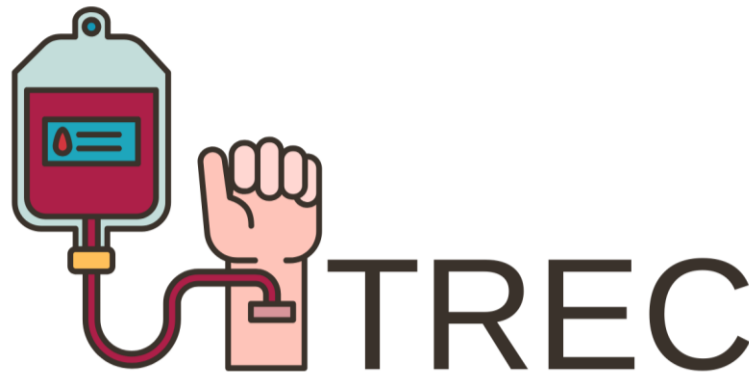


Restrictive versus Liberal Thresholds for Red Blood Cell Transfusion in ExtraCorporeal Membrane Oxygenation – the TREC study



RESEARCH PROTOCOL

Amsterdam University Medical Centers

Amsterdam, the Netherlands

Department of Intensive Care

Prof. Dr. A.P.J. Vlaar

Dr. H.J.S. de Grooth

Dr. M.C.A. Müller

Dr. A.E. Engstrom

P. Wynandts

C.M. Schaap

Dr. S.J. Raasveld

S.F. van Wonderen

Correspondence

Prof. Dr. Alexander P.J. Vlaar

Department of Intensive Care

Amsterdam UMC, location AMC

E: a.p.vlaar@amsterdamumc.nl

Tel: 25846

PROTOCOL TITLE *'Restrictive versus Liberal Thresholds for Red Blood Cell Transfusion in Extracorporeal Membrane Oxygenation'*

Protocol ID	TREC_V9_APR26
Short title	Red blood cell transfusion in ECMO
EudraCT number	<i>Not applicable</i>
ABR number	84295.018.23
Version	9
Date	16-04-2026
Coordinating investigator/project leader	S.F. van Wonderen MD, PhD C.M. Schaap MD, PhD student T.A. van der Meer, MD, PhD student S.J. Raasveld MD, PhD M.C.A. Müller MD, PhD Amsterdam University Medical Centers, location Academic Medical Center (AMC) Department of Adult Intensive Care T: 020-5666342 E: s.vanwonderen@amsterdamumc.nl E: c.m.schaap@amsterdamumc.nl E: t.a.vandermeer@amsterdamumc.nl E: s.j.raasveld@amsterdamumc.nl E: m.c.muller@amsterdamumc.nl
Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder)	Prof. dr. A.P.J. Vlaar Internist-intensivist Amsterdam University Medical Centers, location Academic Medical Center (AMC) Meibergdreef 9, 1105 AZ Amsterdam ,the Netherlands Department of Adult Intensive Care E: a.p.vlaar@amsterdamumc.nl

Amsterdam University Medical centers, Amsterdam, the Netherlands	Dr. A.H.G. Driessen Cardiothoracic surgeon Amsterdam University Medical Centers, location Academic Medical Center (AMC) Meibergdreef 9, 1105 AZ Amsterdam ,the Netherlands Department of Cardiothoracic Surgery E: a.h.driessen@amsterdamumc.nl
Amsterdam University Medical centers, Amsterdam, the Netherlands	Prof. dr. J.P. Simao Henriques Cardiologist Amsterdam University Medical Centers, location Academic Medical Center (AMC) Meibergdreef 9, 1105 AZ Amsterdam ,the Netherlands Department of Cardiology E: a.h.driessen@amsterdamumc.nl
Amsterdam University Medical centers, Amsterdam, the Netherlands	Dr. S. Eberl Cardiothoracic anaesthetist Amsterdam University Medical Centers, location Academic Medical Center (AMC) Meibergdreef 9, 1105 AZ Amsterdam ,the Netherlands Department of Anesthesiology E: s.eberl@amsterdamumc.nl
University Medical Centers Groningen (UMCG), Groningen, the Netherlands	Dr. W.M. van den Bergh Neurologist-intensivist University Medical Center Groningen Hanzeplein 1, 9713 GZ Groningen, the Netherlands Department of Critical Care E: w.m.van.den.bergh@umcg.nl
Erasmus Medical Center, Rotterdam, the Netherlands	Dr. C.L. Meuwese Cardiologist-intensivist Erasmus Medical Center Doctor Molewaterplein 40, 3015 GD Rotterdam, the Netherlands

