Received: 13 November 2019 | Accepted: 28 November 2019
DOI: 10.1111/aas.13518

ORIGINAL ARTICLE

acta Anaesthesiologica Scandinavica

Hyperoxia and antioxidants during major non-cardiac surgery and risk of cardiovascular events: Protocol for a 2 × 2 factorial randomised clinical trial

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Funding information
The trial is supported by an unrestricted grant from the Scandinavian Society of Anaesthesiology and Intensive Care Medicine (SSAI), a research grant from the Danish Society of Anaesthesiology and Intensive Care Medicine (DASAIM) as well as internal institutional funding.

Background: Myocardial injury after non-cardiac surgery occurs in a high number of patients, resulting in increased mortality in the post-operative period. The use of high inspiratory oxygen concentrations may cause hyperoxia, which is associated with impairment of coronary blood flow. Furthermore, the surgical stress response increases reactive oxygen species, which is involved in several perioperative complications including myocardial injury and death. Avoidance of hyperoxia and substitution of reactive oxygen species scavengers may be beneficial. Our primary objective is to examine the effect of oxygen and added antioxidants for prevention of myocardial injury assessed by area under the curve for troponin measurements during the first three post-operative days.

Methods: The VIXIE trial (VitamIn and oXygen Interventions and cardiovascular Events) is an investigator-initiated, blinded, 2 × 2 factorial multicentre clinical trial. We include 600 patients with cardiovascular risk factors undergoing major non-cardiac surgery. Participants are randomised to an inspiratory oxygen fraction of 0.80 or 0.30 during and for 2 hours after surgery and either an intravenous bolus of vitamin C and an infusion of N-acetylcysteine or matching placebo of both. The primary outcome is the area under the curve for high-sensitive cardiac troponin release during the first three post-operative days as a marker of the extent of myocardial injury.

Secondary outcomes are mortality, non-fatal myocardial infarction and non-fatal serious adverse events within 30 days.

Perspective: The current trial will provide further evidence for clinicians on optimal administration of perioperative oxygen in surgical patients with cardiovascular risks and the clinical effects of two common antioxidants.

1 | INTRODUCTION

Many patients undergoing major surgery are at risk of developing a post-operative cardiovascular event. In patients older than 45 years of age, at least 5% is estimated to experience a cardiac complication such as non-fatal myocardial infarction (MI), heart failure, atrial fibrillation, ventricular tachycardia or death.[1] Perioperative MI is the most common of these and the number of patients who experience a post-operative MI may be underestimated: A large international study measuring the post-operative cardiac troponin found more than 60% of patients with MI to have no specific symptoms.[2]

Current evidence on optimal dosage of perioperative oxygen treatment is sparse. The World Health Organisation and Center for Disease Control and Prevention (CDC) suggest an inspiratory oxygen fraction (FiO_2) of 0.80 in intubated patients to reduce the risk of surgical site infection (SSI).[3] This suggestion is based upon a subgroup reported in a meta-analysis and the level of evidence is considered of moderate certainty for the outcome of SSI and does not consider possible harm regarding mortality and myocardial injury. Some clinicians and researchers advocate a lower inspiratory oxygen fraction in the perioperative setting.[4] One of the arguments is that hyperoxia is not documented safe for all patients. Especially patients with cardiovascular risk factors may not benefit or be harmed from hyperoxia due to the risk of reduced coronary artery blood flow caused by supranormal oxygen concentrations, which is seen when 100% oxygen is administered to patients in a test setting.[5,6] A post-hoc analysis from the PROXI trial (investigating perioperative FiO_2 of 0.80 vs 0.30) found that perioperative hyperoxia was associated with an increased long-term risk of mortality, myocardial infarction and other cardiac events.[7,8]

1.1 | Rationale for trial interventions

The surgical stress response occurring from tissue injury results in several unwanted processes, one of which is oxidative stress. Oxidative stress is defined as a state where an excess of reactive oxygen species (ROS) is produced and cannot be neutralised due to the lack of antioxidative agents. Oxidative stress has been associated with myocardial injury, sepsis, acute respiratory distress syndrome, pulmonary oedema, kidney failure and death.[9-11] Several conditions can increase oxidative stress during surgery. One of these is the liberal use of oxygen with tissue hyperoxia, but it has not been established to which extend hyperoxia has a clinical effect through ROS formation.

