BMJ Open Study protocol and pilot results of an observational cohort study evaluating effect of red blood cell transfusion on oxygenation and mitochondrial oxygen tension in critically ill patients with anaemia: the INsufficient Oxygenation in the Intensive Care Unit (INOX ICU-2) study

Meryem Baysan ^{1,2,3} Mendi S Arbous,^{1,3} Egbert G Mik,⁴ Nicole P Juffermans,⁵ Johanna G van der Bom^{2,3}

To cite: Baysan M, Arbous MS, Mik EG, *et al.* Study protocol and pilot results of an observational cohort study evaluating effect of red blood cell transfusion on oxygenation and mitochondrial oxygen tension in critically ill patients with anaemia: the INsufficient Oxygenation in the Intensive Care Unit (INOX ICU-2) study. *BMJ Open* 2020;**10**:e036351. doi:10.1136/bmjopen-2019-036351

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2019-036351>).

Received 13 December 2019

Revised 06 April 2020

Accepted 17 April 2020

© Author(s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Dr Johanna G van der Bom; j.g.van_der_bom@lumc.nl

ABSTRACT

Introduction The recently developed protoporphyrin IX-triple state lifetime technique measures mitochondrial oxygenation tension (mitoPO₂) in vivo at the bedside. MitoPO₂ might be an early indicator of oxygen disbalance in cells of critically ill patients and therefore may support clinical decisions regarding red blood cell (RBC) transfusion. We aim to investigate the effect of RBC transfusion and the associated changes in haemoglobin concentration on mitoPO₂ and other physiological measures of tissue oxygenation and oxygen balance in critically ill patients with anaemia. We present the protocol and pilot results for this study.

Methods and analysis We perform a prospective multicentre observational study in three mixed intensive care units in the Netherlands with critically ill patients with anaemia in whom an RBC transfusion is planned. The skin of the anterior chest wall of the patients is primed with a 5-aminolevulinic acid patch for 4 hours for induction of mitochondrial protoporphyrin-IX to enable measurements of mitoPO₂, which is done with the COMET monitoring device. At multiple predefined moments, before and after RBC transfusion, we assess mitoPO₂ and other physiological parameters of oxygen balance and tissue oxygenation. Descriptive statistics will be used to describe the data. A linear mixed-effect model will be used to study the association between RBC transfusion and mitoPO₂ and other traditional parameters of oxygenation, oxygen delivery and oxygen balance. Missing data will be imputed using multiple imputation methods.

Ethics and dissemination The institutional ethics committee of each participating centre approved the study (reference P16.303), which will be conducted according to the 1964 Helsinki declaration and its later amendments. The results will be submitted for publication in peer-reviewed journals and presented at scientific conferences.

Strengths and limitations of this study

- The study includes consecutive, critically ill patients with severe anaemia receiving red cell transfusions that ensure generalisability to patients in whom oxygenation monitoring at the cellular level is most relevant.
- In addition to all the macrocirculatory measurements for tissue oxygenation in critically ill patients, the study will examine bedside oxygenation at the cellular level with the mitochondrial oxygenation tension (mitoPO₂).
- Measurements are done at various timepoints before and after red blood cell transfusion that will generate data on whether changes in cellular oxygenation parameters—as assessed with mitoPO₂—precede changes in macrocirculatory measurements for tissue oxygenation.
- The diagnostic value of mitoPO₂ cannot be validated against a gold standard, because this is the first test to measure cellular oxygenation in patients.
- Patients in need of an immediate transfusion cannot be included, because the measurement can only be performed 4 hours after application of the patch.

Trial registration number NCT03092297.

INTRODUCTION

Adequate tissue oxygen delivery is one of the cornerstones of therapy in critical care medicine. However, direct measurements of tissue oxygen oxygenation that would inform the clinical assessment of critically ill patients at the bedside are lacking.¹ Currently, we

monitor tissue oxygenation by measuring global oxygen delivery,¹² and one of the most important determinants of global oxygen delivery is haemoglobin (Hb). In clinical practice, Hb is used as a surrogate marker for the need for red blood cell (RBC) transfusion to treat an oxygen deficit, but personalised Hb concentrations at which oxygen deficits exist are still uncertain. Furthermore, transfusion does not necessarily improve tissue oxygenation, indicating that other oxygenation parameters are needed to determine the need for RBC transfusion.³

A series of new techniques have been developed to quantify oxygen content in the microcirculation and within the cell.^{1 2 4} The protoporphyrin IX-triple state lifetime technique (PpIX-TSLT) measures mitochondrial oxygen tension (mitoPO₂) through the oxygen-dependent optical properties of protoporphyrin IX (PpIX).^{5 6} Thus far, the potential of PpIX-TSLT as an early indicator of oxygen deficit in the cell has been shown in animal models,⁷⁻⁹ where after the feasibility has been studied in healthy volunteers,^{10 11} patients undergoing gastroscopy¹² and in a neurosurgical patient.¹³ Its ability to measure mitoPO₂ has also been validated in vivo in various organs and tissues.^{5 8 9 14} However, whether PpIX-TSLT can be used to measure cellular oxygenation in critically ill patients has not been assessed yet.

