondence with the CEIm.

5. Signed acceptance of the protocol.
6. Curriculum vitae of the principal investigator and other members of the research team.
7. Record of signatures of research team members.
8. Delegation of tasks within the research team.
9. Reporting of serious adverse events.
10. Contract between the sponsor and the research team.
11. Copies of data collection forms.

Documentation will be archived following Good Clinical Practice guidelines.

7.6 Case Report Form (CRF) / Data Recordin

For the purposes of this protocol, a CRF refers to a paper form, an electronic data record, or both, depending on the data collection method used in this study. An electronic CRF in Excel format will be used for this study. A CRF must be completed for each patient included in the study. The data included in the CRF are the exclusive property of the Sponsor and must not be made available to third parties in any form, except for authorized representatives of the Sponsor or competent health authorities, without written authorization from the Sponsor

The investigator will have the final responsibility for collecting and reporting all clinical, safety, and analytical data entered into the CRF and any other data collection documents (source documents), ensuring they are accurate, authentic/original, attributable, complete, consistent, legible, current, and enduring, and available when needed. The CRF will be signed by the investigator or an authorized team member to attest to the accuracy of the contained data. Any corrections made to CRF annotations or source documents must be dated, initialed, and explained (if necessary) and must not obscure the original annotation.

7.7 Monitorin

- Given that only 2 centers (Madrid and Barcelona) are participating in the study, it is planned to have 2 monitors located in each of the respective centers/cities. The monitor cannot, under any circumstances, be part of the investigative team.
- The objectives of study monitoring will be to ensure that:
- The rights and integrity of the subjects are protected.
- Trial data is accurate, complete, and verifiable with source documents.
- The trial is conducted in compliance with the approved protocol, following GCP and current regulations.

Monitoring Plan:

- Given the characteristics of the study, a risk-focused monitoring approach will be implemented. The variables collected in the CRF will be reviewed with special emphasis on safety variables and adverse effects. Verification and validation of the collection of key variables will be performed.
- Three monitoring visits will be scheduled (2 during the study and one final closing visit) during which the following checks/verifications will be conducted:

• Critical variables to be monitored:

• Verification of the collection of:

I. Informed Consent Verification

II. Confirmation of eligibility criteria

III. Correct randomization and administration of study drug

IV. Verification that identified data as critical variables. Review of source documents

V. Review of safety assessments (AEs, SAEs)

• **Specific verifications to be performed at each visit:**

a) Written informed consent was obtained before each subject's participation in the trial.

b) The investigator recruits only subjects who meet the selection criteria.

c) The approved protocol is being followed correctly.

d) The time and storage conditions of iNO are acceptable, and supplies are sufficient during the trial; it is only administered at the doses specified in the protocol.

e) Source documents and other trial records are accurate, complete, up-to-date, and properly archived.

f) Accuracy and integrity of data entered into the CRF with respect to source documents and other trial-related records.

g) All adverse events have been reported appropriately and within the timelines specified in GCPs, the protocol, the Ethics Committee (CEIm), and relevant legal requirements.

h) The investigator maintains essential documents (Investigator File).

i) Reporting any protocol deviations and taking necessary actions to prevent recurrence of detected deviations.

• Conduct a final study closure visit to ensure essential documentation (Investigator File).

7.8. Insurance

Although the study could be considered of low intervention according to Article 2 of Royal Decree 1090/2015, the sponsor will procure insurance since the use of iNO could be on the boundary of its indication.

7.9 Publication Policy

The results of this clinical trial may be published in specialty journals in the first quartile.

8. Budget - Financial Report and Financing Plan

This study has received a research grant from Air Liquide (France), which will sponsor it. The total amount received will be 75,000 euros, distributed equally between the two participating centers. This amount will cover the costs of IRB evaluation, insurance for this clinical trial, and specific disposables required:

- Adhesive conductive tape covers and flow sensors for IET measurements.
- Hypertonic saline solution used for perfusion studies.

Cost for each center will be:

• 50 units of 10 ml 20% saline solution ---------- 50€.
• 50 units of 10 ml 10% saline solution ---------- 50€.
• 25 Adhesives (belt covers) ---------- 75 euros per unit ------- 1875 €
• 25 Flow sensors ---------- 86.282 euros per unit ------ 2157 €

Total 4132 €

Additionally, for the reference center, the following costs are included:

• Evaluation by the Ethics Committee (CEIm) ---------- 1000 €
• Medical insurance ---------- approximate cost --- 6000 €.

Total 7000 €

The therapeutic gas nitric oxide and the disposable products necessary for an iNO therapy session will be provided by Air Liquide at no cost to the participating centers. The product sheet is provided as additional documentation.

9. References

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APPENDIX I

Contraindications for the performance of a Transesophageal Echocardiogram:
Esophageal Pathology: stenosis, trauma, tumor, scleroderma, Mallory-Weiss syndrome, diverticulum. Esophagitis, history of dysphagia, upcoming gastrointestinal surgery, active upper gastrointestinal bleeding, esophagectomy, symptomatic hiatal hernia, prior thoracic radiotherapy.