A depletion of the body's oxygen scavengers may be replaced by antioxidants such as vitamin C or N-acetylcysteine (NAC), and this may be associated with reduced morbidity and mortality.[12-15] Vitamin C blocks extracellular ROS and can prevent hyperoxia-induced coronary vasoconstriction.[6] NAC increases the levels of the body's own antioxidant, glutathione, which is the main intracellular antioxidant.[13]

1.2 | Rationale for trial outcomes

Myocardial injury after non-cardiac surgery (MINS) has been defined as an elevated post-operative high-sensitive cardiac troponin T (hsTnT) between 20 and 65 ng/L with an absolute change ≥5 ng/L or a hsTnT ≥65 ng/L regardless of change.[16] The definition for high-sensitive cardiac troponin I (hsTnI) is less described and based on troponin cut-offs from available studies. MINS as well as MI are both syndrome diagnoses which require careful considerations of the diagnostic concentration of cardiac troponin.[17] Absence of clinical symptoms in patients with myocardial injury could cause clinicians to consider MINS or AUC of plasma concentrations of troponin as the only surrogate outcomes for MI, but it must be emphasised that MINS is associated with a more than threefold increase in 30-day mortality and that anticoagulants and statins can improve outcome.[14,16] Because MINS is a dichotomous outcome based on low thresholds, there is a risk of underestimating the impact of large troponin elevations as well as repeated troponin elevations over days. The chosen AUC of troponin concentrations in our trial is, therefore, more sensitive and provides detailed information about the degree of myocardial injury, and it has also been used in several major trials.[18,19]

1.3 | Aim and objectives

The aim of this trial is to evaluate the risks and benefits of perioperative hyperoxia and antioxidant therapy when given to high-risk patients during major non-cardiac surgery in a 2 × 2 factorial randomised clinical trial (RCT), with special emphasis on myocardial injury. We hypothesise that hyperoxia (FiO_2 0.80) will increase the degree of myocardial injury as compared to normoxia (FiO_2 0.30), and separately, that antioxidants will reduce the degree of myocardial injury as compared with placebo. Our primary objective is to examine the effect of added oxygen and added antioxidants for prevention of myocardial injury after non-cardiac surgery, assessed by area under the curve of troponin concentration during the first three post-operative days. Our secondary objectives are to evaluate their effects on mortality, non-fatal myocardial infarction and non-fatal serious adverse events within 30 days.

2 | MATERIALS AND METHODS

2.1 | Trial design

In the VIXIE trial (VitamIn and oXygen Interventions and cardiovascular Events), we investigate the clinical effect of two interventions simultaneously that may minimise the risk of myocardial injury after non-cardiac surgery. The VIXIE trial is an investigator-initiated, blinded, multicentre, randomised clinical 2 × 2 factorial trial. Patients will be randomised for two perioperative interventions: 0.80 vs 0.30 inspiratory oxygen fraction as well as antioxidants or placebo. Patients do not receive remuneration. The protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 statement.[20]

2.2 | Screening and randomisation

We screen all patients who are scheduled for surgery and fulfil the age and general anaesthesia criteria. Patients are eligible if they fulfil all the inclusion criteria and none of the exclusion criteria in Table 1. A medical doctor verifies patient eligibility and an investigator obtains informed consent. The investigator gives the patient a unique patient ID in REDCap and after this, the unblinded trial personnel randomise the patient in REDCap and prepares the study drugs. The computer-generated randomisation process uses block randomisation of varying and blinded sizes. The randomisation is stratified by centre and previous MI/angina. We randomise patients in a 1:1:1:1 allocation ratio to receive 80% oxygen/antioxidants, 80% oxygen/placebo, 30% oxygen/antioxidants or 30% oxygen/placebo.