We aim to investigate the effect of RBC transfusion on mitoPO₂ and the association between mitoPO₂ and other physiological parameters of oxygen balance and tissue oxygenation in anaemic critically ill patients. In this report, we describe the study protocol of this observational study, the study programme in terms of pilot and main study and how preliminary results of the pilot study resulted in adjustment of the main study design.

METHODS AND ANALYSIS

This manuscript was written in accordance with the Standard Protocol Items Recommendations for Interventional Trials.

Study design and setting

The INSufficientOxygenation in ICU patients-2 is a multicentre observational cohort study running in three academic hospital intensive care unit (ICU) departments in the Netherlands: Leiden University Medical Center, Amsterdam University Medical Center and Erasmus University Medical Center Rotterdam. The participating ICUs are all ICUs with a mixed medical and surgical patient population. Enrolment for this study started in May 2017 for the pilot study solely in Leiden University Medical Center, after which enrolment for the main study started in March 2018. The pilot study was designed to investigate whether crucial components of the main study—such as the measurement of mitoPO₂ in critically ill patients with anaemia and inclusion of eligible patients—were feasible and aimed to predict an appropriate sample size for the full-scale project and to improve the study design. The study was registered in ClinicalTrials.gov

prior to study enrolment. Informed consent is asked by a study team member or a trained treating physician when a critically ill patient is admitted to the ICU, regardless of having a RBC transfusion decision. This procedure, of already having the informed consent instead of asking for informed consent when the decision to transfuse is made, was chosen to minimise the delay of RBC transfusion while waiting for informed consent, in case a blood transfusion is needed at some point during ICU admittance. Participant and legal representatives can always decline study participation at any time for any reason. Data collected up to that point will be used, and no further data will be collected.

Patient and public involvement

The public and patients were not involved in the preparation and design of the study. The results of the study will be disseminated to study participants via a letter after the study has ended.

Eligibility criteria

Critically ill patients with anaemia with an arterial catheter in situ receiving a single RBC unit to correct a Hb threshold of 10 g/dL or lower are included. Patients younger than 18 years, in need of an emergency RBC transfusion, without a legal representative, with a brown plaster allergy, with photodermatosis and/or porphyria or pregnant women are excluded. Furthermore, patients with expected admittance <24 hours, without a central venous catheter in situ and without sufficient Dutch language comprehensibility are excluded. Due to logistics, patients can only be included during working hours (08:00 to 18:00) from Monday to Friday.

Study procedures

In the Leiden University Medical Center, the local transfusion guideline states that transfusion should be guided by the Hb concentration, American Society of Anesthesiologist Classification, age of patient, cardiac state, pulmonary state and volemic state,^{15 16} resulting in varying transfusion thresholds between 6.5 g/dL and 9.7 g/dL. In the Amsterdam University Medical Center and Erasmus University Medical Center Rotterdam, the local transfusion guideline states that an Hb threshold below 7 g/dL should be used in all patients.¹⁷ Treating physicians immediately inform the investigators when they decide to give RBC transfusion to critically ill patients with anaemia during working hours. After assessment of eligibility criteria by the investigator and presence of informed consent, the patient is included in the study. The anterior chest wall of the patient is cleaned with alcohol, and a 5-aminolevulinic acid (ALA) containing patch (Alacare, Photonamic GmbH, Wedel, Germany) is placed on the sternum. The RBC transfusion is given 4 hours after administration of this patch to secure that enough PpIX is formed. In the participating centres, the time between the decision to transfuse and the actual transfusion is between 2 and 3 hours; thus, for patients included in our

study, the delay to transfusion will be on average 1–2 hours longer than in standard care. After extensive discussion about the effect of delay on the patient, we concluded that 1–2 hour delay in RBC transfusion, which is only one of the many interventions ICU patients receive, would not significantly affect the outcome. To not influence the clinical management in these critically ill patients, the treating physician, not the researcher, will always have the final say in timing of the RBC transfusion. If clinical judgement is that the patient is in need of RBC transfusion before 4 hours of PpIX formation, the patient will be excluded from the study. Each RBC transfusion is given over a period of 30–60 min.

