Protocol amendment

Version 1.1

Biomarkers of oxidative stress in trauma patients receiving a liberal or restrictive oxygen strategy: A study on TRAUMOX2 patients

Date: 03/08/2022
ClinicalTrials.gov number: -

TABLE OF CONTENS

General information

Protocol title: Biomarkers of oxidative stress in trauma patients receiving a liberal or restrictive oxygen strategy: A study on TRAUMOX2 patients

Protocol date: 03/08/2022

Version number: 1.1

Investigators:

Tobias Arleth, MD, Ph.D.-student
Primary investigator
Afdeling for Bedøvelse, Operation og TraumeCenter, HovedOrtoCentret
Rigshospitalet, Copenhagen, Denmark
E-mail: tobias.arleth@regionh.dk
Phone: +45 35 45 95 02

Josefine Bækgaard, MD, Ph.D.
Investigator
Afdeling for Bedøvelse, Operation og TraumeCenter, HovedOrtoCentret
Rigshospitalet, Copenhagen, Denmark

Lars Simon Rasmussen, MD, Ph.D., DMSc, professor
Investigator
Afdeling for Bedøvelse, Operation og TraumeCenter, HovedOrtoCentret
Rigshospitalet, Copenhagen, Denmark

Peter Østrup Jensen, cand.scient, Ph.D., associate professor
Investigator
Afdeling for Klinisk Mikrobiologi
Rigshospitalet, Copenhagen, Denmark
E-mail: peter.oestrup.jensen@regionh.dk

Claus Ernst Moser, MD, Ph.D., professor
Investigator
Afdeling for Klinisk Mikrobiologi
Rigshospitalet, Copenhagen, Denmark
E-mail: claus.moser@regionh.dk

Jacob Steinmetz, MD, Ph.D., professor
Sponsor
Afdeling for Bedøvelse, Operation og TraumeCenter, HovedOrtoCentret
Rigshospitalet, Copenhagen, Denmark
Danish Air Ambulance
E-mail: jacob.steinmetz@regionh.dk

Study site:	**Rigshospitalet** Afdeling for Bedøvelse, Operation og TraumeCenter, HovedOrtoCentret Inge Lehmanns Vej 6, opgang 6, 1. sal 2100 København Ø E-mail: hoc.rigshospitalet@regionh.dk Phone: +45 35 45 34 74
Study information:	The trial will be carried out in accordance with the protocol and the applicable laws in this field. The trial will be registered at ClinicalTrials.gov.

Abbreviations

ATLS	Advanced Trauma Life Support
ICH-GCP	International Conference on Harmonisation-Good Clinical Practice
CRP	C-Reactive Protein
CPAP	Continuous Positive Airway Pressure
ICU	Intensive Care Unit
MDA	Malondialdehyde
PEEP	Positive End Expiratory Pressure
REDCap	Research Electronic Data Capture
ROS	Reactive oxygen species
RR	Respiratory rate
H_{start}	Time at arrival to the trauma centre at Rigshospitalet
H8	Time at 8 hours of intervention
H24	Time at 24 hours after trauma
H48	Time at 48 hours after trauma
TRAUMOX2	Short title for the study “Comparing Restrictive vs. Liberal Oxygen Strategies for Trauma Patients: The TRAUMOX2 Trial”
TBI	Traumatic brain injury

1. Background and rationale

In trauma care, supplemental oxygen is often administered in the pre- and intrahospital setting to treat and prevent hypoxaemia as recommended by the Advanced Trauma Life Support (ATLS) manual.[1] Supplemental oxygen is often administered in a non-consistent manner[2,3] and very often without even being prescribed.[4] The expected result of a liberal oxygen administration is hyperoxaemia[5], and this is often seen in mechanically ventilated patients, including trauma patients.[6–8] In recent years, the thought that excessive supplemental oxygen is always necessary in trauma care has been challenged due to studies showing an association between liberal oxygen administration and increased risk of major pulmonary complications and mortality in surgical and ICU patients, including trauma patients.[9–12]

Several mechanisms may explain why excessive oxygen administration may be harmful, such as upregulation of oxidative stress and the formation of resorption atelectases.[13,14] At normal physiological circumstances, reactive oxygen species (ROS) are essential factors in cellular signalling and normal metabolism.[15] However, at pathophysiological circumstances, when the formation of ROS is excessive and the normal antioxidant defence is insufficient, the imbalance favouring ROS is named oxidative stress which is believed to result in cell injury and cell death.[16] The pathophysiological upregulation of oxidative stress in relation to supplemental oxygen is thought to be mediated through a hyperoxia-induced formation of ROS.[17,18] A prolonged exposure to hyperoxia may play a key role in the subsequent inflammatory response, destruction of the alveolar-capillary barrier, impaired gas exchange, and pulmonary oedema.[19]