TABLE 1 Inclusion and exclusion criteria in the VIXIE trial

Inclusion criteria
All the listed criteria (1.-4.) must be met.
1. Age 45 y or above
2. Elective or emergency surgery in general anaesthesia
3. Scheduled for abdominal, orthopaedic or vascular surgery with expected duration of surgery of 1 hour or more
4. Fulfil any one of the following five criteria:
a. History of coronary artery disease including angina
b. History of stroke
c. Undergoing vascular surgery
d. History of peripheral arterial disease
e. Any two of the following eight criteria
(i) Emergency surgery
(ii) Current or previous daily smoking
(iii) History of hypertension
(iv) Diabetes mellitus requiring medical treatment
(v) History of transient cerebral ischaemia
(vi) Plasma creatinine >175 µM
(vii) Age 70 y or above
(viii) History of congestive heart failure
Exclusion criteria
1. Pregnancy (A routine HCG will not be measured if women are 50 y or older)
2. Inability to give informed consent
3. Pre-operative arterial oxygen saturation (SpO2) below 90% without oxygen supplementation
4. Drug allergy involving any of the interventional drugs
5. Surgery within the last 30 d prior to the current operation
6. Previous use of bleomycin (due to the risk of pulmonary fibrosis)

2.3 | Registration and approvals

The trial is registered at clinicaltrials.gov (identifier: NCT03494387) and at the European Medicines Agency in the European Clinical Trials Database (EudraCT nr: 2017-002670-39).

The VIXIE trial has been approved by the Danish Medicines Agency (Case no. 2017064658), the regional ethics committee (journal no. H-17039073) and the Danish Data Protection Agency (journal no.2012-58-0004) before inclusion of first patient.

2.4 | Setting

Recruitment commenced in April 2018 in four centres in Denmark and we expect to have last patient last visit in December 2019. Centre no. 1: Bispebjerg and Frederiksberg Hospital, which is also a sponsor and trial coordinating site. Centre no. 2: The Department of Anaesthesia, Centre of Cancer and Organ Dysfunction at Rigshospitalet. Centre no. 3: The Department of Anaesthesia, Centre of Head and Orthopaedics at Rigshospitalet. Centre no. 4: The Department of Anaesthesiology at Herlev Hospital.

2.5 | Standard of care

Patients in VIXIE receive the same standard of care as patients outside the trial. During anaesthesia induction, 100% oxygen is administered. The oxygen intervention begins immediately after intubation, and patients receive a positive end-expiratory pressure (PEEP) of 5 cmH_2O, the PEEP will be increased to 8 cmH_2O if the patient is obese (BMI ≥ 30 kg/m^2). An alveolar recruitment manoeuvre is performed with adherence to local guidelines if clinically significant atelectasis is suspected intraoperatively. The FiO_2 is increased to (or kept at) 0.80 in the minutes just before expected extubation.

Patients scheduled for elective or emergency surgery should avoid prolonged pre-operative fasting and are given pre-operative carbohydrate drinks following local guidelines. We aim for normovolaemia and avoid excess fluid administration. A baseline intraoperative crystalloid infusion of 2-5 mL/kg/h is recommended, preferably Ringer's lactate or acetate. Blood transfusion follows regional guidelines, which state that patients will receive blood transfusion when haemoglobin level is below 4.3 mmol/L or if the patient has clinical symptoms of anaemia. Patients with chronic heart disease receive blood transfusion if haemoglobin level is below 5.0 mmol/L. Intraoperative arterial hypotension is defined as a mean arterial blood pressure below 60 mm Hg or systolic blood pressure below 90 mm Hg. This is treated with fluid boluses or vasopressors according to the clinical and objective evaluation of circulatory state. Inotropes are considered after adequate fluid optimisation and vasopressors in patients with suspected reduced contractility. Mean arterial pressure should be above 60 mm Hg at any time. Early post-operative oral intake of fluids and solids is recommended. A multimodal opioid-sparring analgesic strategy is recommended including regional and local analgesia where possible.[21,22]

2.6 | Interventions

Timeline and interventions are illustrated in Table 2.

