Inhaled Nitric Oxide ReDuce postoperatIve pulmoNAry complicaTions in patiEnts with recent COVID-19 infection (INORDINATE)

Statistical Analysis Plan

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Version History

Date (dd-mm-yyyy)	Version	Reason for the update
22-02-2024	1.0	First draft of SAP based on original trial protocol

ABSTRACT

Background

This a priori statistical analysis Plan describes the analyses for INORDINATE

Methods:

INORDINATE (Inhaled Nitric Oxide ReDuce postoperatIve pulmoNAry complicaTions in patiEnts with recent COVID-19 infection) aims to determine whether the inhalation of high-concentration nitric oxide (NO) during surgery will decrease postoperative pulmonary complications in surgical patients with recent (within 7 weeks) SARS-CoV2 infection history. The study is a single center parallel, two-arm, superior, randomized clinical trial. In total, 660 adult patients with recent SARS-CoV-2 infection schedule for surgery under general anesthesia and mechanical ventilation will be included. After anesthesia induction, patients will be mechanically ventilated and randomly assigned to inhaled 80 ppm NO or placebo until the end of mechanical ventilation or leave the operation room(OR), whichever comes first. The primary outcome is a composite measure of postoperative pulmonary complications (PPCs), recorded as a composite endpoint within the first seven postoperative days. Secondary endpoints include postoperative 30-day all-cause mortality, severity of PPCs scaled by Clavien-Dindo classification, unplanned ICU admission, postoperative length of hospital stay, thrombotic events (including deep venous thrombosis and pulmonary embolism); and postoperative

comprehensive complication index (CCI). The main analyses will focus on the primary and secondary outcomes for recent infected patients undergoing surgery with mechanical ventilation. The analysis will use an intention-to-treat approach to compare treatment arms.

Conclusions:

This documents provides a detailed statistical analysis plan for INORDINATE.

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Trial registration:

ClinicalTrials.gov identifier: NCT05721144. Registered on February, 07, 2023.

Keywords:

COVID-19, Nitric Oxide, Surgery, Prognosis, Inhalation, Perioperative Management, Pulmonary complications, Statistical analysis plan

Background

It has been reported that a history of SARS-CoV-2 infection is associated with a transiently elevated risk of postoperative complications [1–3]. An updated recommendation suggested postponing surgery for at least seven weeks following SARS-CoV-2 infection, thereby reducing the risk of postoperative complications and 30-day mortality to baseline levels (similar to those without a history of SARS-CoV-2 infection) [4–6]. Postoperative pulmonary complications (PPCs) were the most common and frequent complications in patients with a history of COVID-19 infection [2, 7]. Even mild PPCs is associated with increased incidence of postoperative short-term mortality, ICU admission, and prolonged length of ICU and/or hospital stays[15]. Various strategies have been proposed to prevent or mitigate PPCs but with limited efficacy.

In recent years, there has been growing interest in the use of inhaled nitric oxide (iNO) as a potential prophylactic or therapeutic intervention for PPCs. iNO, a selective pulmonary vasodilator, has demonstrated its ability to improve oxygenation, reduce pulmonary hypertension [8], and modulate inflammation in various clinical settings, including pulmonary hypertension, acute lung injury, and ARDS [9-12]. Moreover, iNO has been shown to have a favourable safety profile, with few serious adverse events reported [13]. For patients underwent cardiac surgeries, iNO has been shown to dilate pulmonary blood vessels [14],

reduce pulmonary arterial pressure and lower pulmonary vascular resistance [15-20], and significantly improve oxygenation and patients prognosis [21, 22]. Particularly in patients undergoing aortic dissection surgeries, low-dosed iNO was shown to improve oxygenation, thereby reducing the duration of mechanical ventilation and ICU stay [23]. Additional benefits include a reduction in postoperative complications related to inflammatory responses among patients undergoing knee replacement surgery [24].

