Biomarkers of oxidative stress in trauma patients receiving a liberal or restrictive oxygen strategy: A sub-study on TRAUMOX2 patients – Statistical Analysis Plan

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1. Introduction

The international ATLS (Advanced Trauma Life Support) guidelines recommend that all severely injured trauma patients receive supplemental oxygen[1] based on very limited evidence.[2,3] Thus, supplemental oxygen is commonly used in trauma patients[4–12], although it may lead to hyperoxaemia which has been associated with pulmonary complications and increased mortality.[13,14] The TRAUMOX2 trial[15] randomises adult patients with suspected major trauma to eight hours of a restrictive or liberal oxygen strategy and explores differences in mortality and pulmonary complications. This sub-study of the TRAUMOX2 trial explores whether biomarkers may explain the development of such complications.

Several mechanisms may explain why excessive oxygen administration may be harmful, such as upregulation of oxidative stress and the formation of resorption atelectases.[16,17] Under normal physiological conditions, reactive oxygen species (ROS) are essential factors in cellular signalling and normal metabolism.[18] However, during pathiological situations, when the formation of ROS is excessive and the normal antioxidant defence is insufficient, there is an imbalance favouring ROS. This is called oxidative stress which is believed to result in cell injury and cell death.[19] The oxidative stress in relation to supplemental oxygen is thought to be mediated through a hyperoxia-induced formation of ROS.[20,21] A prolonged exposure to hyperoxia may play a key role in the inflammatory response, destruction of the alveolar-capillary barrier, impaired gas exchange, and pulmonary oedema.[22] ROS are very volatile molecules. Therefore, biomarkers such as antioxidants, DNA/RNA damage, lipid peroxidation and protein oxidation are indirect measures of the level of oxidative stress. This is explained in more detail in the protocol amendment on biomarkers of oxidative stress to the TRAUMOX2 trial.[23]

In this sub-study of the TRAUMOX2 trial, we aim to analyse biomarkers of oxidative stress that may relate to the pathophysiology of possible complications related to hyperoxaemia. We will include patients allocated to either a liberal or restrictive oxygen treatment in the TRAUMOX2 trial at the Copenhagen study site.[15] The primary outcome will be the level of the biomarker malondialdehyde (MDA) at the 24-hour mark after randomisation in the TRAUMOX2 trial since MDA has previously been used as a biomarker for oxidative stress in patients and animals.[24–26] This document presents the statistical analysis plan of the current sub-study of the TRAUMOX2 trial.

2. Methods

This statistical analysis plan of the sub-study on biomarkers of oxidative stress in the TRAUMOX2 trial has been written according to the *Guidelines for the Content of Statistical Analysis Plans in Clinical Trials*.[27] It aims to give an overview and justification of the chosen variables and statistical methods used. The database system used for this trial is REDCap.[28] The plan is prepared while the trial is still including patients and thus before the database is exported for analysis by the trial statistician. All analyses of the primary, secondary and exploratory secondary outcomes will be conducted according to this publication. The steering committee of the study approved this statistical analysis plan on the 5th of September 2023.

This sub-study of the TRAUMOX2 trial is part of the approval for the TRAUMOX2 trial and has been approved by the Danish Research Ethics Committee (H-21018062), the Danish Medicines Agency (EudraCT 2021-000556-19) and the Knowledge Centre on Data Protection Compliance, Health Science and Innovation in the Capital Region of Denmark.

2.1 Trial design

The biomarker sub-study is 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-group, superiority, outcome assessor- and analyst-blinded, randomised, controlled trial TRAUMOX2. Therefore, it is mandatory that the included patients undergo the full intervention of either the liberal or restrictive oxygen strategy in TRAUMOX2 since data in this study depends on the entire TRAUMOX2 intervention.