2.6.1 | Oxygen intervention

The intervention will start after intubation and consist of either FiO_2 0.80 or 0.30 given during and for 2 hours after surgery. FiO_2 may be increased to 0.80 just before extubation at the attending anaesthetist's discretion.

Immediately after extubation, patients will receive oxygen therapy during transfer to the post-anaesthesia care unit (PACU) using a non-rebreathing mask (High Concentration Oxygen Mask, Intersurgical Ltd) with a flow of either 15 L/min oxygen (the 80% oxygen group) or 10 L/min oxygen (the 30% oxygen group). During this transfer, the 30% oxygen group will receive a higher FiO_2 than allocated, because a flow of at least 10 L/min is required through the face mask to avoid hypercapnia, and because ambient air cannot be given during the transfer to PACU (which commonly lasts less than 10 minutes). Oxygen therapy with face mask during transfer to PACU is chosen to avoid unblinding of patients using nasal oxygen only in the 30% oxygen group and to ensure that patients in the 80% oxygen group receives adequate oxygen at all time.

In the PACU, the patients will receive a mixture of 14/2 L/min vs 2/14 L/min of oxygen/air in the 80% and 30% group respectively. This intervention will continue for the entire duration of PACU stay, and a minimum of 2 hours, after which oxygen will be administered at the attending physician's discretion.

The oxygen administration can be increased from the allocated concentration at any time during the intervention, if clinically

TABLE 2 Trial Period Overview

		Trial period										
		Enrolment	Allocation	Post-allocation								
											Follow-up	
	TIMEPOINT			0-4 hours prior to surgery	Start of surgery	4 hours after start of surgery	2 hours post extubation	POD 1	POD 2	POD 3	30 days	365 days
ELIGIBILITY	Eligibility screen	X										
ELIGIBILITY	Informed consent	X										
ELIGIBILITY	Allocation		X									
INTERVENTIONS	Oxygen intervention				X	—	X					
INTERVENTIONS	Antioxidant intervention: Vitamin C i.v. bolus			X								
INTERVENTIONS	Antioxidant intervention: NAC-infusion				X	X						
ASSESSMENT	Baseline variables	X										
ASSESSMENT	Intraoperative variables				X	X						
ASSESSMENT	Primary outcome							X	X	X		
ASSESSMENT	Secondary outcomes										X	
ASSESSMENT	Adverse and Serious Adverse Events										X	
ASSESSMENT	Mortality										X	X

POD, postoperative day; NAC, N-acetylcysteine.

required to achieve the following minimum levels: Arterial oxygen saturation measured by pulse oximetry (SpO_2) ≥94%, except for patients with chronic obstructive pulmonary disease or body mass index ≥40 kg/m^2, in which a target of SpO_2 88%-92% will be used.

2.6.2 | Antioxidant intervention

The antioxidant intervention consists of 3 g vitamin C given intravenously pre-operatively (time limit: 0-4 hours before anaesthesia start). An infusion of NAC 100 mg/kg will be started after induction of anaesthesia and administered over 4 hours. Matching placebo of each medication will be administered to the patients in the placebo group.

2.7 | Blinding

Unblinded trial personnel receive the unique patient ID from the investigator and then perform the randomisation in REDCap. They inform the investigator of the oxygen intervention (80% or 30% oxygen) via a note in an envelope, and the investigator gives this written information to the attending anaesthesia personnel who administrates the intervention. The anaesthesia personnel keep the patients blinded for the oxygen intervention and keep the anaesthesia monitor out of sight from the surgeons. PACU personnel is also not possible to blind, but they are informed to make sure that patients are not informed about the intervention.

The unblinded trial personnel also prepares the antioxidant interventions and matching placebo. The antioxidants/placebo have the same label stating that it contains either the active drug or isotonic saline. The vitamin C has a slightly yellow colour, and therefore, we use syringes with a dense orange colour, in which vitamin C is indistinct from saline. The NAC solution is colourless and similar in appearance to saline. Blinding for the antioxidant intervention applies to investigators, surgeons, anaesthesia personnel and patients.