ROS are very volatile molecules. Therefore, biomarkers within antioxidants, DNA/RNA damage, lipid peroxidation and protein oxidation are indirect measurements of the level of oxidative stress.[20,21] Forsberg et al. measured malondialdehyde (MDA) and total antioxidant capacity (TAC) as biomarkers of oxidative stress in patients (N = 30) undergoing laryngeal surgery under general anaesthesia randomised to THRIVE (Transnasal Humidified Rapid-Insufflation Ventilatory Exchange, FiO_2 1.0, 70 L min^{-1}) or mechanical ventilation (FiO_2 0.4, tidal volume of 6 mL kg^{-1}, RR of 12 min^{-1} and PEEP of 5 cm H_2O) and both interventions were associated with an increase in MDA levels.[22] Another study by Dursun et al. assessed the TAC and total oxidant status (TOS) as biomarkers of oxidative stress in neonates (N = 37) using either CPAP or synchronized intermittent mechanical ventilation (SIMV) and found a significant increase in TOS in the CPAP group compared to the SIMV group.[23] In contrast, Lång et al. randomised 27 and 38 severe TBI patients on mechanical ventilation in an ICU in an FiO_2 group of 0.40 and 0.70 for a maximum of 14 days, respectively, and found no difference in biomarkers of ROS and IL-6 at day 1.[24] A systematic review on effects of different perioperative oxygen concentrations on oxidative stress conclude that higher FiO_2 may be associated with elevated oxidative stress during surgery where MDA was the most commonly reported biomarker of oxidative stress.[25] Therefore, we find it beneficial to add more knowledge to the development of biomarkers of oxidative stress in patients receiving supplemental oxygen in the trauma population.

In this study, we wish to analyse biomarkers of oxidative stress that may relate to the pathophysiological mechanism. We will include patients allocated to either liberal or restrictive oxygen treatment during the study "Comparing Restrictive vs. Liberal Oxygen Strategies for Trauma Patients: The TRAUMOX2 Trial" at Rigshospitalet. TRAUMOX2 (Danish Research Ethics Committee number: H-21018062, ClinicalTrials.gov number: NCT05146700, EudraCT number: 2021-000556-19) is a multicentre RCT investigating a liberal oxygen strategy compared to a restrictive oxygen strategy the first eight hours after trauma. We will primarily focus on MDA as a biomarker of oxidative stress since this has proven to be an often used biomarker in patients and animals.[25–27] Other studies have found the 24-hour mark to be able to detect

differences in MDA.[27,28] Therefore, we hypothesize that trial participants receiving a liberal oxygen strategy will have a significantly higher level of MDA at the 24-hour mark (H24) post-trauma compared to trial participants receiving a restrictive oxygen strategy.

2. Study objectives

2.1 Primary objective of the study

The primary objective of this trial is to assess whether there is a significant difference in MDA as a biomarker of oxidative stress in trauma trial participants receiving a liberal versus restrictive oxygen strategy for eight hours after trauma at the 24-hour mark after initiation of the TRAUMOX2 intervention (H24).

2.2 Secondary objectives of the study

The secondary objectives are to assess whether there are significant differences in other biomarkers of oxidative stress, inflammation and anti-inflammation in trauma trial participants receiving a liberal versus restrictive oxygen strategy for eight hours after trauma at H_{start}, H8, H24 and H48.

2.3 Hypothesis

We hypothesize that trial participants receiving a liberal compared to a restrictive oxygen strategy for eight hours will have significantly higher levels of MDA at the 24-hour mark (H24) post-trauma.

3. Methods

3.1 Study design and setting

This will be a prospective, single centre study on trauma patients at Rigshospitalet, Copenhagen, Denmark. The patients will be assessed for eligibility for this study once they are included in the international, multicentre, parallel-grouped, superiority, outcome assessor- and analyst-blinded, randomised, controlled trial "Comparing Restrictive vs. Liberal Oxygen Strategies for Trauma Patients: The TRAUMOX2 Trial" (EudraCT number: 2021-000556-19, ClinicalTrials number: NCT05146700, Danish Research Ethics Committee number: H-21018062). Therefore, it is mandatory that a trial participant is included and undergoes the full intervention of either the liberal or restrictive oxygen strategy in TRAUMOX2 since data in this study depends on the TRAUMOX2 intervention. This protocol will serve as a protocol amendment to the TRAUMOX2 trial.

3.2 Inclusion criteria

- Included in the TRAUMOX2 study

3.3 Exclusion criteria

- Excluded from the TRAUMOX2 study
- Trial participants discharged prior to 24 hours post-trauma
- A major protocol violation in TRAUMOX2

3.4 Recruitment and consent

The trial participants eligible for inclusion in this trial will be temporarily incompetent because of the acute and severe condition related to their traumatic injuries. They may need multiple interventions and perhaps even surgery. Symptoms will include severe pain and impaired consciousness and early complications are circulatory failure and respiratory failure requiring emergency intubation. Some of these trial participants are expected to die. The venous blood samples that are drawn at this early time in this trial are pivotal to be taken immediately in the early phase due to increase the knowledge on the acute phase response. Therefore,

we cannot delay enrolment and need to use the consent procedures for emergency research. To make clinical trials with the goal of improving the treatment of traumatic injuries it is necessary to include unconscious and incompetent trial participants as no clinically relevant animal model exists.