	Department of Critical Care E: c.meuwese@erasmusmc.nl
Maastricht Universitair Medisch Centrum Plus (MUMC+), Maastricht, the Netherlands	Dr. M.C.G. van de Poll Surgeon-intensivist Maastricht Universitair Medisch Centrum Plus P. Debyelaan 25, 6229 HX Maastricht, the Netherlands Department of Critical Care E: marcel.vande.poll@mumc.nl
Leids Universitair Medisch Centrum (LUMC), Leiden, the Netherlands	Drs. C.V. Elzo Kraemer Internist-intensivist Albinusdreef 2, 2333 ZA Leiden, the Netherlands Department of Critical Care E: c.v.elzo_kraemer@lumc.nl
Universitair Medisch Centrum Utrecht (UMCU), Utrecht, the Netherlands	Dr. H.J.S. De Grooth Anesthesiologist-intensivist Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands Department of Critical Care E: h.j.s.degrooth@umcutrecht.nl
St. Antonius Ziekenhuis, Nieuwegein, the Netherlands	Drs. E. Scholten Anesthesiologist-intensivist Koekoekslaan 1, 3435 CM Nieuwegein, the Netherlands Department of Critical Care E: e.scholten@antoniuziekenhuis.nl
Medisch Spectrum Twente (MST), Enschede, the Netherlands	Dr. J.W. Vermeijden Anesthesiologist-intensivist Koningstraat 1, 7512 KZ Enschede, the Netherlands Department of Critical Care E: J.Vermeijden@mst.nl
Radboudumc, Nijmegen, the Netherlands	Dr. T. Frenzel Anesthesiologist-intensivist

	Geert Grooteplein Zuid 10, 6525 GA Nijmegen, the Netherlands Department of Critical Care E: Tim.Frenzel@radboudumc.nl
Hôpital Erasme, Bruxelles, Belgium	Dr. F.S. Taccone Internist – Intensivist Hôpital Erasme, Brussels Lenniksebaan 808, 1070 Bruxelles, Belgium Department of Critical Care: E: fabio.taccone@ulb.be
University Hospital Leuven, Leuven Medical ICU, Belgium	Prof. Dr. G. Hermans Internist – Intensivist University Hospitals Leuven Herestraat 49, 3000 Leuven, Belgium Department of Medical Intensive Care E: greet.hermans@uzleuven.be
University Hospital Leuven, Leuven Surgical ICU, Belgium	Prof. dr. D. Dauwe Cardiologist – Intensivist University Hospitals Leuven Herestraat 49, 3000 Leuven, Belgium Department of Surgical Intensive Care E: dieter.dauwe@uzleuven.be
C.H.U. de Charleroi, Bruxelles, Belgium	Dr. M. Piagnerelli Intensivist C.H.U. de Charleroi, Bruxelles, Université Libre de Bruxelles Chau. de Bruxelles 140, 6042 Charleroi, Belgium Department of Intensive Care E: michael.piagnerelli@chu-charleroi.be
Karolinska Institutet, Karolinska, Sweden	Dr. L.M. Broman Intensivist Karolinska Universitij Hospital, Stockholm, Sweden Karolinska Universitetssjukhuset Eugeniavägen 3, Solna, Hälsovägen 13, 171 76 Stockholm, Zweden