2.2 Randomisation and blinding

Patients are randomised 1:1 in variable block sizes of four, six and eight, stratified by including base (prehospital base or trauma centre) and tracheal intubation (yes/no) at inclusion to either a liberal oxygen strategy (control) or restrictive oxygen strategy (intervention). The restrictive group receives the lowest dosage of oxygen ($\geq$21%) that ensures an SpO_2 of 94%. The liberal group receives 12-15 L O_2/min or FiO_2 = 0.6-1.0. The randomisation table was generated outside of REDCap by a statistician otherwise not affiliated with the study. The allocation sequence list and specific block size are only known by this statistician and will remain concealed from the investigators. Prior to randomisation, consent is obtained either by proxy (from another physician) or otherwise according to national legislation. Immediately afterwards, randomisation is done by opening an envelope with information on allocation. Each envelope contains a study ID that matches a study ID on the randomisation list generated by the statistician. Subsequently, informed consent is confirmed from the patient or a relative in case the patient cannot give consent. A more detailed description of the interventions and consent procedure is presented in the protocol of the TRAUMOX2 trial, the protocol of the TRAUMOX2 biomarkers of oxidative stress, and on the study website.[15,23,29]

The treating staff will be aware of the patients' randomisation group, and due to the nature of oxygen treatment, patients and relatives will often be aware of the allocated oxygen strategy as well. While the trial is including patients for the sub-study, members of the research team will also be aware of the patients' oxygen allocation strategy.

2.3 Sample size calculation

In a study by Forsberg et al.[30], patients were treated with THRIVE (Optiflow to 70 L min^{-1} of 100 % oxygen when apnoea occurred) and MDA levels were measured before and after the intervention. The MDA levels before THRIVE were 11.2 µM (SD 3.1) and after THRIVE were 12.7 µM (SD 3.1). Assuming these two time points represent the restrictive (before THRIVE) and liberal group (after THRIVE) in the TRAUMOX2 trial, 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 standard deviation (SD) will be approximately 3 µM. With an estimated number of 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%. However, the number of patients will depend on the TRAUMOX2 study enrolment rate in daytime working hours at Rigshospitalet. Hence, recruitment for this study terminates when the last TRAUMOX2 patient is included.

2.4 Statistical interim analysis and stopping guidance

No interim analyses will be conducted for the sub-study, but the TRAUMOX2 trial has a DMSC which conducts a 1st and 2nd interim analysis for the main study. The sub-study will follow the decisions made by the DMSC and steering committee regarding the TRAUMOX2 trial.

2.5 Timing of variables, outcome assessments and final analysis

The registered variables at baseline, during the intervention and during admission (in-hospital) are presented in Tables 1, 2 and 4. In addition to the variables collected during the intervention (Table 2), a figure displaying the median hourly SpO_2 with interquartile ranges for both groups will be made. The primary and secondary outcomes are presented in Table 3. In addition to Table 3, a figure showing each selected biomarker's median values according to randomisation group with interquartile ranges at H_{start} (baseline), H8 (8-10 hours after randomisation), H24 (24 hours after randomisation ± 3 hours) and H48 (48 hours after randomisation ± 3 hours) will be made. The exploratory secondary outcomes (in-hospital variables) at day 30 are presented in Table 4.

3. Statistical principals

3.1 Confidence intervals and P values

We will use a significance level of 5% and thus 95% confidence intervals (CI) on our estimates. Significance tests will be two sided. The secondary outcomes and exploratory secondary outcomes will be adjusted for multiple testing by having their *P*-values assessed using a significance level lower than 5% according to the Benjamini-Hochberg method.[31] Hence, for the *P*-values of the exploratory secondary outcomes it will be indicated whether they are significant while controlling the false discovery rate at 5%.

3.2 Adherence and protocol deviations

For all TRAUMOX2 trial participants, it is possible to deviate from the protocol if clinically justified by the treating physician. These reasons should always be documented in REDCap.

A major protocol violation will be defined as:

• Restrictive O_2 group, during the intervention period

For non-intubated trial participants: Supplemental oxygen ≥3 L O_2/min AND an SpO_2 ≥98% recorded at two consecutive hourly time points on the data collection sheet.
For intubated trial participants: FiO_2 >0.4 AND an SpO_2 ≥98% recorded at two consecutive hourly time points on the data collection sheet.