The guidelines pertaining to the management of critically ill adults with Coronavirus Disease 2019 [25] recommended administration of inhaled oxide as a rescue therapy for COVID-19 patients experiencing refractory hypoxemia [26]. Studies have proposed NO inhalation as an effective strategy for COVID-19 treatment [27], as SARS-CoV-2 modulates endogenous NO levels and availability upon entering host cells. It was demonstrated that NO can reduce SARS-CoV-2 viral load by 95% within 24 hours and over 99% within 72 hours [28]. Despite its theoretical plausibility and potential, the clinical evidence supporting the efficacy of NO in treating COVID-19-related pulmonary complications has primarily originated from observational studies [29, 30] or small trials [31], yielding inconsistent reported results. Recently, a phase II study demonstrated an improvement of PaO_2/FIO_2 with high-dose NO (at 80ppm) at 48 hours compared with usual care in adults with acute hypoxemic respiratory failure due to COVID-19 [32]. Although the clinically important hard outcomes did not differ, the exploratory results suggested that

inhaled NO leads to a steeper reduction in plasma viral load. In addition, at 80 ppm (relatively high dose), iNO was safe and well-tolerated.

The rationale for using iNO in treating surgical patients with COVID-19 is well established and lies in several key mechanisms. There are however very limited study exploring whether inhaled NO can improve postoperative lung function, especially in non-cardiac surgeries. Furthermore, no randomised controlled trial is available evaluating iNO in surgical patients with a history of recent SARS-CoV-2 infection [33].

INORDINATE is a single-center, randomized placebo controlled trial that aims to determine if intraoperative inhalation of 80 ppm NO will reduce the postoperative pulmonary complications in surgical patients with recent (within 7 weeks) SARS-CoV-2 infection.

The trial commenced in Febrary17, 2023 and the estimated timeline for completion of patient accrual was 12 months. Duration of participation for each patient is 30 days (last follow-up). The total study duration is estimated to be approximately 13 months. This statistical analysis plan details the planned analyses for INORDINATE to facilitate the transparency of data analyses. The trial protocol has previously been published [34].

STUDY OVERVIEW

Ethics and Study Design

Single centre, randomized superiority trial of inhaled nitric oxide during intraoperative mechanical ventilation compared to standard care with placebo nitrogen inhalation in a tertiary teaching hospital. The study protocol has been approved by the institutional review board (IRB) at Xijing Hospital (KY20232058-F1) and has been registered on ClinicalTrials.gov as INORDINATE trial with the identifier NCT05721144. The informed consent will be collected by investigators for all patients before inclusion in the trial. No interim analyses is planned.

Study population

Adult participants (18 years of age or older) recently infected with SARS-CoV-2 within 7 weeks and scheduled for surgery under general anaesthesia with mechanical ventilation are potentially eligible for INORDINATE.

Table 1. Eligibility Criteria

Inclusion	1. Patients who are ≥ 18 years old; 2. Patients who are diagnosed COVID-19 via RT-PCR, rapid antigen or clinical symptoms verified by trained professionals; 3. Surgery should have taken place within 7 weeks of diagnostics of COVID-19; 4. Patients should have understood and signed the informed consent.

Exclusion	1. The healthcare professionals, including surgeons, anesthesiologists, and other personnel responsible for the patient's treatment, or the investigators, have chosen to abstain from participating in the study. Their decision is grounded in the belief that the patient may not derive significant benefits from inhaled nitric oxide (iNO) 2. ASA ≥ 4 or life expectancy < 24h; 3. Females during pregnancy or lactation; 4. Patients with severe liver diseases (defined as Child Pugh score ≥ 12); 5. Patients with severe respiratory failure, requiring mechanical ventilation or ECMO support before surgery; 6. Patients with severe kidney dysfunction (eGFRC ≤ 30 ml/min/1.73m^2) or requiring any of ordinary renal replacement therapy, hemodialysis or peritoneal dialysis; 7. Patients who have been participating in a different clinical trial within a month.

Randomization and Masking

Eligible participants will be randomly allocated at 1:1 ratio to either NO inhalation or placebo. The randomization process will utilize permuted-block randomisation, with stratification based on the predicted risks of PPCs using the Assess Respiratory Risk in Surgical Patients in Catalonia (ARISCAT) index (< 45 [low and medium risk], ≥ 45 [high risk]), as well as the time interval from infection to surgery(≤ 10d, 11-28d, 29-49d). Random blocks of 4 or 6 will

be used for randomization. The allocation will be concealed by a web-based system that was accessed no earlier than the morning of the surgery.