	Perioperative Medicine and Intensive Care E: lars.broman@regionstockholm.se
Kepler Universitäts Klinikum, Linz, Austria	Prof. dr. J. Meier Intensivist Kepler Universitätsklinikum GmbH, Med Campus II, Krankenhausstraße 7a, 4020 Linz, Austria Department of Intensive Care E: jens.meier@kepleruniklinikum.at
University Hospital Vienna, Vienna, Austria	Dr. N. Buchtele Internist-Intensivist Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria Department of Medicine I E: nina.buchtele@meduniwien.ac.at
Sponsor (in Dutch: verrichter/opdrachtgever)	Amsterdam University Medical Centers, location Academic Medical Center (AMC) Meibergdreef 9, 1105 AZ, Amsterdam, the Netherlands. 020-5669111
Subsidising party	ZonMw reference number 10390032310031
Independent expert(s)	Prof. dr. J. Horn Department of Intensive Care Amsterdam University Medical Center Location Amsterdam Medical Center Meibergdreef 9, 1105 AZ Amsterdam The Netherlands
Laboratory sites	<i>Not applicable</i>
Pharmacy	<i>Not applicable</i>

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
<u>Head of department:</u> Prof. dr. A.P.J. Vlaar Department of Intensive Care Amsterdam University Medical Center, location Amsterdam Medical Center Meibergdreef 9, C3-425 1105 AZ Amsterdam, Nederland Telephone: +31-20-5662808 E-mail: a.p.vlaar@amsterdamumc.nl		
<u>Principal investigator</u> Prof. dr. A.P.J. Vlaar		
<u>Site investigator</u>		

TABLE OF CONTENTS

1. INTRODUCTION AND RATIONALE	15
2. OBJECTIVES	17
3. STUDY DESIGN	18
3.1 Study design	18
3.2 Regimens	19
3.3 Stratification	19
3.4 Allocation assignment and duration	19
3.5 Compliance	19
3.6 Flowchart	19
4. STUDY POPULATION	21
4.1 Population (base)	21
4.2 Inclusion criteria	21
4.3 Exclusion criteria	21
4.4 Sample size calculation	21
5. TREATMENT OF SUBJECTS	23
5.1 Investigational product/treatment	23
5.1.1 Duration of intervention	23
5.1.2 Measurement of hemoglobin	23
5.1.3 Threshold events & transfusion strategy adherence	24
5.1.4 Temporary protocol suspensions	24
5.1.5 Other products used in bleeding problems	25
5.2 Use of co-intervention (if applicable)	25
5.3 Escape medication (if applicable)	25
6. INVESTIGATIONAL PRODUCT	26
6.1 Name and description of investigational product(s)	26
6.2 Summary of findings from non-clinical studies	26
6.3 Summary of findings from clinical studies	26
6.4 Summary of known and potential risks and benefits	26
6.5 Description and justification of route of administration and dosage	26
6.6 Dosages, dosage modifications and method of administration	26
6.7 Preparation and labelling of Investigational Medicinal Product	26
6.8 Drug accountability	26
7. NON-INVESTIGATIONAL PRODUCT	27
7.1 Name and description of non-investigational product(s)	27
7.2 Summary of findings from non-clinical studies	27
7.3 Summary of findings from clinical studies	27
7.4 Summary of known and potential risks and benefits	27
7.5 Description and justification of route of administration and dosage	27
7.6 Dosages, dosage modifications and method of administration	27
7.7 Preparation and labelling of Non Investigational Medicinal Product	28
7.8 Drug accountability	28
8. METHODS	29

8.1	Study parameters/endpoints	29
8.1.1	Main study parameter/endpoint	29
8.1.2	Secondary study parameters/endpoints (if applicable)	29
8.1.3	Other study parameters (if applicable)	31
8.2	Randomization, blinding and treatment allocation	32
8.3	Study procedures	32
8.3.1	Extracorporeal membrane oxygenation [standard medical treatment]	32
8.3.2	Blood sampling [standard measurements]	34
8.3.3	Follow-up of subjects	35
8.4	Withdrawal of individual subjects	35
8.5	Replacement of individual subjects after withdrawal	35
8.6	Follow-up of subjects withdrawn from treatment	36
8.7	Premature termination of the study	36
9.	SAFETY REPORTING	37
9.1	Temporary halt for reasons of subject safety	37
9.2	AEs, SAEs and SUSARs	37
9.2.1	Adverse events (AEs)	37
9.2.2	Special considerations for assessment of AE and non-reportable findings in the ICU setting	37
9.2.3	Serious adverse events (SAEs)	37
9.2.4	Suspected unexpected serious adverse reactions (SUSARs)	39
9.2.5	Procedures for recording, reporting, causality and follow-up	39
9.3	Data Safety Monitoring Board (DSMB)	40
10.	STATISTICAL ANALYSIS	41
10.1	Primary study parameter(s)	41
10.2	Secondary study parameter(s)	42
10.3	Other study parameters	42
10.4	Interim analysis	42
11.	ETHICAL CONSIDERATIONS	43
11.1	Regulation statement	43
11.2	Recruitment and consent	43
11.2.1	Recruitment	43
11.2.2	Consent procedure	44
11.3	Objection by minors or incapacitated subjects (if applicable)	46
11.4	Benefits and risks assessment, group relatedness	46
11.5	Compensation for injury	47
11.6	Incentives (if applicable)	47
12.	ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION	48
12.1	Handling and storage of data and documents	48
12.2	Monitoring and Quality Assurance	48
12.3	Amendments	48
12.4	Annual progress report	49
12.5	Temporary halt and (prematurely) end of study report	49