• Liberal O_2 group, during the intervention period

For non-intubated trial participants: Supplemental oxygen <3 L O_2/min at two consecutive hourly time points on the data collection sheet.
For intubated trial participants: FiO_2 <0.4 at two consecutive hourly time points on the data collection sheet.

For the main paper, data on protocol deviations and major protocol violations will be presented in Table 2.

3.3 Analysis populations

In the analyses we will exclude patients that:

- Are expected to be discharged within 24 hours after trauma centre admission
- Sustain a major protocol violation during the intervention
- Are discharged from Rigshospitalet or die within 24 hours post-trauma

Please see Figure 1. If consent is withdrawn by a patient, no data after the day of withdrawal will be collected; please see section 4.3 regarding withdrawal and follow-up.

4. Trial population

4.1 Screening, inclusion and follow-up

Screening, inclusion and follow-up is conducted as stated in the sub-study trial protocol.[23] A CONSORT[32] flow diagram is presented in Figure 1.

4.2 Baseline patient characteristics

Baseline variables for all analysed patients are presented in Table 1. Data will be summarised with numbers and percentages for categorical variables and medians with interquartile ranges (IQRs) for continuous variables. According to the CONSORT 2010 statement[32], differences in baseline variables between the restrictive oxygen group and liberal oxygen group will not explored.

4.3 Withdrawal and follow-up

Since this is an acute medical study, patients are always included upon the discretion of an including physician. Patient or next-of-kin consent is sought after inclusion in all cases. After inclusion, patients are at any time allowed to refrain from giving consent or withdraw consent they have given previously. If consent is withdrawn, patients are followed up until this time point according to Danish law. However, patients may at any time request to have their blood samples destroyed.

5. Analysis

5.1 Outcome definitions

5.1.1 Primary outcome and secondary outcomes

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

The secondary outcomes will be the levels of MDA at H_{start}, H8 and H48, superoxide dismutase (SOD), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), intercellular adhesion molecule-1 (ICAM-1), vascular endothelial growth factor (VEGF) and granulocyte colony-stimulating factor (G-CSF) at H_{start}, H8, H24 and H48.

The details of all primary and secondary outcomes are presented in Table 3.

5.1.2 Exploratory secondary outcomes

Exploratory secondary outcomes (in-hospital variables) are presented in Table 4.

5.2 Analysis methods

5.2.1 Primary outcome

The primary outcome is compared between the two groups using the difference between the median values; 95% CI and *P*-values are estimated in a non-parametric bootstrap approach. The patients, retaining the up-to-four measurements per patient, will be sampled with replacement using 10000 bootstrap replicates. Inference is then done through the empirical distribution in these bootstrap samples of the outcome measure, i.e. a 95% CI for the difference between median values is calculated by the 2.5% and 97.5% percentile of the collection of these differences calculated on the bootstrap samples. The comparison will be adjusted for the stratification variables by weighting the calculated medians by a propensity score (see section 2.2 for the stratification variables). A propensity score is the quotient between the theoretical probability of the randomisation (i.e. 50%) and the estimated probability in the sample based on baseline covariates: stratification variables and age, sex, ISS and the first available GCS score after trauma. Propensity scores will be re-calculated within each bootstrap replicate. An additional analysis for the primary outcome will be made with the same approach, but will include further adjustments, including the stratification variables, age, sex, ISS and the first available GCS score after trauma. Evaluation of significance will be based on the *P*-value.

5.2.2 Secondary outcomes

The secondary outcomes will be analysed similarly to the primary outcome and adjusted for the same covariates.