Intervention

All participants will receive perioperative management based on our institutional standard practice, which includes various components such as preoperative evaluation and preparation, general anaesthesia with or without neuraxial anaesthesia and/or nerve blocks, mechanical ventilation, inotropic drugs and vasopressors, postoperative sedation and analgesia, diuretics, intravenous fluids, antibiotics and invasive monitoring. This management regimen will encompass a range of interventions and monitoring, including but not limited to non-invasive or invasive arterial pressure, electrocardiogram, central venous pressure, cardiac output, pulse oximetry, temperature, urine output, arterial blood gases, coagulation monitoring, and frequent routine laboratory examinations. No additional interventions or laboratory tests beyond the standard perioperative management will be conducted on participants. All perioperative management will be at the discretion of the treating anesthesiologist and in accordance with existing protocols for patients undergoing surgery and equal for both groups. Predicted body weight will be calculated as 50 + 0.91*(height [cm] -152.4) for male and 45.5 + 0.91*(height [cm] -152.4) for female. Patients will be randomized into one of the following interventions (Figure 1):

Inhalation of NO:

After randomization, patients assigned to NO group will receive NO inhalation at a concentration of 80 parts per million (ppm) through the respiratory circuit of a mechanical ventilator. The intervention will commence immediately after tracheal intubation and will persist until either extubation or the patient exits the operation room, whichever happens first.

Placebo group:

After randomization, patients assigned to placebo group will receive standard care and inhalation with the NO vehicle gas, aka nitrogen through the respiratory circuit of a mechanical ventilator. The intervention will commence immediately after tracheal intubation and will persist until either extubation or the patient exits the operation room, whichever happens first.

For the intervention details, please refer to the published protocol [34].