Four hours after induction of PpIX, the ALA patch is removed, and mitoPO₂ is measured with a probe that is placed lightly on the part of the skin where PpIX was induced and that is attached to the COMET monitor (Photonics Healthcare, Utrecht, the Netherlands, CE marked but not yet approved by the U.S. Food and Drug Administration). The mitoPO₂ measurement is validated first by occlusion of the microcirculation, by applying local pressure on the measurement probe. In doing this, an immediate drop in mitoPO₂ level is expected, as well as a fast recovery after release of the pressure on the microcirculation.¹³ After this validation procedure, mitoPO₂ is measured once per minute for 5 min to obtain a mean mitoPO₂. The measurement of mitoPO₂ is thus a non-invasive measurement and can be safely used in humans.¹³ To minimise the effect of different light conditions on the PpIX utilisation during all measurements, the room lights are turned off. Directly following the mitoPO₂ measurement, the ALA patch is re-placed on the skin to protect the exposed skin against phototoxicity. After completion of the last measurement, after the last timepoint, the exposed skin is protected from sunlight for an additional 24 hours. During the pilot study, we had eight timepoints for mitoPO₂ measurements: before transfusion, at the end of transfusion, 15, 30, 60, 120 and 180 min after transfusion and 24 hours after transfusion. The RBC transfusion is neither unnecessary delayed nor administered for the purpose of the study, and only the treating physicians have the final decision to administer blood. Besides these study interventions, care was delivered according to local standard and protocolised practice in the three participating centres.

Description of the PpIX-TSLT measurement

The PpIX-TSLT measurement has been described in detail elsewhere.⁵ In short, PpIX is a porphyrin synthesised in the mitochondria as a final precursor of heme. Conversion of PpIX to haem is a rate limiting step, so exogenous administration of ALA, a precursor of PpIX, leads to intramitochondrial accumulation of PpIX. In skin, 4 hours is needed to induce a PpIX level with a valid delayed fluorescence signal.¹³ After photoexcitation of PpIX with a pulse of light of 515 nm from the COMET monitor, PpIX, normally in a singlet state, turns into its triplet state. The spontaneous relaxation process of PpIX

to ground state results in emission of delayed fluorescence (630–700 nm), but excited PpIX can transfer its energy to oxygen and fall back to the ground state without emission of light. Due to such oxygen-dependent quenching, the delayed fluorescence lifetime is a measure of mitoPO₂, that is, the more oxygen is present, the shorter is the extinction time of the delayed fluorescence signal. With every increase or decrease of skin temperature in degrees Celsius, an increase or decrease in this oxygen-dependent quenching constant is seen.¹⁸ The detection of delayed fluorescence is a delicate process, since the signal in itself is weak, which is overcome by a pulsed excitation with a q-switched laser in combination with time-gated detection.⁵ After calculating the delayed fluorescence lifetime from the acquired signal, the mitoPO₂ of the measurement site can be calculated using the Stern-Volmer equation.⁵ The necessary calibration constants, the quenching constants, were validated in rat skin,^{14 19} rat liver⁸ and rat heart.⁹ The measurement location is limited to the epidermis layer since topical ALA skin penetration is possible until a depth of maximum 0.6 mm.^{13 20} Therefore, it is not expected that body fat tissue will affect the mitoPO₂ measurement. However, the possible effect of oedema on mitoPO₂ measurement cannot be ruled out.¹¹ There is a risk of PpIX clearance with each measurement, possibly leading to a decline in fluorescence signal, which is also known as photobleaching.²¹ However, the COMET uses a light dose less than 2 mJ/cm² per measurement. Therefore, the influence of PpIX photobleaching on the mitoPO₂ measurement, even after repeated measurements, is expected to be negligible and appeared no practical problem in previous studies until now.^{22 23}

Primary aim

To assess whether crucial components of the main study—such as the measurement of mitoPO₂ in critically ill patients with anaemia—are feasible, we performed a pilot study in 20 critically ill patients with anaemia. There is no non-invasive golden standard of mitoPO₂ measurement to compare the COMET performances with. Hence, feasibility is pragmatically determined by the following two parameters: a PpIX-TSLT signal quality >25% and a within-subject variability <5 mm Hg. Signal quality is measured with the COMET monitor device, in which a higher signal quality corresponds to a stronger PpIX signal and a more sensitive mitoPO₂ measurement. Signal-to-noise ratio (SNR) in time domain lifetime measurements (as implemented in the COMET) is commonly defined as the ratio of maximal signal amplitude (at the start of the decay) to the maximum signal of the noise (peak-to-peak). Since SNR is not regarded as an intuitive measure for the end user of the COMET device, the software converts SNR to signal quality using a non-linear relationship in which, for example, an SNR of 100 provides a signal quality of almost 80%. In a previous study, it was shown that for PpIX-based delayed fluorescence measurements, an SNR >20 limits the noise-induced potential error in mitoPO₂ measurements below

2% over the physiological PO₂ range.²⁴ A signal quality of 25% corresponds to an SNR of 20.²⁴ Therefore, we chose to (arbitrarily) define 25% as the minimum required signal quality for a feasible measurement. Within-subject variability is defined as the variation of mitoPO₂ within the subject at each measurement timepoint. The mean mitoPO₂ with its corresponding SD is calculated per participant at each timepoint. The mean SD per timepoint therefore represents the within-subject variability of the study population per timepoint. Pragmatically, a within-subject variability up to 5 mm Hg is defined as a feasible measurement to account for possible measurement errors.