5.2.3 Exploratory secondary outcomes

Exploratory secondary outcomes (Table 4) will be analysed in logistic regression models adjusted for the same covariates as indicated above for the primary outcome. Evaluation of significance will be adjusted according to the Benjamini-Hochberg method.[31]

5.2.4 Subgroup analyses

The following subgroup analyses related to the primary outcome will be performed:

- Injury severity score >15
- For the H_{start} sample: Obtained 1 hour after time of randomisation

5.3 Missing data

If patients withdraw their consent once they regain consciousness, data up until that day is collected and stored according to Danish law. If data is available for time-dependent outcomes (e.g., outcomes that are specified to be assessed 7 or 30 days after inclusion), the assessments will be done dependent on the timing of withdrawal of consent. In case a time-dependent outcome occurs within the period of available data for patients who withdraw consent, the outcome will be registered. If an outcome does not occur within the period of available data for a time-dependent outcome, the outcome is registered as missing data. Missing outcome data, which primarily covers the patients who withdraw consent, is adjusted for using an inverse probability weighting method.

5.4 Harms

5.4.1 Adverse Events and Serious Adverse Events

The Adverse Event and Serious Adverse Event registration on these patients in the sub-study are already followed for events as part of the TRAUMOX2 trial.

5.5 Statistical software

SAS (version 9.4) and RStudio 4.3.0 will be used for all analyses.

6. Status

This sub-study of the TRAUMOX2 trial began recruiting the 3rd of October, 2022. As of the 5th September 2023, 88 patients have been included. The last patient will be included the 12th of September 2023.

7. 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. Eskesen TG, Baekgaard JS, Steinmetz J, Rasmussen LS. Initial use of supplementary oxygen for trauma patients: A systematic review. *BMJ Open*. 2018;8(7):1-7. doi:10.1136/bmjopen-2017-020880

3. Hansen TE, Christensen RE, Baekgaard J, Steinmetz J, Rasmussen LS. Supplemental Oxygen for Traumatic Brain Injury: A Systematic Review. *Acta Anaesthesiol Scand*. Published online 2021:1-10. doi:10.1111/aas.14019

4. 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

5. Leitch P, Hudson AL, Griggs JE, Stolmeijer R, Lyon RM, ter Avest E. Incidence of hyperoxia in trauma patients receiving pre-hospital emergency anaesthesia: results of a 5-year retrospective analysis. *Scand J Trauma Resusc Emerg Med*. 2021;29(1):4-11. doi:10.1186/s13049-021-00951-w

6. 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.

7. 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

8. Baekgaard JS, Abback PS, Boubaya M, et al. Early hyperoxemia is associated with lower adjusted mortality after severe trauma: Results from a French registry. *Crit Care*. 2020;24(1):1-11. doi:10.1186/s13054-020-03274-x

9. Baekgaard J, Siersma V, Christensen RE, et al. A high fraction of inspired oxygen may increase mortality in intubated trauma patients – A retrospective cohort study. *Injury*. Published online 2021. doi:10.1016/j.injury.2021.09.015

10. Kondo Y, Gibo K, Abe T, Fukuda T, Kukita I. Association of prehospital oxygen administration and mortality in severe trauma patients (PROMIS): A nationwide cohort study. *Medicine (Baltimore)*. 2019;98(27):e16307. doi:10.1097/MD.0000000000016307

11. 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

12. 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

13. 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

14. 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

15. Baekgaard J, Arleth T, Siersma V, et al. Comparing restrictive versus liberal oxygen strategies for

trauma patients — the TRAUMOX2 trial: protocol for a randomised clinical trial. *BMJ Open*. 2022;12:e064047. doi:10.1136/bmjopen-2022-064047

16. 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

17. 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

18. Magder S. Reactive oxygen species: Toxic molecules or spark of life? *Crit Care*. 2006;10(1):1-8. doi:10.1186/cc3992

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

20. 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

21. 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

22. 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

23. Arleth T, Bækgaard J, Rasmussen LS, Jensen PØ, Moser CE, Steinmetz J. Protocol amendment - Biomarkers of oxidative stress in trauma patients receiving a liberal or restrictive oxygen strategy: A study on TRAUMOX2 patients. www.traumox2.org. Published 2022. Accessed July 6, 2023. https://www.traumox2.org/wp-content/uploads/2022/11/App.-14-Protocol-amendment-TRAUMOX2-Biomarkers-of-oxidative-stress-v1.1-03-08-2022.pdf