12.6	Public disclosure and publication policy	49
13.	STRUCTURED RISK ANALYSIS	50
13.1	Potential issues of concern	50
13.2	Synthesis	51
14.	REFERENCES	52

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)
AE	Adverse Event
AKI	Acute Kidney Injury
aPTT	Activated Partial Thromboplastin Time
AR	Adverse Reaction
ARDS	Acute Respiratory Distress Syndrome
ABG	Arterial Blood Gas
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CE	Conformité Européenne
CV	Curriculum Vitae
DO₂	Oxygen delivery
DSMB	Data Safety Monitoring Board
ECMO	Extracorporeal Membrane Oxygenation
ECPR	Extracorporeal Cardiopulmonary Resuscitation
eCRF	Electronic Case Report Form
ESICM	European Society of Intensive Care Medicine
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
Hb	Hemoglobin
IB	Investigator's Brochure
IC	Informed Consent
ICU	Intensive Care Unit
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
OR	Operating Room

P/F	Arterial oxygen pressure/ inspired oxygen fraction ratio (PaO₂/FiO₂)
PCs	Platelet Concentrates
RBC	Red Blood Cells
RRT	Renal Replacement Therapy
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
TACO	Transfusion Associated Circulatory Overload
TRALI	Transfusion-related Acute Lung Injury
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
VV	Veno-venous
VA	Veno-arterial
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: In patients supported with extracorporeal membrane oxygenation (ECMO), transfusion of red blood cells (RBC) is very common. This is possibly due to the application of liberal thresholds and the lack of evidence-based guidelines. Although RBC transfusion can be lifesaving, it is also a risk-bearing intervention with substantial risk for morbidity and mortality in this critically ill population. Also, with increasing scarcity, RBC transfusions are becoming more expensive. Furthermore, in the past decades it has been shown in several critically ill patient populations – not on ECMO – that maintaining a restrictive hemoglobin (Hb) threshold for RBC transfusion is non-inferior, including in cardiothoracic surgery, acute myocardial infarction and septic shock. Therefore, we hypothesize that a restrictive transfusion threshold for RBC is safe to apply in patients on ECMO in comparison with a liberal transfusion threshold.

Objective: The primary objective of this trial is to study in a prospective randomized comparison whether a restrictive RBC transfusions strategy is non-inferior compared to a liberal strategy in patients on ECMO with respect to 90-day mortality.

Study design: Prospective multi-center randomized controlled non-inferiority trial.

Study population: Patients, 18 years or older, receiving ECMO.

Intervention (if applicable): Restrictive RBC transfusion threshold: in case the Hb transfusion trigger of 7.0 g/dL (4.3 mmol/L) is reached, 1 RBC unit at a time will be transfused. The aimed Hb target range of the restrictive/intervention group will be 7.1 – 9.0 g/dL (4.3 – 5.6 mmol/L). Liberal RBC transfusion threshold: in case the Hb transfusion trigger of 9.0 g/dL (5.6 mmol/L) is reached, 1 RBC unit at a time will be transfused. Target range of the liberal group is defined as Hb 9.1 – 11.0 g/dL

Main study parameters/endpoints: The primary outcome parameter is 90-day all-cause mortality. Secondary outcomes include: 1) proportion of patients on ECMO exposed to allogeneic RBC transfusion; 2) RBC volume infused per patient during ECMO; 3) reasons for RBC transfusion other than Hb triggers; 4) transfusion reactions; 5) time on ECMO; 6) length of hospital- and ICU-stay; 7) in-ICU morbidity; 8) quality of life (QoL) up to 12 months; 9) costs related to a) transfusion, b) transfusion-related sequelae, c) medical consumption (up to 12 months) and d) productivity loss (up to 12 months).