Hence, we consider all trauma patients to be without ability to consent to participation ("uden handleevne").

The study fulfills the following criteria from "komitéloven" regarding research in acute situations:

§ 12:
"*Komiteen kan tillade, at et sundhedsvidenskabeligt forskningsprojekt, der angår kliniske forsøg med lægemidler, gennemføres uden indhentelse af samtykke efter §§ 3-5, hvis der i stedet indhentes stedfortrædende samtykke fra forsøgsværgen, og hvis forskningsprojektet efter sin karakter kun kan gennemføres i akutte situationer, hvor forsøgspersonen ikke er i stand til at afgive et informeret samtykke, og det ikke er muligt at indhente et stedfortrædende samtykke efter §§ 3 og 4.*

Stk. 2. Den forsøgsansvarlige skal snarest muligt efterfølgende søge at indhente informeret samtykke eller stedfortrædende samtykke efter §§ 3 og 4."

The proxy consent from the study guardian in TRAUMOX2 will be considered sufficient on behalf of inclusion in this study. Once a trial participant is included in TRAUMOX2 pre-hospital or in-hospital, the trial participant fulfils the inclusion criteria for this study. Trial participants will be eligible for inclusion when arriving to the hospital from Monday to Friday 8:00-15:00.

Hereafter, consent will be sought by the trial participants' next-of-kin as soon as-possible, and when possible, consent will be sought by the trial participant. If the trial participant is not able to consent within 30 days, consent from the next-of kin will be accepted as the final consent. In details, consent will be obtained in the following two steps:

1. Consent will be sought by the trial participant's next-of-kin as soon as possible (as early as possible after admission in an undisturbed environment) by the investigator. Efforts will be taken to ensure the conversation proceeds in an undisturbed room. Furthermore, at the initial conversation the next-of-kin will be informed about having the right to have a "bisidder" and have this relative next to the next-of-kin's side before the conversation proceeds if necessary. If it requires an additional waiting time, the investigator will respect this and return at a later point once the "bisidder" is present. Additionally, the trial participant's next-of-kin will be informed of his/her right to take time to think of his/her choice ("betænkningstid"). This will be set at 24 hours. Until a decision has been reached, the trial participant will continue to be included.

2. Furthermore, the investigator will judge if the trial participant becomes capable of consenting himself/herself within the admission, and if this is the case, consent will be sought. Efforts will be taken to ensure the conversation proceeds in an undisturbed room. Furthermore, at the initial conversation the trial participant will be informed about having the right to have a "bisidder" and have this relative next to the trial participant's side before the conversation proceeds if necessary. If it requires an additional waiting time, the investigator will respect this and return at a later point once the "bisidder" is present. Additionally, the trial

participant will be informed of his/her right to take time to think of his/her choice ("betænkningstid"). This will be set at 24 hours. Until a decision has been reached the trial participant will continue to be included. If the trial participant does not become capable of consenting himself/herself 30 days after the trauma, trial participant consent will no longer be sought.

Some trial participants are expected to remain in the state of being without the ability to consent to participation due to their injuries (continuated need of intubation) and for some of these trial participants, their next-of-kins are not possible to identify and contact. At day 30 after inclusion, no further attempts will be carried out to obtain informed consent. In these situations, the trial participant will be considered as continuing in the study. Thus, the proxy consent from a legally appointed study guardian will be the consent on behalf of the trial participant.

All informed consent forms will be obtained by a member attached to the TRAUMOX2 research team (medical doctor, nurse or medical student). The signed informed/proxy consent form from the trial participant/trial participant's next-of-kin will be available in the electronic secure database, REDCap (a secure research electronic data capture system). The consent forms will be digital, and all signatures will be written on a smart phone or tablet (via a signature pen) using REDCap. This electronic data system has dedicated functionalities for written consent and is considered completely safe for data management and storage. It will be made clear, both verbally and in a written manner, that participation is voluntary and can be withdrawn at any time without affecting the treatment of the trial participant. It will also be made clear that the informed/proxy consent from the trial participant/trial participant's next-of-kin contains a permission for the personnel involved in the study to gather information from the trial participant's medical record. Lastly, it will also be made clear that the informed consent from the trial participant/trial participant's next-of-kin contains a permission for the personnel involved in the study, The Good Clinical Practice Unit (monitor of the study) and the Danish Medicines Agency (due to their legislative control inspection of the study) to gather information/data from the trial participant's medical record.

According to the standard informed consent form from the National Ethics Committee regarding competent participants, the participant can choose to be informed about secondary findings due to their treatment at the hospital. However, the purpose of this trial is not to generate new knowledge about the specific participant, so we find that this question is redundant, and have omitted the question from the consent form to spare the participant from making unnecessary decisions.

3.5 Peripheral venous blood sample obtainment and storage

Trial participants will have 18 ml of peripheral venous blood obtained in blood collection tubes (e.g. tubes coated with EDTA and a tube for serum analyses) at four different time points during admission, yielding a total of 72 ml blood. See "*Figure 1 – Participant timeline"*. The trial blood samples will be sent to the Department of Clinical Immunology and centrifuged at 3000 rpm and pipetted in smaller tubes for later analyses. The blood will be stored in a research biobank at minus 80 degrees Celsius established for this study.