24. 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

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. 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

27. Gamble C, Krishan A, Stocken D, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. *JAMA*. 2017;318(23):2337-2343. doi:10.1001/jama.2017.18556

28. 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

29. The TRAUMOX2 Trial website. www.traumox2.org

30. 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

31. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. *J R Stat Soc*. 1995;57(1):289-300. doi:10.1111/j.2517-6161.1995.tb02031.x

32. Schulz KF, Altman DG, Moher D, The CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *Lancet*. 2010;375(9721):1136. doi:10.1016/S0140-6736(10)60456-4

33. Ringdal K, Coats T, Lefering R, et al. *The Utstein Trauma Template for Uniform Reporting of Data Following Major Trauma: Data Dictionary. Version 1.1.1*.; 2008.

34. CDC: National Healthcare Safety Network. *Pneumonia (Ventilator-Associated [VAP] and Non-Ventilator-Associated Pneumonia [PNEU]) Event*.; 2021. https://www.cdc.gov/nhsn/pdfs/pscmanual/6pscvapcurrent.pdf

35. Ranieri VM, Rubenfeld GD, Thompson BT, et al. Acute respiratory distress syndrome: The Berlin definition. *JAMA*. 2012;307(23):2526-2533. doi:10.1001/jama.2012.5669

Tables

TABLE 1 - BASELINE REGISTRATIONS	
Variable	**Definition**
Age	Calculated from birth year
Sex	Genotypic
Height	Measured in cm
Weight	Measured in kg
Active smoker (y/n)	
Comorbidities prior to trauma (y/n)	
Lung disease	Chronic obstructive pulmonary disease (COPD), asthma, lung fibrosis, or positive COVID-19 test on the day of admission, other
Cardiovascular disease	Hypertension, angina, atrial fibrillation, heart failure, coronary artery disease, other
Other	
Mechanism of injury*	Traffic (motor vehicle accident, motorcycle accident, bicycle accident, pedestrian, other), shot, stab, struck, fall (0-2 metres, 2-4 metres, >4 metres), blast/explosion, other, unknown
Dominating type of injury	Blunt or penetrating
Site of inclusion	Pre-hospital or in-hospital
Airway at inclusion**	Non-intubated or intubated
Use of pre-hospital or in-hospital supplemental oxygen prior to randomisation (y/n)	
Highest SpO_2 measured prior to randomisation	number
Time with supplemental oxygen treatment before randomisation	minutes
Indication for supplemental oxygen treatment before randomisation	Lifesaving (SpO_2 <85%), avoiding hypoxaemia (SpO_2< 90%), routine treatment independent of SpO_2
Supplemental oxygen administration form before randomisation	Nasal cannula, non-rebreather mask, intubated
Initial oxygen flow or initial FiO_2	number
Active treatment of pneumonia on admission (y/n)	Evaluated through patient medical charts
Intubation (y/n)	Pre-hospital, Trauma Centre
Pre-hospital information	
First vital signs***	
Systolic blood pressure	mmHg
Diastolic blood pressure	mmHg
Pulse	bpm
Respiratory rate	number / min
SpO_2	%
Body temperature	Celsius

Glasgow Coma Scale score	number 3-15
Trauma centre information	
Type of transport to the trauma centre	Ground ambulance, helicopter, combination of ground ambulance and helicopter, private vehicle, walk-in, police, other
Time from trauma to trauma centre arrival	minutes
Surgery performed in the trauma centre (y/n)	
If yes, type of surgery	Neurosurgery, Cardiothoracic surgery, Abdominal surgery, Orthopaedic surgery, Urological surgery, Vascular surgery, Gynaecological surgery, other
First vital signs***	
Systolic blood pressure	mmHg
Diastolic blood pressure	mmHg
Pulse	bpm
Respiratory rate	number / minute
SpO_2	%
Body temperature	Celsius
Glasgow Coma Scale score	number 3-15
Destination after trauma centre treatment	Operating Room, Intensive Care Unit, Ward
Injury details	
Injured body region(s)	Head, Neck, Face, Spine, Thorax, Abdomen, Pelvis, Extremity, Other(s)
Abbreviated Injury Score (AIS) codes	numbers
Injury Severity Score (ISS)	number