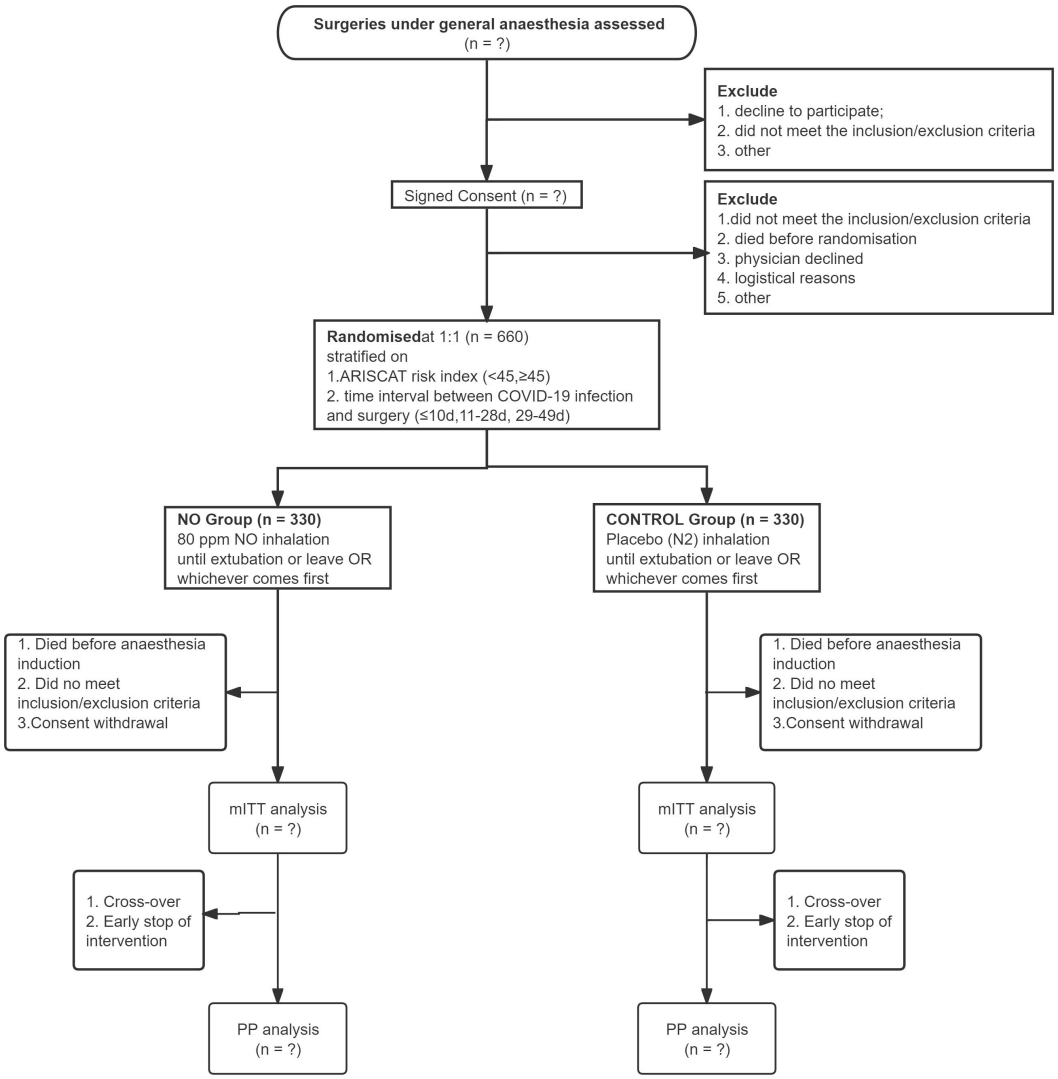

Figure 1. Flowchart

Data collection

A prespecified and designed case report form (CRF) will be used for data collection. All data collected for the trial will be entered into the REDCap application by trained research staff from the clinical chart source data. Access to the data will be restricted and granted by the principal investigator (PI) to authorized investigators within the study team. To ensure the quality

and integrity of collected data, various measures are implemented. After completing the data collection (Table 2), the database will be locked and only the PI (C.L.)and the statistician (Z.Z and C.L) responsible for the analyses will have access to it.

STUDY OUTCOMES

Primary Endpoint

The primary endpoint is a composite measure of postoperative pulmonary complications (PPCs) occurring within the first seven days after surgery. Components include respiratory infection, respiratory failure, pleural effusion, atelectasis, pneumothorax, bronchospasm, aspiration pneumonitis, and pneumonia (detailed definition please refer to the published protocol [34]).

Secondary Endpoints

Secondary endpoints include postoperative 30-day all-cause mortality, severity of PPCs scaled by Clavien-Dindo classification, unplanned ICU admission, postoperative length of hospital stay, thrombotic events (including deep venous thrombosis and pulmonary embolism); and postoperative comprehensive complication index (CCI). Details on definition can be found in the published study protocol [34]. Other measurements are non-pulmonary complications including stroke, cardiac infarction, ventricular dysfunction, delirium, acute kidney injury, surgical revision for bleeding,

hypotension/hypertension, arrhythmia, hyper-responsiveness.

Safety Outcomes

Safety outcomes include hypotension (SBP < 180 mmHg or a rise ≥ 30% of baseline lasting for 5 min), arrhythmia (including bradycardia (HR 100 bpm), or new-onset arrhythmia requiring anti-arrhythmic drugs), airway hyper-responsiveness (airway peak pressure > 40 cm H2O), the count of NO concentration adjustments, and massive bleeding (hemorrhage > 1000 ml).

Table 2. Summary table with all follow-up examinations

	Screening Phase	Study Phase				
		Treatment	Post treatment follow-up			
	Before surgery	In OR	PACU discharge	ICU discharge	Hospital discharge	30 d post
Evaluation						
Inclusion/Exclusion Criteria	×	×				
Previous Medical/Surgical History	×					
Informed Consent	×					
Prior Medication History	×					
Patient Demography	×					
Clinical Examination	×					
Vital Signs	×					
Randomization		×				
Lab Testing	×					
Treatment						
Investigational		×				

Intervention					
Compliance	×				
Outcome evaluation					
Primary				×	
Secondary		×	×	×	×
Safety					
AE/SAE recording (if any)	×	×	×	×	×

STATISTICAL ANALYSIS PLAN

Scope of the Analysis plan

This Statistical Analysis Plan (SAP) presents the analyses for the INORDINATE trial. The final manuscript will include follow-up data until leaving hospital for the trial and analyses will follow this SAP strictly.

Sample Size Consideration

The sample size for this study has been calculated based on the incidence of postoperative pulmonary complications of incidence of PPCs is reported to be approximately 20% for all surgical populations [62, 63] and increase up to 39.5% after COVID-19 infections[5]. The study assumes a 30% PPC incidence in the control group. We anticipate a 30% relative reduction (from 30% to 20%) in PPCs incidence for the iNO group. At 5% significance level and on consideration of 10% dropouts, the sample size needed to achieve 80% power is thus calculated to be 660 (330 per group) using a

two-sided Chi-squared test with continuity correction.