3.5.1 Blood samples on arrival to the trauma centre (H_{start})

When the trial participant arrives to the trauma centre, 18 ml blood will be obtained by a laboratory technician ("bioanalytiker"), a health care professional or a member of the TRAUMOX2 research team in blood collection tubes (e.g. tubes coated with EDTA and a tube for serum analyses) as the first set of trial

venous blood samples. The trial blood samples will be sent to the Department of Clinical Immunology and centrifuged at 3000 rpm and pipetted in smaller tubes for later analyses. The blood will be stored in a research biobank at minus 80 degrees Celsius established for this study.

3.5.2 Blood samples after the TRAUMOX2 intervention (H8)

When the eight hours of TRAUMOX2 intervention is complete (restrictive or liberal oxygen treatment), 18 ml blood will be obtained by a laboratory technician ("bioanalytiker"), a health care professional or a member of the TRAUMOX2 research team in blood collection tubes (e.g. tubes coated with EDTA and a tube for serum analyses) as the second set of trial venous blood samples. The trial blood samples will be sent to the Department of Clinical Immunology and centrifuged at 3000 rpm and pipetted in smaller tubes for later analyses. The blood will be stored in a research biobank at minus 80 degrees Celsius established for this study. It is considered acceptable if the blood samples are obtained up to two hours after the TRAUMOX2 intervention has ended.

3.5.3 Blood samples 24 hours after initiation of the TRAUMOX2 intervention (H24)

When 24 hours have passed by after initiation of the TRAUMOX2 intervention, 18 ml blood will be obtained by a laboratory technician ("bioanalytiker"), a health care professional or a member of the TRAUMOX2 research team in blood collection tubes (e.g. tubes coated with EDTA and a tube for serum analyses) as the third set of trial venous blood samples. The trial blood samples will be sent to the Department of Clinical Immunology and centrifuged at 3000 rpm and pipetted in smaller tubes for later analyses. The blood will be stored in a research biobank at minus 80 degrees Celsius established for this study. It is considered acceptable if the blood samples are obtained at ±3 hours in the period around the 24-hour mark of initiation of the TRAUMOX2 intervention.

3.5.4 Blood samples 48 hours after initiation of the TRAUMOX2 intervention (H48)

When 48 hours have passed by after initiation of the TRAUMOX2 intervention, 18 ml blood will be obtained by a laboratory technician ("bioanalytiker"), a health care professional or a member of the TRAUMOX2 research team in blood collection tubes (e.g. tubes coated with EDTA and a tube for serum analyses) as the fourth and final set of trial venous blood samples. The trial blood samples will be sent to the Department of Clinical Immunology and centrifuged at 3000 rpm and pipetted in smaller tubes for later analyses. The blood will be stored in a research biobank at minus 80 degrees Celsius established for this study. It is considered acceptable if the blood samples are obtained at ±3 hours in the period around the 48-hour mark of initiation of the TRAUMOX2 intervention.

3.5.5 Establishment of a research biobank

The blood will be stored in the Department of Clinical Immunology where a research biobank designated for this study will be established. The blood will be stored until analyses have been performed on all trial participants. When the analyses have been performed, an additional amount of blood may be left. Normally, additional blood is destroyed after all study related analyses have been performed. However, we will apply the Danish data authorities for an approval to save the additional blood in a biobank for potential future research. This is also written in the trial participant- and next-of-kin information. All standard procedures on proper storage in a biobank at the Department of Clinical Immunology will be followed.

3.6 Blood sample (biomarkers) analyses

In total, 72 ml blood will be collected from a trial participant during the study. With the assistance from Department of Clinical Microbiology, we wish to determine the blood levels of biomarkers of oxidative

stress in trial participants randomised to either a liberal or restrictive oxygen strategy. In specific, we want to measure biomarkers of oxidative stress in plasma and/or serum, e.g.:

- Lipid peroxidation: Malondialdehyde (MDA) and lipid hydroperoxides
- Antioxidants: Total antioxidant capacity (TAC), glutathione (GSH) and superoxide dismutase (SOD)
- DNA/RNA damage: 8-hydroxydeoxyguanosine (8-OHdG)
- Protein oxidation: Protein carbonyl groups (PCs) and 3-nitrotyrosine

Additional biomarkers include, but are not to restricted to, e.g.:

- Inflammation: Leukocytes (and differential blood count), C-reactive protein (CRP), IL-1, IL-2, VEGF, procalcitonin, G-CSF and TNF-alpha
- Anti-inflammation: IL-10 and ICAM

3.7 Outcome measurements

3.7.1 Primary outcome

The primary outcome will be the level of malondialdehyde (MDA) at H24.