The main aim of the study is to assess the effect of RBC transfusion on mitoPO₂ and to describe the association between mitoPO₂ and traditional parameters used to measure oxygenation, oxygen delivery and oxygen balance (ie, central venous oxygen saturation, arterial oxygen saturation, arterial oxygen tension, central venous-to-arterial carbon dioxide difference, cardiac output and lactate).

Secondary aims

Other aims include safety of mitoPO₂ measurements, for which adverse and serious adverse events are recorded, and the description of the association of cutaneous mitoPO₂ with vital organ functions. Vital organ function is assessed by troponin and ischaemic changes on an ECG, 24-hour urine creatinine, Glasgow Coma Scale and Intensive Care Delirium Screening List score and the arterial oxygen tension/fraction inspired oxygen ratio.

Data collection

Data is extracted from the hospital's electronic patient dossier system by the coordinating investigator and coded to ensure anonymity of the patients. Recorded data include age, sex, comorbidities, body mass index, reason of ICU admission and Acute Physiology and Chronic Health Evaluation (APACHE) II²⁵ and IV^{26 27} score. The reason of ICU admission is recorded in accordance with the APACHE IV scoring system.²⁶ With each mitoPO₂ measurement, the following parameters are assessed: heart rate, blood pressure, central venous pressure, peripheral oxygen saturation, body temperature, cardiac output, arterial lactate, central venous oxygen saturation, arterial oxygen saturation, arterial oxygen tension and central venous-to-arterial carbon dioxide difference. Cardiac output is assessed by a limited invasive method, the Vigileo/FloTracsystem.²⁸ Information regarding use and dosage of noradrenalin, adrenalin, phenylephrine, dobutamine, dopamine, milrinone and enoximone are collected with each mitoPO₂ measurement. In addition, Sequential Organ Failure Assessment (SOFA) score,²⁹ the need for mechanical ventilation and the amount of fractional inspired oxygen on the day of transfusion and 1 day after transfusion are also recorded. Data regarding RBC transfusion are recorded as well, including timing of RBC transfusion, infusion duration,

number of RBC transfusions, history of RBC transfusions and product code of the RBC units. Clinical outcome data are extracted from the electronic health record in the 3-month follow-up time and include ICU length of stay, hospital length of stay, ICU mortality and in-hospital mortality. Critically ill patients will be observed during study participation and afterwards for the possible side effects of the ALA plaster. We will collect and report data regarding adverse events, serious adverse events and suspected unexpected serious adverse reactions to gain insight in the safety of the mitoPO₂ measurement.

Missing data

After the last recruitment, data will be reviewed to check for outliers and missing data. Multiple imputation will be used to handle missing data.³⁰

Statistical analysis of the pilot study

Descriptive statistics was used to describe the characteristics of the study population. The mitoPO₂ at the different timepoints before and after transfusion were described with medians and IQRs or as means with SD depending on the distribution of the data. For each timepoint, the mean of all five measurements of mitoPO₂ was calculated with a corresponding SD for each patient. These patient-specific means were used to calculate the population mean at each timepoint. The SDs of the patient-specific means at each timepoint were used to calculate the within-subject variability, which is the mean of these SDs at each timepoint. Signal quality of the mitoPO₂ measurement was described using the mean and SD of the signal quality.

Results of the pilot study

The pilot study was performed between May and August 2017. During the 3-month study period, 233 RBC transfusions were given to 114 patients (figure 1). After assessment of eligibility criteria, 20 patients were included in the pilot study.

In two study participants, no consecutive mitoPO₂ measurements were possible due to discharge from the ICU. The delayed fluorescence signals were detected in all but one patient who had a large amount of red-coloured chlorhexidine on the chest after cardiothoracic surgery, despite cleaning the skin with alcohol. A large amount of red-coloured chlorhexidine possibly influences the delayed fluorescence detection, thereby leading to a low signal quality. The demographic data of the 18 included patients in the analysis can be found in table 1.

The mean mitoPO₂ before start of RBC transfusion was 70.5 mm Hg (SD 13 mm Hg), which largely corresponded with the mean mitoPO₂ value in healthy volunteers (mean 66.3 mm Hg, SD 16 mm Hg).¹¹ Since there is no known normal range of mitoPO₂, interpretation needs to be performed cautiously. Reliable signals were detectable in all remaining patients (n=18), with a signal quality above 25% during all the measurements (table 2). The within-subject variability of mitoPO₂ was 3.0 mm Hg

Figure 1 Flow diagram of enrolment of study participant for the pilot study of the INOX ICU-2 study. ICU, intensive care unit; INOX-ICU-2, INSufficient OXYgenation in ICU patients-2; RBC, red blood cell.

before RBC transfusion, 5.8 mm Hg 30 min after RBC transfusion, 3.6 mm Hg 2 hours after RBC transfusion, 7.3 mm Hg 3 hours after RBC transfusion and 8.7 mm Hg 24 hours after transfusion (table 3). No adverse or serious adverse events regarding the ALA patch were observed by the patient, family, nurse, investigators or physician during the mitoPO₂ measurements and 1 day after the measurements.