We also apply author blinding in a process, where two versions of the manuscript will be written under code: One where 'group A' is assumed to be 80% oxygen and 'group B' is assumed to be 30% oxygen and vice versa. The antioxidant intervention will likewise be presented under code. All authors will approve the final version before unblinding.

2.8 | Outcome measures

The primary outcome is the degree of myocardial injury as assessed by the area under the curve (AUC) for hsTnT (Combas 8000, cobas e 801 module, Diagnostics Roche) or hsTnI (ADVIA Centaur XP, Siemens) in ng/L in absolute plasma concentrations measured during the first three in-hospital post-operative days.

We will report the following secondary outcomes within 30 days:

1. All-cause mortality
2. Non-fatal MI, as defined by the fourth universal definition[17]
3. Non-fatal serious adverse events (SAE) according to the International Consensus of Harmonised tripartite for Good Clinical Practice (ICH-GCP) guidelines.[23]

The following explorative outcomes will be reported

1. Surgical site infection, as defined by CDC.[24]
2. Pneumonia as defined by CDC with the presence of radiologic findings consistent with pneumonia and fever, leucopoenia or leucocytosis or altered mental status in patients aged 70 or older.[24]
3. Sepsis as defined by the joint task force by the European Society of Intensive Care Medicine and the Society of Critical Care Medicine.[25]
4. Acute respiratory failure defined as the need for controlled ventilation or the presence of arterial oxygen saturation of 90% or less despite oxygen therapy.
5. Acute kidney injury as defined by Kidney Disease Improving Global Outcomes (KDIGO) guidelines.[26]

Pre-operative baseline troponin is measured within 30 days before anaesthesia induction (Table 3). The post-operative troponin levels are measured at morning rounds at approximately 8 AM every day, which means that there is approximately 24 hours between each measurement. Patients discharged from hospital less than 3 days after surgery will only have post-operative troponin measurements done corresponding to the number of days of post-operative hospitalisation. Values from hsTnI and hsTnT will be processed together because they react similarly although hsTnI values increase to higher levels with ischaemic lesions.[27] Moreover, we have stratified for centre, so patients with hsTnI measurements will be equally distributed among the intervention groups. The lowest detectable values are 3 ng/L (hsTnT at centre no. 1), 13 ng/L (hsTnT at centre no. 2 and 3) and 6 ng/L (hsTnI at centre no. 4). If the measured troponin is below the lowest detectable value, we will use the following values in the primary outcome analysis: 2.5, 12.5, 12.5 and 5.5 ng/L at centre no. 1-4 respectively.

Patients with no data on the primary outcome will be excluded from the intention-to-treat (ITT) analysis population. This is defined as the absence of any post-operative troponin measurements within 7 days after surgery. A number of measures will be taken in case there are some, but not all, troponin missing in the calculation of the primary outcome (Table 3). The primary outcome will be presented in a modified intention-to-treat analysis (mITT), in which we analyse all randomised patients with exclusion of patients who withdraw from the trial, fulfil an exclusion criterion, do not undergo surgery or have no post-operative troponin measured.

2.9 | Withdrawal and discontinuation of the trial

If patients withdraw their consent to participate in the trial, they will not receive the interventions, and they will not be replaced. We will, however, ask for permission to collect data from the medical

TABLE 3 Measures to account for missing data in primary outcome analysis

	Pre-operative troponin	POD1	POD2	POD3	Data replacement
	0-30 d before Sx	Day of Sx + 1 d	Day of Sx + 2 d	Day of Sx + 3-7 d	
Example 1	Not done	Measured	Measured	Measured	Baseline value replaced by: 2.5 ng/L (centre no. 1) 12.5 ng/L (centre no. 2 and 3) 5.5 ng/L (centre no. 4)
Example 2	Measured	Not done	Measured	Measured	Linear regression
Example 3	Measured	Measured	Not done	Measured	Linear regression
Example 4	Measured	Measured	Measured	Not done	Last observation carried forward
Example 5	Measured	Not done	Not done	Not done	Patient excluded from analyses
Example 6	Measured	Measured	Patient discharged	Patient discharged	Primary outcome assessed as AUC for in-hospital days
Example 7	Measured	Patient discharged	Patient discharged	Measured	Ambulatory measurement if patient is discharged before POD1