*Following the Utstein template for reporting[33]
mechanism of injury
**A supraglottic airway is also considered intubation and can be noted in the CRF separately
***Including time and date of vital sign measurement

TABLE 2 - DATA COLLECTED SPECIFICALLY DURING INTERVENTION PERIOD	
Variable	**Definition**
Time from trauma to randomisation	minutes
Time from randomisation to H_{start} blood sample	minutes
For every hour during the 8-hour intervention:	
Patient location	Pre-hospital, Trauma Centre, Operating Room, Examination Room, Post-Anaesthesia Care Unit, Intensive Care Unit, Ward
SpO_2	median
Type of supplemental oxygen	None, nasal cannula, non-rebreather mask, intubated*
Oxygen flow (L/min)	median
FiO_2	median
1st ABG** *(at hour 1 ± 30 minutes after randomisation)*	
PaO_2	kPa or mmHg
Haemoglobin	mmol/L
Lactate	mmol/L
2nd ABG*** *(at hour 6 ± 2 hours after randomisation)*	
PaO_2	kPa or mmHg
Haemoglobin	mmol/L
Lactate	mmol/L
Episodes of hypoxaemia	Number of times with SpO2 <90%
Major protocol violation*** (y/n)	
Protocol deviation (y/n)	

*A supraglottic airway is also considered intubation and can be noted in the CRF separately
**Arterial blood gas
***A major protocol violation will be defined as:

• Restrictive O_2 group, during the intervention period

For non-intubated trial participants: Supplemental oxygen ≥3 L O_2/min AND an SpO_2 ≥98% recorded at two consecutive hourly time points on the data collection sheet.
For intubated trial participants: FiO_2 >0.4 AND an SpO_2 ≥98% recorded at two consecutive hourly time points on the data collection sheet.

• Liberal O_2 group, during the intervention period

For non-intubated trial participants: Supplemental oxygen <3 L O_2/min at two consecutive hourly time points on the data collection sheet.
For intubated trial participants: FiO_2 <0.4 at two consecutive hourly time points on the data collection sheet.

TABLE 3 - PRIMARY OUTCOME AND SECONDARY OUTCOMES	
Primary outcome	**Unit**
Malondialdehyde (MDA) at H24	µM (median)
Secondary outcomes	
Malondialdehyde (MDA) at H_{start}, H8 and H48	µM (median)
Superoxid dismutase (SOD) at H_{start}, H8, H24 and H48	ng/mL (median)
Interleukin-6 (IL-6) at H_{start}, H8, H24 and H48	pg/mL (median)
Interleukin-8 (IL-8) at H_{start}, H8, H24 and H48	pg/mL (median)
Interleukin-10 (IL-10) at H_{start}, H8, H24 and H48	pg/mL (median)
Intercellular adhesion molecule-1 (ICAM-1) at H_{start}, H8, H24 and H48	pg/mL (median)
Vascular endothelial growth factor (VEGF) at H_{start}, H8, H24 and H48	pg/mL (median)
Granulocyte colony-stimulating factor (G-CSF) at H_{start}, H8, H24 and H48	pg/mL (median)

H_{start}, H8, H24 and H48 are evaluated at:
H_{start}: Closest possible time from time of randomisation upon arrival to the trauma centre
H8: After the 8-hour intervention has ended and up to 2 hours after
H24: At 24 hours ± 3 hours after time of randomisation
H48: At 48 hours ± 3 hours after time of randomisation

TABLE 4 - EXPLORATORY SECONDARY OUTCOMES (IN-HOSPITAL VARIABLES)