The within-subject variability in this study seems to tally with the observed within-subject variability in healthy volunteers.¹¹ However, there were no data on the course of within-subject variability over time in the healthy volunteers. The observed increase in the within-subject variation in mitoPO₂ values over time may have been due to decreased measurement sensitivity, despite having adequate signal quality. Alternatively, other interventions that were performed besides a RBC transfusion in critically ill patients may have contributed to the variation. To assess this further, a separate study will assess the 24-hour course of mitoPO₂ in healthy volunteers and in critically ill patients who receive minimal ICU interventions and no RBC transfusion.

Sample size estimation

To the best of our knowledge, no previous data on mitoPO₂ values in critically ill patients with anaemia were available. We used data from our pilot study among 20 subjects to calculate the mean and SD of mitoPO₂ in the target population to subsequently calculate the sample size. With a p value for statistical significance of 5% and a statistical power of 90%, a mean mitoPO₂ of 67.9 mm Hg

Table 1 Demographic and admission characteristics of the study participants

Characteristic	Values are median (IQR) or number (%)
Age in years, mean±SD	65.3±13.9
Male sex	13 (72.2)
BMI, mean±SD	26.3±4.2
Admission reason (according to APACHE IV)	
Surgical	7 (38.9)
System	1 (14.3)
Cardiovascular	6 (85.7)
Respiratory	0 (0)
Digestive	0 (0)
Neurosurgery	0 (0)
Genitourinary	0 (0)
Trauma	0 (0)
Miscellaneous	0 (0)
Non-surgical	11 (61.1)
Cardiovascular	1 (9.1)
Respiratory	5 (45.5)
Sepsis	3 (27.3)
Neurological	1 (9.1)
Miscellaneous	1 (9.1)
Chronic comorbidities (according to APACHE IV)	
Chronic cardiovascular insufficiency	0 (8.3)
Chronic obstructive lung disease	1 (8.3)
Chronic respiratory insufficiency	1 (8.3)
Chronic renal insufficiency	1 (8.3)
Haematological malignancy	4 (33.3)
Immunological deficiency	2 (16.7)
Diabetes mellitus	3 (25)
Days already admitted to ICU	4 (2–11)
APACHE II score	23.5 (20–30)
APACHE IV score	86 (73–103)
SOFA score	8 (4–11)
Baseline Hb in g/dL	7.7 (7.3–7.9)
Baseline Ht in L/L	0.237 (0.220–0.246)
Number of patients using vasopressors	9 (50)
Number of patients with a central venous catheter in place	16 (88.9)
Number of red cell transfusions	1.0 (1–1)
Number of patients receiving RBC before inclusion	16 (88.9)

Continued

Table 1 Continued

Characteristic	Values are median (IQR) or number (%)
Number on mechanical ventilation during study	5 (27.8)
Baseline fractional inspired oxygen in %	
Mechanically ventilated patients	45 (40–50)
Non-mechanically ventilated patient	28 (21–42)
Mortality in ICU during follow-up	5 (27.8%)

APACHE, Acute Physiology and Chronic Health Evaluation; BMI, body mass index; Hb, haemoglobin; Ht, haematocrit; ICU, intensive care unit; RBC, red blood cell; SOFA, Sequential Organ Failure Assessment.

and a SD of 13 mm Hg, we need 150 subjects to show a change in mitoPO₂ of at least 13 mm Hg after RBC transfusion, taking into account an expected 10% attrition rate.

Statistical analysis of the main study

Descriptive statistics will be used to describe the characteristics of the study population. The following baseline characteristics of the study population will be shown: age in years, sex, body mass index, reason of admission according to APACHE IV criteria,²⁶ chronic comorbidities according to APACHE IV criteria,²⁶ days of admission to the ICU before inclusion, APACHE II score, APACHE IV score, baseline SOFA score, baseline Hb, baseline haematocrit and the number of red cell transfusions given.

Linear mixed-effect models will be used to examine the effect of RBC transfusion on mitoPO₂ and on other traditional parameters of oxygenation, oxygen delivery and oxygen balance.³¹ The following parameters will be added to the linear mixed-model: age (continuous), sex (dichotomous), vasopressor use (dichotomous), inotropic therapy (dichotomous), skin temperature (continuous), body temperature (continuous), fluid balance (continuous), mechanical ventilation (dichotomous), Hb level

Table 2 Overview of signal quality of the mitoPO₂ measurements

Measurement moment	Median in %	IQR
Before RBC transfusion	35.25	33.00–48.00
At the end of RBC transfusion	35.89	32.38–42.08
15 min after RBC transfusion	37.70	32.75–46.57
30 min after RBC transfusion	41.00	37.44–67.67
60 min after RBC transfusion	37.08	35.50–38.50
120 min after RBC transfusion	37.92	32.58–48.93
180 min after RBC transfusion	41.80	35.85–63.36
24 hours after RBC transfusion	46.00	34.00–64.50

mitoPO₂, mitochondrial oxygen tension; RBC, red blood cell.