3.7.2. Secondary outcomes

The secondary outcomes will be the levels of biomarkers at H_{start}, H8, H24 and H48, e.g.:

- Antioxidants: Gluthathione (GSH), superoxide dismutase (SOD) and total antioxidant capacity (TAC)
- Lipid peroxidation: MDA at H_{start}, H8 and H48 and lipid hydroperoxides
- DNA/RNA damage: 8-hydroxydeoxyguanosine (8-OHdG)
- Protein oxidation: Protein carbonyl groups (PCs) and 3-nitrotyrosine
- Inflammation: Leukocytes (and differential blood count), C-reactive protein (CRP), IL-1, IL-2, VEGF, procalcitonin, G-CSF and TNF-alpha
- Anti-inflammation: IL-10 and ICAM

3.7 Participant timeline and duration of study participation

Trial participants will be enrolled and have the first set of blood samples obtained on arrival to the trauma centre (H_{start}). The second set will be obtained after eight hours of TRAUMOX2 intervention is complete (H8). At the 24-hour mark of initiation of the TRAUMOX2 intervention, a third set of blood samples will be obtained (H24). At the 48-hour mark of initiation of the TRAUMOX2 intervention, a fourth and final set of blood samples will be obtained (H48). After blood samples at H48 have been obtained and analysed for biomarkers, the study participation is considered complete. As long as the trial participants are enrolled in the study, the study personnel have the permission to access the trial participants' medical record.

3.8 Data collection and storage

Data will be stored in the electronic, web-based, secure, interface named REDCap.[29] This data management system is secure, fully compliant with all regulatory guidelines and a complete audit-trail for data entry validation. Trained members of the research team will be responsible for data collection and entry into REDCap using local electronic clinical registries. All consent forms will be stored in REDCap. Therefore, the electronic case report form (eCRF) will be digital. The REDCap database will be set up from Rigshospitalet in the Capital Region of Denmark.

3.8.1 Data points collection (eCRF) in REDCap

- Trial participant characteristics: REDCap ID from the TRAUMOX2 study, name, unique trial participant identifier (Danish Central Personal Register Number ("CPR nummer"), age, sex, height, weight
- Date and time for obtainment of the blood samples at $H_{start,}$ H8, H24 and H48
- Analysed levels of the biomarkers at $H_{start,}$ H8, H24 and H48

Since the trial participants are included in TRAUMOX2, a complete list of data on the trial participants will be stored and available in the separate TRAUMOX2 REDCap database.

3.8.2 Data law management

The laws on "General Data Protection Regulation" (GDPR; in Danish: "databeskyttelsesforordningen og databeskyttelsesloven") will be followed. The data management is registered internally at Region Hovedstadens own data management center Knowledge Centre on Data Protection Compliance, Health Science and Innovation in a data platform called "Pactius". The investigators of the trial will handle all information confidentially (*tavshedspligt*).

3.9 Withdrawal from the study

In accordance with the Helsinki Declaration, trial participant's/next-of-kin consent can be withdrawn at any time, for any reason. Likewise, the investigator can also withdraw trial participants from the trial at any time. The reason for withdrawal will be noted in the eCRF.

3.10 Statistical analysis

In the study by Forsberg et al.[22] the intervention with THRIVE (Optiflow to 70 L min^{-1} of 100 % oxygen when apnoea occurred) could represent the liberal oxygen strategy intervention in TRAUMOX2 in relation to measuring MDA levels. Furthermore, the trial participant group before the THRIVE intervention could represent the trial participant group with the restrictive oxygen strategy. The MDA levels before THRIVE was 11.2 µM (SD 3.1) and after THRIVE was 12.7 µM (SD 3.1). Based on these results, in our study we aim to detect a difference of 2 µM in MDA level between the restrictive group and the liberal group at H24. We assume that the SD will be around 3 µM. With 120 trial participants, we will have at least 90% power to detect this difference at the 5% significance level and a drop-out rate of 5%.

Continuous variables will be reported using means with standard deviations (SD) or medians with interquartile ranges (IQR) depending on the distribution of the data. Categorical variables will be reported as proportions with percentages. For the primary outcome, an absolute difference estimate will be calculated with a 95% confidence interval between the two groups of oxygen strategy (liberal vs. restrictive). The statistical test for the primary outcome will be an unpaired t-test when comparing the MDA levels in the liberal and restrictive group at H24. For the secondary outcomes, each biomarker chosen for analysis will be evaluated at each time point ($H_{start,}$ H8, H24 and H48) and compared between groups calculated with an absolute difference estimate with 95% confidence interval and a comparison will be made with an unpaired t-test. We will also do a secondary analysis of the primary and secondary outcomes on trial participants included only prehospital. A p-value of <0.05 will be considered statistically significant.

4. Safety management

All trial participants in this study will be followed for Adverse Events and Serious Adverse Events via the TRAUMOX2 main study according to the TRAUMOX2 protocol (Danish Research Ethics Committee number: H-21018062).

The risk of adverse reactions for the trial participants in relation to this study are minimal and only related to the collection of blood samples from a peripheral vein. This is a routine task performed by health care professionals. In the case of an adverse event related to this, the investigator will be responsible for recording these events in the trial participant's eCRF.