Table illustrates calculations for the primary outcome of myocardial injury in patients with at least 3 days post-operative admission (example 1-5) and patients discharged before day 3 (example 6-7).

Abbreviations: POD, post-operative day; Sx, surgery.

records. If the patients withdraw their consent after the intervention, the same procedure as above applies. The interventions will be stopped if a patient experiences a suspected unexpected serious adverse reaction (SUSAR).

2.10 | Data registration

All data will be collected in electronic case reports forms in the web-based database REDCap (Research Electronic Data Capture, The REDCap Consortium, Vanderbilt University), which is a secure software platform provided by the Capital Region of Denmark. Baseline data include age, BMI, ASA class, type of surgery, pulmonary and cardiovascular disease, diabetes, alcohol consumption, medication and routine blood analyses.

2.11 | Follow-up

Follow-up is 30 days after the day of surgery, where the electronic medical record will be reviewed for trial outcomes. The research team will perform a 30-day telephone call to assess additional events. If the patient does not respond, we will repeat attempts by phone, email, spouse telephone or family physician. If the patient is still admitted at the time of the 30-day follow-up, we will use medical records at that time point with additional follow-up in case of any ongoing SAE.

The trial also includes a 1-year follow-up of all-cause mortality, MI and readmissions, and this is performed using medical records.

2.12 | Monitoring

The local Good Clinical Practice Unit (GCP unit) is monitoring the trial at each centre, following a monitoring plan developed in collaboration with the sponsor. The monitoring plan adheres to the International Conference on Harmonisation of Good Clinical Practice (ICH-GCP) standards and verifies informed consent in all patients as well as primary and secondary outcomes and protocol adherence in selected patients. The primary outcome is also verified using double data entry by a blinded investigator who has not been involved with the trial interventions. SAEs will be reported to the sponsor in accordance with the ICH-GCP.[23]

The primary investigators from each centre, LNJ, JW and the sponsor of the trial constitute the steering committee and will supervise the overall conduct of the trial.

The committee will ensure adequate recruitment, follow-up and decide on the local conduct of the trial.

2.13 | Adverse events and serious adverse events

Adverse events (AEs) are defined as any untoward medical occurrence in a patient administered a medicinal product (here oxygen, vitamin C, NAC or placebo) and which does not necessarily have a causal relationship with this treatment. SAEs are defined in accordance with the ICH-GCP as any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity.[28]

AEs are collected in the following categories:

- Surgical wound-related
- Urinary tract infection
- Other infection
- Post-operative nausea and vomiting
- Respiratory
- Circulatory
- Gastrointestinal tract
- Other AE

We report the collected SAEs by the following categories:

- Reoperation
- Circulatory, including MI categorised as SAE
- Major bleeding
- SSI categorised as SAE
- Pneumonia categorised as SAE
- Other infection
- Gastrointestinal
- Other SAE

2.14 | Sample size and power considerations

We will include 600 patients. The sample size is based on the following estimations related to the primary outcome: In the Air Versus Oxygen in ST-Segment-Elevation Myocardial Infarction (AVOID) trial, the 3-day AUC for cardiac troponin was median 1996 [IQR 766-4426] µg/L in the control group. Assuming an estimated standard deviation of (4,426-766)/1.5 = 2440 µg/L and a mean of 1996 µg/L, it would require a total of 578 patients to detect a 33% reduction in AUC for troponin for ST-segment elevation myocardial infarction (STEMI) patients with 90% power and 5% type 1 error. Troponin release in high-risk surgical patients is lower than in STEMI patients, and thus we have estimated the troponin release from patients having routine post-operative troponin screening regardless of cardiovascular risk (at Danish hospitals, unpublished data) in which the 2-day median AUC was approximately 34 [IQR 12-63] ng/L with an estimated standard deviation of (63-12)/1,5 = 34 ng/L, thus requiring a total of 420 patients to detect a 33% reduction in AUC with 90% power and 5% type 1 error. The median AUC in our trial is expected to be larger, because patients at low risk are not included.