Table 3 The within-subject variability of mitochondrial oxygen tension per measurement moment

Measurement moment	Mean mitoPO ₂ (mm Hg)	Mean SD of mitoPO ₂ (mm Hg)
Before RBC transfusion	70.5	3.0
At the end of RBC transfusion	68.1	3.4
15 min after RBC transfusion	69.5	3.5
30 min after RBC transfusion	68.4	5.8
60 min after RBC transfusion	68.1	4.6
120 min after RBC transfusion	70.0	3.6
180 min after RBC transfusion	67.5	7.3
24 hours after RBC transfusion	90.0	8.7

The within-subject variability is stable until 2 hours after RBC transfusion, after which the within-subject variability increases above the pragmatic margin of 5 mm Hg. mitoPO₂, mitochondrial oxygen tension; RBC, red blood cell.

(continuous), peripheral oxygen saturation (continuous), cardiac index (continuous) and partial arterial oxygen pressure (continuous). Skin temperature at the measurement site is measured by the COMET device and taken into account in the analysis, since it is a known factor influencing PpIX formation.²⁰ Oedema at measurement site is taken into account in the analysis by a pragmatic proxy measurement: the fluid balance. The choice of the above factors was determined by the fact that the mitoPO₂ reflects a balance between oxygen delivery and oxygen consumption.⁵ Oxygen delivery to the tissue can be impaired by anaemia, arterial oxygen desaturation, low cardiac output and, to a lesser degree, by low partial pressure of oxygen.³² The dependence of mitoPO₂ values on the fractional inspired oxygen concentration has been demonstrated in animal studies.^{7 24} Furthermore, oxygen delivery and extraction can be influenced by vasopressor and/or inotropic therapy due to vasoconstriction or vasodilatation of the microcirculation.³³ Oxygen consumption is influenced by the level of metabolic rate, which is influenced for example by hyperthermia, an inflammatory state, stress, pain and mechanical ventilation.³² The analysis will be performed in the whole population and in subgroups of Hb level before transfusion (Hb level ≤7 g/dL or Hb level >7 g/dL, as well as in subgroups according to disease severity and/or presence of shock).

ETHICS AND DISSEMINATION

Institutional review board approval

The institutional ethics committee of each participating centre approved the study (reference P16.303), which is conducted according to the 1964 Helsinki declaration and its later amendments. Amendments are changes made to the study after a favourable opinion by the accredited institutional ethics committee has been given. All amendments first have to be approved by the institutional ethics

committee of Leiden University Medical Center before any changes can be made and communicated to the other participating centres. The latest amendment is study protocol version 5.0 of 27 February 2019, which has been approved by the institutional ethics committee. This research is financially supported by Grant PPOC-16-31 by Sanquin Research, Amsterdam, the Netherlands.

Data handling and dissemination plan

All patients are assigned a random patient identification code after inclusion, which is generated by the electronic case report form Castor Electronic Data Capture. The code book is stored digitally, with restricted access, and encrypted with a password. All handling of personal data complies with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation. Extracted study data will be coded and entered in the electronic case report form Castor and stored for further publication. To ensure data quality, a data dictionary was made before start of the study and implemented digitally in the electronic case report, including range checks. Only the coordinating investigator, the principal investigators, the study monitor and Health and Youth Care Inspectorate will have access to the final dataset. Multiple times a year, the study will be monitored, and source data verification will be applied as depicted in the monitor plan in the study protocol, via the independent monitor pool of Leiden University Medical Center. No data safety monitoring board was set up, as it was deemed unnecessary by an independent expert. The study protocol was published before start of the study on clinicaltrials.gov. The results of the study will be published in (inter-)national scientific journals and guidelines. The International Committee of Medical Journal Editors authorship criteria will be used to define authors in these publications.

Current status of the main study

One of the main results of the pilot study was that measurement of mitoPO₂ levels using the PpIX-TSLT in critically ill patients receiving RBC transfusions was feasible, since the signal quality was above 25% during all timepoints, and the within-subject variability was acceptable in the first 2 hours after RBC transfusion. However, the results of the pilot study led to three changes in the main study design.

First, having a central venous catheter in place was dismissed as an inclusion criterion, due to the slow inclusion rate and no influence on the primary outcome.

At the end of the pilot study, we noticed that multiple patients were not included in the study, due to not having a written informed consent in time (42 out of 114 transfused patients). A second important result of the pilot study was therefore a change in the consent procedure. Previous research had already shown that approximately 10% of critically ill patients can make an autonomous decision regarding research participation, thus most decisions are made by their substitute decision makers

who are already overwhelmed by the medical information concerning their relative and/or loved one. Furthermore, studies have also shown that obtaining contact with the substitute decision makers can be difficult, especially in narrow time windows for inclusion.^{34 35} The same was seen in our study, and this resulted in a low accrual rate and was possibly influencing the generalisability of our study. We noticed that nearly all substitute decision makers decided positively on participation the second day of admittance of their loved one, once the first shock had decreased. We therefore introduced deferred consent in our study. In principal, we still ask all ICU patients, or their substitute, admitted to the ICU and expected to stay at the ICU for longer than 24 hours for participation in our study. However, if no substitute can be reached in person for written informed consent, while the patient meets the eligibility criteria, the patient is included in the study and deferred informed consent is sought within 48 hours after inclusion.