Datasets for analyses

All statistical analyses will be conducted on an intention-to-treat basis, with patients analyses according to their assigned treatment arms (Figure 1). No or minimal losses to follow-up for the primary and secondary outcomes are anticipated. Complete‐case analysis will be carried out for all the outcomes. However, if more than 5% of missing data were found for the primary outcome, a sensitivity analysis using multiple imputations and estimating‐equation methods will be carried out.

Intent-to-treat population (ITT):

The intention-to-treat (ITT) population is defined as all randomized patients. Patients in this population will be analyzed in the arm allocated by randomization, regardless of eligibility criteria and treatment received, drop out, or switched treatments during the trial.

The modified intention-to-treat (m-ITT) population will exclude participants whose surgical procedure was canceled, who did not meet the eligibility after randomization, who withdraw consent and without evaluable efficacy endpoints.

As-treated:

The as-treated population is defined based on the treatment they actually received during the trial. This takes into account the deviations from the

assigned treatment regimen, aka.the cross-overs from the assigned treatment to the other treatment.

Per-protocol population (PP):

The per-protocol (PP) population includes all patients randomized to their randomization arm and without major protocol deviations. In particular, the analysis will be restricted to patients finished the full-term intervention as assigned. Participants with treatment interruptions, or early discontinuations of NO inhalation will be excluded from PP analysis. A list of exclusion will be validated by the steering committee before freezing the database.

Stopping rules

The patient's participation in the study is terminated if the patient (or trusted person) withdraws consent. In the event that the investigator temporarily or permanently discontinues participation in the study for any reason that would be in the best interest of the patient, particularly in the case of serious adverse events suspected to be related to the intervention strategy being tested, the patient remains in the analysis to respect the intention-to-treat principle, and his or her data are collected until 30 days after surgery (the final folllw-up).

No interim analysis was plans, therefore there is no prespecified early termination rules for the study due to efficacy or futility.

Statistic significance and software

Hypothesis tests will be two‐sided with a significance level of 0.05. No Type I error corrections were conducted for multiple tests which will be interpreted as exploratory results. Analyses will be performed using the R program(R Foundation for statistical computing platform) version 4.1.0 or higher.

Distribution of Subjects

Based on all subjects randomized, the number and percentage of subjects who completed the study and withdrew early, as well as the number and percentage of subjects who withdrew early for various reasons, will be summarized if presented. Listing will be presented for the reasons for early termination. Subjects who signed the informed consent but were not randomized will be summarized with listing of reasons provided. Subject distribution flow chart will be presented.

Protocol Deviation

Based on all the randomized subjects, major protocol deviation will be summarized (if there is any), and listing will be provided for all.

General Approach

INORDINATE is designed to detect whether intraoperative administration of up

to 80 ppm NO can reduce PPCs from 30% to 20% in participants undergoing surgical procedures under general anaesthesia who have been infected with SARS-CoV-2 within 7 weeks. Hypothesis tests will be two – sided with a significance level of 0.05. Strength of evidence (e.g., confidence intervals around estimates) will be provided. Data will be screened for integrity prior to full analysis.

Baseline and intraoperative Characteristics

Demographic, baseline, and intraoperative characteristics will be summarised using descriptive statistics and will be presented by treatment group. Categorical variables will be reported as counts and percentages. Pertentages will be calculated according to the number of trial participants for whom data are available. Where values are missing, the denominator will be stated and no assumptions or imputations will be made. Continous variables will be summarized in either means and standard deviation or medians and interquartile range, according to the distribution of the variables.

T-test or Wilcoxon rank sum test will be used for comparison of continuous data. $\chi 2$ test or Fisher's exact test will be used for comparison of categorical data. Normality for continuous variables will be assessed via the Shapiro-Wilk normality test and visually using QQ-plots and residual plots. Appropriate variable transformation such as natural logarithm will be applied in case of non-normality. Should normality not be obtained through transformation of the

data, alternative non-parametric methods (such as Wilcoxon rank sum test) will be applied. All statistical tests will be 2-tailed with $p < 0.05$ considered statistically significant. All analyses will be performed on an intention-to-treat basis.

Blinded Analysis

The primary analysis will be completed using blinded data. Treatment groups will be identified using coded identifiers (i.e. treatment A and B). Analyses will be performed and interpreted based on these blinded treatment groups, prior to unblinding.