We have chosen AUC troponin to be the primary outcome because it is a more sensitive measure of the degree of myocardial injury than point estimates of troponin or a dichotomous outcome of myocardial injury. As there are no documented clinical interactions between hyperoxia and antioxidants, we will perform this factorial 2 × 2 trial with only limited adjustment of any potential interaction. Our trial will, therefore, not be sufficiently powered for the test of interaction itself. No interim analyses will be performed.

We will present 95% confidence intervals (CIs) for the primary analysis of mean differences in AUC between intervention groups and a 95% CI not including zero will be considered statistically significant.

A conclusion based upon the primary and secondary outcomes will be agreed upon by the investigators before unblinding of the trial groups.

2.15 | Statistical methods

Participants will be analysed according to their randomisation group in a mITT analysis. In the primary analysis, we will adjust for the factors used in the stratified group allocation (ie centre and previous MI/angina), since these are stratification values. The primary outcome is a continuous outcome measure and will be analysed using an analysis of covariance (ANCOVA) model to examine the group mean differences. The model includes 80% oxygen (yes/no), antioxidants (yes/no), centre (no. 1/2/3/4), previous MI/angina (yes/no) and interaction between 80% oxygen and antioxidants as fixed effects, with the baseline value of troponin as a covariate.

The primary interventions are 80% vs 30% oxygen and antioxidants vs placebo respectively. We will test for interaction between 80% oxygen and antioxidants.

Regardless of the significance of the test of interaction, this trial will not be able to conclude about clinical interaction between hyperoxia and antioxidant intervention due to the lack of power of the test of interaction.

In case of substantial amounts of missing data on the trial outcomes or stratification variables (≥10%), we will analyse missing data by the use of multiple imputation, assuming potentially missing data are missing at random.

2.15.1 | Additional analyses

We will perform subgroup analyses on the primary outcome according to our stratification factors (centre and previous MI/angina) as recommended by the European Medicines Agency.[29] We will perform a secondary analysis to assess the robustness of the primary analysis in which all measured troponin values are set to 0 ng/L if the results are below the detection thresholds (<3, <13, and <6 ng/L respectively).

2.15.2 | Per-protocol analysis

The per-protocol analysis (PP analysis) consists of all mITT patients, excluding patients based on the following:

Oxygen intervention:

- Cumulative time:
  - Oxygen mask used <1 hour
  - FiO_2 <0.60 for >1 hour in 80% oxygen group
  - FiO_2 ≥0.60 for >1 hour in 30% oxygen group
- No available data on FiO_2
- Primary outcome data entry unblinded

Antioxidant intervention:

- Vitamin C intervention completed after surgical incision
- NAC intervention started after surgical incision
- Less than 90% (in mL) of either antioxidant given
- Intervention unblinded

We plan a long-term follow-up study of the risk of mortality, MI and readmissions performed at 1 year after inclusion of the last patient. Data for this analysis will be collected centrally through the Danish National Patient Registry. We plan two other follow-up study: One in which we investigate ischaemic troponin elevations defined as peak troponin levels above the internationally defined thresholds without extracardiac causes; another in which we in detail analyse the intervention effects in the subgroup of patients undergoing vascular surgery.

2.16 | Trial status

The VIXIE trial is active and is expecting last patient last visit in December 2019.