The third important result of the pilot study was the change in the amount of timepoints. Due to logistic problems during the pilot study, the number of timepoints was minimised to 6: before transfusion, at the end of transfusion, 30, 60 and 180 min after transfusion and 24 hours after transfusion. All of these changes were laid down in amendments to the study protocol and reviewed by the institutional review board, which approved these changes. The main study has started in March 2018 and is ongoing. End of recruitment is expected halfway 2020.

Author affiliations

¹Department of Intensive Care, LUMC, Leiden, The Netherlands

²Clinical Transfusion Research, Sanquin Research Clinical Transfusion Research, Leiden, Zuid-Holland, The Netherlands

³Department of Clinical Epidemiology, LUMC, Leiden, Zuid-Holland, The Netherlands

⁴Department of Anesthesiology, Laboratory of Experimental Anesthesiology, Erasmus Medical Center, Rotterdam, Zuid-Holland, The Netherlands

⁵Department of Intensive Care, Amsterdam UMC – Location AMC, Amsterdam, North Holland, The Netherlands

Acknowledgements The authors would like to thank Camila Caram and Yavanna van Oostveen with the data management of this study. Furthermore, we would like to thank the intensive care unit department of Leiden University Medical Center, Amsterdam University Medical Center and Erasmus Medical Center for their help before and during the study.

Contributors All authors were involved in the design of the study. MB and MSA collected data in Leiden University Medical Center, EGM in Erasmus Medical Center and NPJ in Amsterdam Medical Center. MB performed the analysis under supervision of MSA and JGvDB. MB drafted the manuscript. All authors reviewed and edited the manuscript. All authors approved the final manuscript.

Funding This research is financially supported by Grant PPOC-16-31 by Sanquin Research, Amsterdam, the Netherlands.

Competing interests EGM is founder and shareholder of Photonics Healthcare B.V. (Utrecht, The Netherlands). Photonics Healthcare develops and commercialises the COMET measuring system for mitochondrial oxygen measurements. The other authors have no conflicts of interest to declare.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval The institutional ethics committee approved the study (reference P16.303), which was conducted according to the 1964 Helsinki declaration and its later amendments. The study was registered in ClinicalTrials.gov.