5. Study administration

5.1 Ethics

The risks for trial participants in this protocol amendment for TRAUMOX2 are only related to blood collection (72 ml blood collection in total) from a peripheral vein. The first blood sample upon arrival at the trauma centre will be collected as part of the standard blood samples. Therefore, this is not an additional stab to the skin. The next three blood samples at H8, H24 and H48 may potentially be three additional stabs to the skin compared to standard treatment if no other standard blood samples are obtained at the time slot. However, this is a routine task for health care professionals and is not thought to impose a significant risk for the trial participants.

As stated in section 3.4 regarding recruitment and consent, the trial participants in this study are considered temporarily incompetent because of the acute and severe condition related to their traumatic injuries. Symptoms will include severe pain, impaired consciousness, and early complications are circulatory and respiratory failure requiring emergency intubation. Some of these trial participants are expected to die. Current practice is that supplemental oxygen is administered as soon as possible after trauma. Therefore, we need to use the consent procedures for emergency research to gain more knowledge on the biomarkers of oxidative stress in the acute phase in the trauma population. The trial participants are included in TRAUMOX2 already with a proxy consent from a study guardian which is considered sufficient. To make clinical research studies with the goal of improving the understanding of the acute response to traumatic injuries in relation to the use of supplemental oxygen and the investigation of biomarkers of oxidative stress, it is necessary to include unconscious and incompetent trial participants because no clinically relevant animal model exists, and no conscious trial participants have a disease severity that is expected to produce a response suggested in this study.

We think that the risk to the trial participants in this study is minimal. The data obtained at $H_{start,}$ H8, H24 and H48 have the potential of attributing to the general knowledge on the use of supplemental oxygen and its influence on biomarkers of oxidative stress in trauma trial participants within the acute phase. The objective is to clarify if such an association exists. Therefore, this study can serve as a stepping stone for further studies on an explanation of why a liberal oxygen strategy potentially may be harmful. Thus, we find it important and justified to carry out this study.

5.2 Approvals

An application for approval of this study protocol amendment will be sent to the following Danish authorities according to Danish law (since the main study TRAUMOX2 has been approved already):

- The Committee on Health Research Ethics for the Capital Region of Denmark (De Regionale Videnskabsetiske Komitéer for Region Hovedstaden)

5.3 Funding

The study is initiated by a research group of medical doctors in "Afdeling for Bedøvelse, Operation og TraumeCenter, HovedOrtoCentret, Rigshospitalet, København, Danmark" and "Afdeling for Klinisk Mikrobiologi, Rigshospitalet, København, Danmark". An overview of expenses can be seen below.

Budget item	**Expense (DKK)**
1 month salary for a laboratorian worker at the Department of Microbiology	40.000
Multiplex test kits (10 biomarkers) for 120 patients at $H_{start,}$ H8, H24 and H48	144.000
ELISA test kits (1 biomarker) for 120 patients at $H_{start,}$ H8, H24 and H48	72.000
Department of Immunology: Blood samples management	108.000
Overhead	15.700
Total	**379.700**

The study is funded by the Danish Air Ambulance with 329.700 DKK and "Holger og Ruth Hesse's Mindefond" with 50.000 DKK.

5.4 Compensation for participation

The trial participants will not be offered financial compensation for participation in the study.

5.5 Archiving of documents

The subject's files and original data will be stored, as specified in the ICH-GCP Guideline, for 5 years after the study has ended.