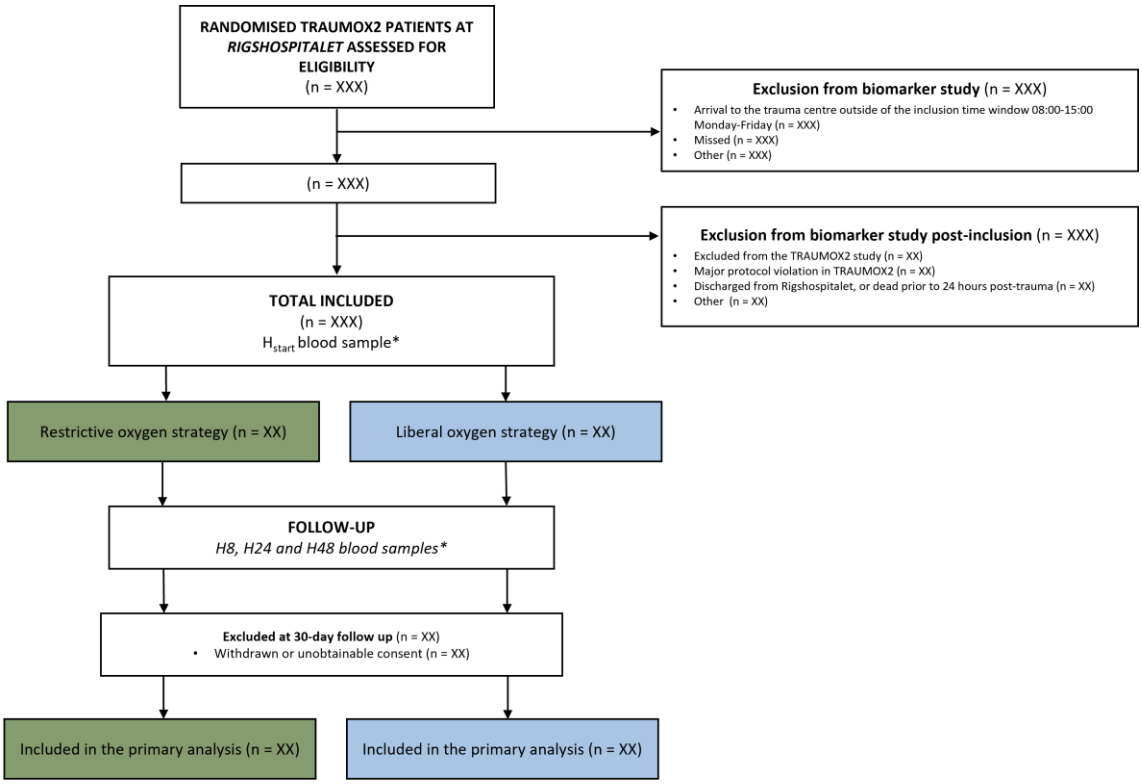
Variable	**Definition**
30-day mortality (y/n)	
Major respiratory complications (pneumonia* and/or ARDS**) within 30 days (y/n)	Assessed by two specialists in anaesthesia, intensive care medicine, emergency medicine or similar using blinded medical records, laboratory and microbiology results, and radiological assessments
Intensive Care Unit admission (y/n)	
Intubation post randomisation (y/n)	During and after intervention
If yes, location, time and date of intubation	Pre-hospital, Trauma Centre, Intensive Care Unit *Mechanical ventilation initiated as part of general anaesthesia on spontaneously breathing patients to facilitate surgery is not included*
Sepsis during hospital admission (y/n)	Clearly stated in the patient's medical record by a physician or assigned as a diagnosis

*Pneumonia is categorised as non-ventilator-associated pneumonia (PNEU) or ventilator-associated pneumonia (VAP), defined by the CDC criteria[34]

**ARDS is categorised as mild, moderate, or severe, defined by the Berlin definition[35]

Figures

CONSORT flow diagram of screening, inclusion and follow-up



** H_{start}, H8, H24 and H48 blood samples refer to the planned blood samples drawn at the start of the study, at eight hours, 24 hours, and 48 hours*