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iD

Meryem Baysan <http://orcid.org/0000-0002-8642-4802>

REFERENCES

- Bronicki RA, Monitoring H. Hemodynamic monitoring. *Pediatr Crit Care Med* 2016;17:S207–14.
- Biedrzycka A, Lango R. Tissue oximetry in anaesthesia and intensive care. *Anaesthesiol Intensive Ther* 2016;48:41–8.
- Nielsen ND, Martin-Loeches I, Wentowski C. The effects of red blood cell transfusion on tissue oxygenation and the microcirculation in the intensive care unit: a systematic review. *Transfus Med Rev* 2017;31:205–22.
- Sadaka F, Aggu-Sher R, Krause K, et al. The effect of red blood cell transfusion on tissue oxygenation and microcirculation in severe septic patients. *Ann Intensive Care* 2011;1:46.
- Mik EG. Special article: measuring mitochondrial oxygen tension: from basic principles to application in humans. *Anesth Analg* 2013;117:834–46.
- Harms FA, Voorbeijtel WJ, Bodmer SIA, et al. Cutaneous respirometry by dynamic measurement of mitochondrial oxygen tension for monitoring mitochondrial function in vivo. *Mitochondrion* 2013;13:507–14.
- Harms FA, Bodmer SIA, Raat NJH, et al. Non-Invasive monitoring of mitochondrial oxygenation and respiration in critical illness using a novel technique. *Crit Care* 2015;19:343.
- Mik EG, Johannes T, Zuurbier CJ, et al. In vivo mitochondrial oxygen tension measured by a delayed fluorescence lifetime technique. *Biophys J* 2008;95:3977–90.
- Mik EG, Ince C, Eerbeek O, et al. Mitochondrial oxygen tension within the heart. *J Mol Cell Cardiol* 2009;46:943–51.
- Harms FA, Stolker RJ, Mik EG. Cutaneous respirometry as novel technique to monitor mitochondrial function: a feasibility study in healthy volunteers. *PLoS One* 2016;11:e0159544.
- Baumbach P, Neu C, Derlien S, et al. A pilot study of exercise-induced changes in mitochondrial oxygen metabolism measured by a cellular oxygen metabolism monitor (PICOMET). *Biochim Biophys Acta Mol Basis Dis* 1865;2019:749–58.
- van Dijk LJD, Ubbink R, Terlouw LG, et al. Oxygen-Dependent delayed fluorescence of protoporphyrin IX measured in the stomach and duodenum during upper gastrointestinal endoscopy. *J Biophotonics* 2019;12:e201900025.
- Ubbink R, Bettink MAW, Janse R, et al. A monitor for cellular oxygen metabolism (comet): monitoring tissue oxygenation at the mitochondrial level. *J Clin Monit Comput* 2017;31:1143–50.
- Harms FA, Bodmer SIA, Raat NJH, et al. Validation of the protoporphyrin IX-triplet state lifetime technique for mitochondrial oxygen measurements in the skin. *Opt Lett* 2012;37:2625–7.
- Smit Sibinga CT, Das PC, Fratantoni JD. *Alternative approaches to human blood resources in clinical practice*. Dordrecht: Kluwer Academic Publishers, 1998: 9–15.
- Centraal BegeleidingsOrgaan(CBO). Richtlijn Bloedtransfusie. Utrecht: CBO, 2011. Available: <http://nvb-trip-symposium.nl/wp-content/uploads/2017/08/Richtlijnbloedtransfusie2011.pdf> [Accessed 04 Dec 2019].
- Hébert PC, Wells G, Blajchman MA, et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. transfusion requirements in critical care Investigators, Canadian critical care Trials Group. *N Engl J Med* 1999;340:409–17.
- Lo LW, Koch CJ, Wilson DF. Calibration of oxygen-dependent quenching of the phosphorescence of Pd-meso-tetra (4-carboxyphenyl) porphine: a phosphor with general application for measuring oxygen concentration in biological systems. *Anal Biochem* 1996;236:153–60.
- Harms FA, de Boon WMI, Balestra GM, et al. Oxygen-Dependent delayed fluorescence measured in skin after topical application of 5-aminolevulinic acid. *J Biophotonics* 2011;4:731–9.
- Wachowska M, Muchowicz A, Firczuk M, et al. Aminolevulinic acid (Ala) as a prodrug in photodynamic therapy of cancer. *Molecules* 2011;16:4140–64.
- Kelty CJ, Brown NJ, Reed MWR, et al. The use of 5-aminolevulinic acid as a photosensitizer in photodynamic therapy and photodiagnosis. *Photochem Photobiol Sci* 2002;1:158–68.
- de Bruijn HS, Kruijt B, van der Ploeg-van den Heuvel A, et al. Increase in protoporphyrin IX after 5-aminolevulinic acid based photodynamic therapy is due to local re-synthesis. *Photochem Photobiol Sci* 2007;6:857–64.
- Mik EG, Stap J, Sinaasappel M, et al. Mitochondrial PO2 measured by delayed fluorescence of endogenous protoporphyrin IX. *Nat Methods* 2006;3:939–45.
- Bodmer SIA, Balestra GM, Harms FA, et al. Microvascular and mitochondrial PO(2) simultaneously measured by oxygen-dependent delayed luminescence. *J Biophotonics* 2012;5:140–51.
- Knaus WA, Draper EA, Wagner DP, et al. Apache II: a severity of disease classification system. *Crit Care Med* 1985;13:818–29.
- Zimmerman JE, Kramer AA, McNair DS, et al. Acute physiology and chronic health evaluation (APACHE) IV: hospital mortality assessment for today's critically ill patients. *Crit Care Med* 2006;34:1297–310.
- Brinkman S, Bakhshi-Raiez F, Abu-Hanna A, et al. External validation of acute physiology and chronic health evaluation IV in Dutch intensive care units and comparison with acute physiology and chronic health evaluation II and simplified acute physiology score II. *J Crit Care* 2011;26:105.e11–105.e18.
- Thiele RH, Bartels K, Gan TJ. Cardiac output monitoring: a contemporary assessment and review. *Crit Care Med* 2015;43:177–85.
- Vincent JL, de Mendonça A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Crit Care Med* 1998;26:1793–800.
- Dong Y, Peng C-YJ. Principled missing data methods for researchers. *Springerplus* 2013;2:222–38.
- Cheng J, Edwards LJ, Maldonado-Molina MM, et al. Real longitudinal data analysis for real people: building a good enough mixed model. *Stat Med* 2010;29:504–20.
- Leach RM, Treacher DF. The pulmonary physician in critical care * 2: oxygen delivery and consumption in the critically ill. *Thorax* 2002;57:170–7.
- Martin C, Papazian L, Perrin G, et al. Norepinephrine or dopamine for the treatment of hyperdynamic septic shock? *Chest* 1993;103:1826–31.
- Burns KEA, Zubrinich C, Tan W, et al. Research recruitment practices and critically ill patients. A multicenter, cross-sectional study (the consent study). *Am J Respir Crit Care Med* 2013;187:1212–8.
- Coppolino M, Ackerson L. Do surrogate decision makers provide accurate consent for intensive care research? *Chest* 2001;119:603–12.