3 | DISCUSSION

All patients undergoing anaesthesia receive oxygen therapy, and high inspiratory oxygen concentrations are commonly used to prevent hypoxia or to improve tissue oxygenation. A Cochrane review from 2015 has found a point estimate of a relative risk of 0.87 for SSI when patients received a FiO_2 of 0.80 in the perioperative phase as compared to 0.30.[30] This review also found a point estimate of a relative risk of death of 1.12 when patients received a FiO_2 of 0.80 in the perioperative phase as compared to 0.30.[30]

The net benefit of hyperoxia on preventing SSI is not clear-cut, and recent studies have raised concerns that it may not be without complications when arterial oxygen concentrations reach supranormal levels. Hyperoxia has been shown to increase peripheral vascular resistance in both healthy subjects and in anaesthetised patients, and hyperoxia can reduce coronary artery blood flow.[5,6,31,32] A post-hoc analysis of the PROXI trial (investigating perioperative FiO_2 of 0.80 vs 0.30) showed that perioperative hyperoxia may be associated with an increased long-term risk of MI and other heart diseases. The 80% oxygen group in the PROXI trial also had significantly increased all-cause long-term mortality (HR 1.30, 95% CI 1.03-1.64).

The PROXI trial follow-up studies included a total of 287 deaths among 1382 patients, but there were only 21 cases of MI.[7,8] The AVOID trial included patients with ST-elevation MI and found that hyperoxia was associated with increased infarct size and in-hospital mortality.[33] These studies raise concerns about association between perioperative hyperoxia and myocardial injury. Cardiac troponin is a strong and independent predictor of myocardial injury and death. Myocardial injury in the post-operative setting is often missed clinically, and the post-operative measurement of troponin can help to identify patients with silent myocardial injury.

Reactive oxygen species are defined as free radicals and other molecules with strong oxidative abilities. ROS are highly reactive and can cause DNA damage and untimely apoptosis. We have chosen to investigate the effect of NAC and vitamin C, mainly because of promising clinical data and because they are the most commonly used antioxidants in previous trials.[15,34] Another reason for choosing NAC and vitamin C is that these antioxidants are distributed in most body compartments: Intravenously administered Vitamin C is transported into the extracellular and intracellular compartments and NAC is a precursor of the body's own intracellular antioxidant, glutathione.[13] NAC is most commonly administered intravenously as 100 mg/kg over 4 hours, and the 3 g bolus of vitamin C intravenously is suggested as the dose necessary to normalise plasma concentrations in antioxidant depletion.[35] The amount of antioxidant required to achieve a protective effect is unknown. Research regarding antioxidant treatment in surgical and critically ill patients has revealed interesting potential but is not yet conclusive. One RCT found that a combination of Vitamin C, hydrocortisone and thiamine reduced hospital mortality from 40.4% to 8.5% (P < .001) in 94 ICU patients.[15]

4 | PERSPECTIVES

The VIXIE trial is a pragmatic randomised trial that will assess potential benefits and harms of perioperative high vs normal inspiratory oxygen fractions with detailed follow-up for relevant clinical outcomes including myocardial injury. If beneficial effects are documented, a combination of optimal oxygen concentration and antioxidants may be suggested. This could lead to a safe and easy treatment to prevent cardiovascular complications in the perioperative period.

CONFLICT OF INTEREST

CP: Reports having received research funding for her department from Merck Sharp & Dohme for research outside the submitted work. FCL: None. EKA: Reports having received direct and indirect research funding from Radiometer, and Norpharma for research outside the submitted work. MVA: None. LSR: None. LNJ: None. JW: None. RC: The Parker Institute, Bispebjerg and Frederiksberg Hospital is supported by a core grant from the Oak Foundation (OCAY-18-774-OFIL). CSM: Reports having received direct and indirect research funding from Ferring Pharmaceuticals, Merck Sharp & Dohme, Radiometer, and Boehringer Ingelheim for research outside the submitted work.

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How to cite this article: Petersen C, Loft FC, Aasvang EK, et al. Hyperoxia and antioxidants during major non-cardiac surgery and risk of cardiovascular events: Protocol for a 2 × 2 factorial randomised clinical trial. *Acta Anaesthesiol Scand*. 2020;64:400–409. https://doi.org/10.1111/aas.13518