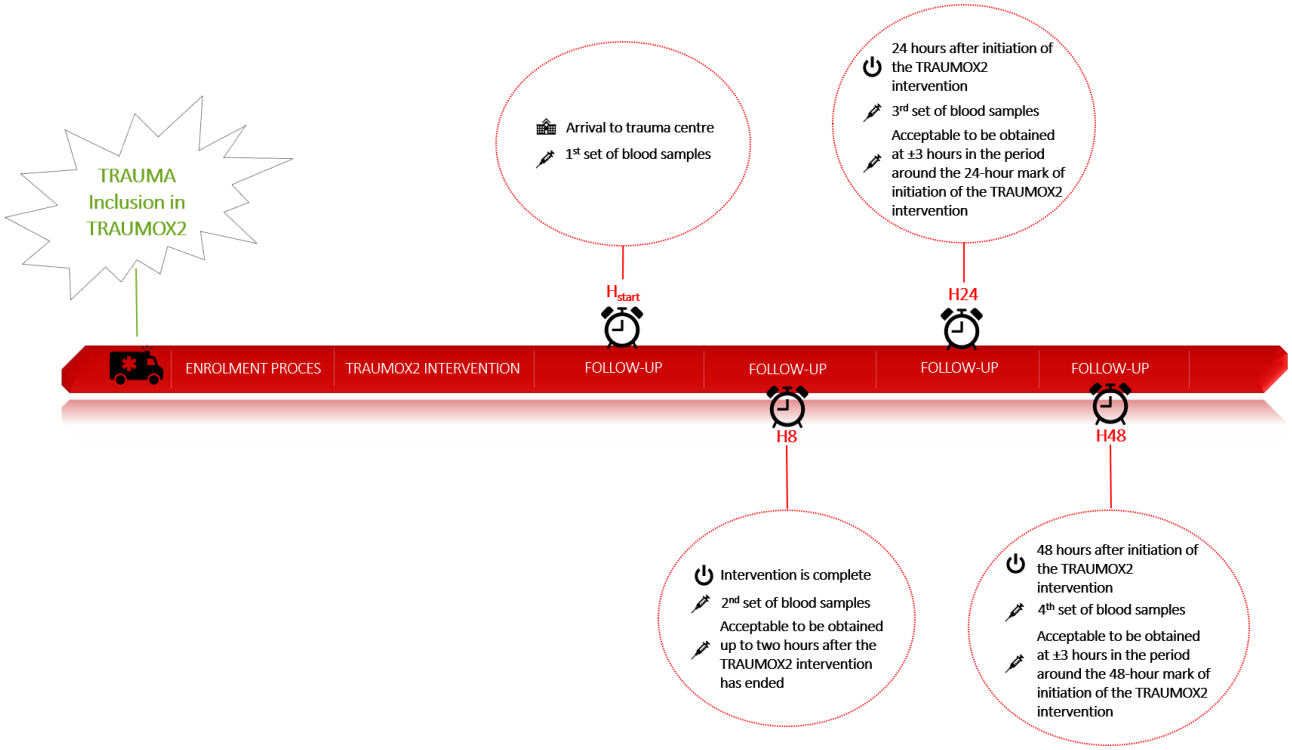
5.6 Study registration

Before first trial participant, first visit, the study protocol will be registered at ClinicalTrials.gov.

5.7 Publication policy

The study results will be submitted to an appropriate international peer-reviewed scientific journal. Both positive, negative, and inconclusive results will be published at ClinicalTrials.gov. Authorship will be granted to persons involved in the study based on the ICMJE criteria for authorship.[30] Tobias Arleth will be listed as first author and Jacob Steinmetz as the last author on the main publication. Investigators are allowed to make proposals to the rest of the group for new studies on biomarkers. New studies should be approved by the whole group and all investigators will be listed as co-authors in new studies. The publication should include the following phrase in funding: "The work presented in this article is supported by Novo Nordisk Foundation grant NNF20OC0063985". The Danish Air Ambulance will also be mentioned in funding with the phrase: "The work presented in this article is supported by the Danish Air Ambulance". Finally, "Holger og Ruth Hesse's Mindefond" will be mentioned.

Figure 1 – Participant timeline



References

1. American College of Surgeons: The Committee on Trauma. *ATLS: Advanced Trauma Life Support (Student Course Manual), 10th Edition*.; 2018. doi:10.1111/j.1365-2044.1993.tb07026.x

2. Hale KE, Gavin C, O'Driscoll BR. Audit of oxygen use in emergency ambulances and in a hospital emergency department. *Emerg Med J*. 2008;25(11):773-776. doi:10.1136/emj.2008.059287

3. McMullan J, Rodriquez CmsD, Ward KW, et al. Prevalence of Prehospital Hypoxemia and Oxygen Use in Trauma Patients. *Mil Med*. 2013;178(10):1121-1125. doi:10.7205/MILMED-D-13-00126

4. Boyle M, Wong J. Prescribing oxygen therapy. An audit of oxygen prescribing practices on medical wards at North Shore Hospital, Auckland, New Zealand. *N Z Med J*. 2006;119(1238):31-35.

5. Tretter V, Zach M-L, Böhme S, Ullrich R, Markstaller K, Klein KU. Investigating Disturbances of Oxygen Homeostasis: From Cellular Mechanisms to the Clinical Practice. *Front Physiol*. 2020;11:1-19. doi:10.3389/fphys.2020.00947

6. Eskesen TG, Baekgaard JS, Christensen RE, et al. Supplemental oxygen and hyperoxemia in trauma patients: A prospective, observational study. *Acta Anaesthesiol Scand*. 2019;63(4):531-536. doi:10.1111/aas.13301

7. Suzuki S, Eastwood GM, Peck L, Glassford NJ, Bellomo R. Current oxygen management in mechanically ventilated patients: A prospective observational cohort study. *J Crit Care*. 2013;28(5):647-654. doi:10.1016/j.jcrc.2013.03.010

8. Helmerhorst HJF, Schultz MJ, Van Der Voort PHJ, et al. Effectiveness and Clinical Outcomes of a Two-Step Implementation of Conservative Oxygenation Targets in Critically Ill Patients: A Before and After Trial. *Crit Care Med*. 2016;44(3):554-563. doi:10.1097/CCM.0000000000001461

9. Staehr-Rye AK, Meyhoff CS, Scheffenbichler FT, et al. High intraoperative inspiratory oxygen fraction and risk of major respiratory complications. *Br J Anaesth*. 2017;119(1):140-149. doi:10.1093/bja/aex128