Missing and outlier Data

We anticipate no missing values in the primary outcome and very low missing values in other variables if any. The demographics and chronic medical history will be filled on site, intraoperative data will be monitored and recorded in the operating rooms, and the follow-ups until discharge of hospital or deaths can be traced back in the electronic health records. If patients are lost during the 30-day follow-ups, no imputation will be conducted for outcomes. Data missing at baseline will be reported as such. In case where low missing rate (<5%) occurred with no trace-backs available, multiple imputation will be performed as a sensitivity analysis.

Outliers will be subject to a confirmation request to the investigators. In

case of confirmation, their value will not be modified, and will be taken into account as it is during the analysis.

Presentation of Data

The baseline demographic characteristics, surgical procedures performed will be summarized descriptively by treatment group.

Quantitative variables will be described using the following descriptive statistics: size, number of missing values, mean and standard deviation (SD), median and interquartile range (IQR), or minimum and maximum as appropriate depending on continuity.

Categorical variables will be summarized using the following descriptive statistics: number of people, number of missing values, frequencies, and percentages for each level of the variable (missing values will not be included in the denominator of the percentage calculation).

Computation of dates

For calculation of durations, the date of index surgery corresponding to the date of randomization or admission to the intensive care unit will be considered as day 1, depending on the nature of the calculated duration. Therefore, the durations will be calculated from the following rule, for example for the postoperative length of hospital stay (in days):

Date of discharge from hospital - date of index surgery +1

For the time interval between SARS-CoV-2 infection and surgery, it will be calculated in days as follow:

Date of index surgery - date of infection.

If only the infection date is reported as the first, middle or last third of the month, the day considered will be the 5^{th}, 15^{th} or 25^{th} of the month.

Primary Analysis

The effects of inhaled NO on incidence of PPCs will be reported as number and percentages and estimated with risk ratio and 95% confidence intervals calculated with Wald's likelihood ration approximation test and with Chi-squared or Fisher's exact test for hypothethesis testing. The unadjusted analysis will estimate risk ratios with 95% confidence intervals by two-by-two table with the use of log-Normal approximation. In addition, the effect size will be expressed as risk differences (95% confidence intervals) with the number needed to treat as the reciprocal of the risk difference, rounded to the nearest integer. This will further be analysed as the generalised linear models (GLM) under binomial family with either log or logit link where appropriate. Odds ratios or relative risks together with 95% confidence intervals will be reported. Further adjustment on stratification variables such as ARISCAT index and time interval from infection to surgery will be made. The point estimates with 95% confidence intervals will be reported.

Sensitivity Analysis

The pre-specified sensitivity analysis will re-estimate the iNO effect on the primary outcome using GLM using a binomial distribution. Additional adjustment will be made for subgroups and any variables showing substantial imbalance across treatment arms at baseline. We assessed the balance of randomised groups on baseline and procedural characteristics using absolute standardised difference, defined as the absolute difference in means, mean ranks, or proportions divided by the pooled standard deviation. Baseline and procedural characteristics with absolute standardised difference >0.15 (1.96*sqrt[1/330+1/330]) were considered to be imbalanced and would be adjusted. The other way of exploring the potential imbalance accross treatment arms with be achieved by constructing a a logistic regression model to estimate the treatment effect and predictors of PPCs. Colinearity and overfitting will be assessed through Pearson's correlation tests and variation inflation factors. If outcome events are observed to be rare, a Poisson regression will be employed. Classic logistic regression will be performed with a consistent number of events, and the number of covariates in the model will be decided based on the number of outcomes. The ARISCAT score and operation duration score used as one randomization stratification will be treated as continuous variables, exploring their continuous impacts on the outcome.

Time-to-event (PPC) within the observation window (7 days and 30 days respectively) will be assessed using Kaplan-Meier curves and compared with log-rank tests. Per-protocol and as-treated analyses of PPCs will serve as sensitivity analyses. If a low missing rate (< 5%) in the primary outcome were presented, an extra sensitivity analyses with multiple imputations would be performed.

In addition to the standard analysis described above, the following analyses will be performed to test the robustness of the trial findings:

(1) the count of positive component events within the composite will be assessed and groups will be compared using a Wilcoxon rank–sum test, and odds ratio with the 95% confidence interval will be assessed with a proportional odds logistic regression;

(2) the effect of inhaled NO on each component will be analyzed using a GLM using Bonferroni correction for multiple comparisons with 99.37% Bonferroni–corrected confidence intervals reported (1 – 0.05/8 = 0.9937);

(3) a multivariate analyses treating different components as a multiple outcome;

(4) the average relative effect test will be conducted to determine if the average of component-specific treatment effects equals to zero and a distinct treatment effect is estimated for each component via GEE;

(5) heterogeneity of treatment effect across components will be assessed by a treatment–by–component interaction test;

(6) patients in the treatment and control groups will be paired by risk profiles.

(7) The postoperative 30-day all-cause mortality will combined with severity of PPCs scaled by Clavien-Dindo classification as a hierarchical composite outcome. This composite endpoint will be analyzed using prioritized generalized pairwise comparison methods, namely the win-ratio method/global rank sum technique [35]. Each patient from the intervention group will be compared to each patient in the placebo group (a total of m x n comparisons where m is the total number of patients in the intervention group and n the number of patients in the standard care group) for the death endpoint and then on the Clavien-Dindo classification. Based on which patient performs better in each pair, the group they belong to would be declared the ‘winner’. This would give us the total number of winners in each group and our test statistic would be based on this. In the case of the win-ratio method, for instance, the statistic would be the number of winners in the intervention group divided by the number of winners in the standard care/placebo group. This approach will allow us to infer if the intervention is significantly better than the standard care having taken into account the clinical priority i.e. treating mortality as a more important outcome than having a better Clavien-Dindo classification. This work will be reported separate to the main trial results.

(8)To enhance the interpretability of the results and provide valuable information for decision-making, the INORDINATE trial will employ

Acceptability Curve Estimation using Probability above Threshold (ACCEPT) analysis [36]. This analysis method involves calculating and plotting the probabilities of the true difference between treatments being above various acceptability thresholds, based on the data obtained from the trial. By using this approach, the limitations of traditional binary trial conclusions, which categorize results as either positive (meeting the trial aim) or negative (not meeting the trial aim), can be overcome. ACCEPT analysis provides a more nuanced understanding of the data and allows for a range of possible threshold values to be considered, thereby facilitating more informed decision-making.

Secondary Analysis

Secondary outcomes will be analyzed according to the intention-to-treat principle. Proportion of patients experiencing secondary endpoints including 30-day all-cause mortality, unplanned ICU admission, thrombotic events (including deep venous thrombosis and pulmonary embolism), non-pulmonary complications (including stroke, cardiac infarction, ventricular dysfunction, delirium, acute kidney injury, surgical revision for bleeding, hypotension/hypertension, arrhythmia, hyper-responsiveness) will be compared using Fisher's exact test or Chi-squared tests depending on incidence rate. Each component of the thrombotic events and non-pulmonary complications will also be compared. The severity of PPCs scaled by

Clavien-Dindo classification will be treated as an ordinal variable; the ordinal regressions will be conducted. Postoperative length of hospital stay will be analysed using Kaplan-Meier curves and compared with log-rank tests. Cox proportional hazard models were utilized for length-of-stay outcomes, incorporating a shared frailty model. The proportionality assumption was visually inspected using Kaplan-Meier plots, log-log plots, and Schoenfield residuals, with no clear evidence of divergence. Non-parametric comparisons were made using Kaplan-Meier curves and between-group log-rank tests.

For continuous outcomes, linear or quartile regression was chosen based on the skewness of the distribution. Models were adjusted for the ARISCAT index and the time interval from infection to surgery as per randomisation strata, treated as fixed effects. Between-group risk differences were estimated and reported with 95% confidence intervals. Adverse reactions were documented and analyzed, with differences in incidence tested using Fisher exact tests. No Type I error corrections were conducted for multiple tests in this context.

Safety Outcome Analysis

Incidence of safety outcomes will be compared as primary analysis. The count of NO concentration adjustments will be modeled using Poisson regression.

Pre-defined Subgroup Analysis

Subgroup analysis will be conducted on age (<65 vs ≥ 65), sex (male vs female), surgery types (cardiac or non-cardiac surgery), BMI (≤ 35, >35), grades of complexity of surgery (minor, intermediate and major), duration of anaesthesia (< 2hr, 2-3 hrs or ≥ 3hrs), history of smoking, one-lung ventilation, ARISCAT (<26, 26-44 or ≥45), and time interval between infections and operations (≤10, 11-28, 29-49d). Effect modification will be assessed by stratification-and-group interactions in the generalised linear models. A forest plot with P-for-interaction will be reported.

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