10. Six S, Jaffal K, Ledoux G, Jaillette E, Wallet F, Nseir S. Hyperoxemia as a risk factor for ventilator-associated pneumonia. *Crit Care*. 2016;20(195):1-8. doi:10.1186/s13054-016-1368-4

11. Chu DK, Kim LHY, Young PJ, et al. Mortality and morbidity in acutely ill adults treated with liberal versus conservative oxygen therapy (IOTA): a systematic review and meta-analysis. *Lancet*. 2018;391(10131):1693-1705. doi:10.1016/S0140-6736(18)30479-3

12. Christensen MA, Steinmetz J, Velmahos G, Rasmussen LS. Supplemental oxygen therapy in trauma patients: an exploratory registry-based study. *Acta Anaesthesiol Scand*. 2021;(July 2020):1-12. doi:10.1111/aas.13829

13. Damiani E, Donati A, Girardis M. Oxygen in the critically ill: Friend or foe? *Curr Opin Anaesthesiol*. 2018;31(2):129-135. doi:10.1097/ACO.0000000000000559

14. Zaher TE, Miller EJ, Morrow DMP, Javdan M, Mantell LL. Hyperoxia-induced signal transduction pathways in pulmonary epithelial cells. *Free Radic Biol Med*. 2007;42(7):897-908. doi:10.1016/j.freeradbiomed.2007.01.021

15. Magder S. Reactive oxygen species: Toxic molecules or spark of life? *Crit Care*. 2006;10(1):1-8.

doi:10.1186/cc3992

16. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol*. 2003;552(2):335-344. doi:10.1113/jphysiol.2003.049478

17. Helmerhorst HJF, Schultz MJ, van der Voort PHJ, de Jonge E, van Westerloo DJ. Bench-to-bedside review: The effects of hyperoxia during critical illness. *Crit Care*. 2015;19(284):1-12. doi:10.1186/s13054-015-0996-4

18. Mach WJ, Thimmesch AR, Pierce JT, Pierce JD. Consequences of Hyperoxia and the Toxicity of Oxygen in the Lung. *Nurs Res Pract*. 2011;2011(18):1-7. doi:10.1155/2011/260482

19. Gore A, Muralidhar M, Espey MG, Degenhardt K, Mantell LL. Hyperoxia sensing: From molecular mechanisms to significance in disease. *J Immunotoxicol*. 2010;7(4):239-254. doi:10.3109/1547691X.2010.492254

20. Frijhoff J, Winyard PG, Zarkovic N, et al. Clinical Relevance of Biomarkers of Oxidative Stress. *Antioxidants Redox Signal*. 2015;23(14):1144-1170. doi:10.1089/ars.2015.6317

21. Dias-Freitas F, Metelo-Coimbra C, Roncon-Albuquerque R. Molecular mechanisms underlying hyperoxia acute lung injury. *Respir Med*. 2016;119(2016):23-28. doi:10.1016/j.rmed.2016.08.010

22. Forsberg I-M, Mkrtchian S, Ebberyd A, et al. Biomarkers for oxidative stress and organ injury during THRIVE compared to mechanical ventilation in adults undergoing microlaryngoscopy - a randomised controlled study. *Acta Anaesthesiol Scand*. 2021;00:1-9. doi:10.1111/aas.13927

23. Dursun A, Okumuş N, Erol S, Bayrak T, Zenciroɨlu A. Effect of Ventilation Support on Oxidative Stress and Ischemia-Modified Albumin in Neonates. *Am J Perinatol*. 2015;33(2):136-142. doi:10.1055/s-0035-1560044

24. Lång M, Skrifvars MB, Siironen J, et al. A pilot study of hyperoxemia on neurological injury, inflammation and oxidative stress. *Acta Anaesthesiol Scand*. 2018;62(6):801-810. doi:10.1111/aas.13093

25. Oldman AH, Martin DS, Feelisch M, Grocott MPW, Cumpstey AF. Effects of perioperative oxygen concentration on oxidative stress in adult surgical patients: a systematic review. *Br J Anaesth*. 2021;126(3):622-632. doi:10.1016/j.bja.2020.09.050

26. Rakhshandeh H, Asgharzade S, Khorrami MB, Forouzanfar F. Protective Effect of Capparis spinosa Extract Against Focal Cerebral Ischemia-Reperfusion Injury in Rats. *Cent Nerv Syst Agents Med Chem*. 2021;21. doi:10.2174/1871524921666210625112356

27. Nagato AC, Bezerra FS, Lanzetti M, et al. Time course of inflammation, oxidative stress and tissue damage induced by hyperoxia in mouse lungs. *Int J Exp Pathol*. 2012;93(4):269-278. doi:10.1111/j.1365-2613.2012.00823.x

28. Ottolenghi S, Maria Rubino F, Sabbatini G, et al. Oxidative stress markers to investigate the effects of hyperoxia in anesthesia. *Int J Mol Sci*. 2019;20(21):1-12. doi:10.3390/ijms20215492

29. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010

30. International Committee of Medical Journal Editors. Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals. Published 2019. Accessed June 18, 2021. http://www.icmje.org/icmje-recommendations.pdf