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Anemia as well as RBC transfusion have been associated with increased morbidity and mortality in critically ill patients. In addition, several risk factors for transfusion-related sequelae are present in patients on ECMO, a vulnerable patient population in which morbidity and mortality rate is already high. Nevertheless, transfusion in this patient population is not uncommon, possibly adding to this high rate of morbidity. Therefore, it is utterly important

to only transfuse when necessary in this vulnerable patient group. Participation in this trial will result in minimal burden. Blood samples needed for the Hb values can be measured in the regular blood samples following standard hospital protocol in the monitoring of patients on ECMO. The study's intervention will only be performed during ICU admission. As the quality of life assessment can be done by email or phone, no extra visits are needed, which offers an accessible option for the participants. Since as well transfusion and anemia are known to have high incidence in this patient population, no extra risks will be added from either one of them.

1. INTRODUCTION AND RATIONALE

Extracorporeal membrane oxygenation (ECMO) is used as a supportive method in case of temporary and potentially reversible cardiac or respiratory failure, refractory to conventional therapies (1). Over the past decades, application of ECMO has been increasing worldwide (2). As ECMO is generally used as a 'last resort' therapy, the population is vulnerable, and many complications can occur. Anemia occurs in >90% of the patients on ECMO, caused by many different patient-related, disease-related, and ECMO-related factors (3). Nevertheless, rationale for the recommended hemoglobin (Hb) thresholds for red blood cell (RBC) transfusion in this patient population is limited. This was recently confirmed by the members of the European Society of Intensive Care Medicine (ESICM), who concluded in their clinical practice guideline that no recommendation on transfusion thresholds can be made, since solid evidence is missing (4). The panel stated that this area is a research priority.

This lack of evidence-based guidelines may explain the high variance in Hb thresholds applied, as well as the thresholds in use being relatively liberal (5,6). As a result, transfusion of RBC is very common. Observational studies describe that almost 9 out of 10 patients receiving ECMO receive at least one RBC transfusion, and the total amount is very high (7,8). These numbers are even more remarkable when comparing to other patient populations in the Intensive Care Unit (ICU), in which 1 out of 4 patients receives RBC with way lesser amounts (9). One of the main arguments for using a liberal transfusion threshold in ECMO is the hypothesis that in patients receiving ECMO, tissue hypoxemia can develop due to decreased pulmonary oxygen intake (e.g. in pneumonia as indication for veno-venous [VV] ECMO), or decreased cardiac output (e.g. in myocardial infarction as indication for veno-arterial [VA] ECMO). By providing a larger Hb buffer, it is assumed that the oxygen delivery (DO_2) will be preserved and the incidence of tissue hypoxemia will be reduced (10). However, evidence to either confirm or refute this hypothesis is lacking. Since ECMO ensures oxygenation and can provide a blood flow of up to 7 L/min, it can be assumed that ECMO fully compensates for the possible decrease in DO_2 .

Although RBC transfusion can be lifesaving, it is also a risk-bearing intervention with substantial risk for morbidity and mortality in this critically ill population (11). In similar patient populations without ECMO, maintaining a restrictive RBC transfusion strategy (Hb 7.0 g/dL) has been proven non-inferior to a more liberal practice (Hb 9.0 g/dL). This includes randomized controlled trials (RCTs) in septic shock patients (comparable to patients on VV ECMO), cardiothoracic surgery patients, and even patients suffering from acute myocardial infarction and anemia (comparable to patients on VA ECMO) (12–15). Although these conclusions are

promising, they cannot directly be translated to patients supported by ECMO, although underlying conditions are similar. Moreover, RBC transfusions are expensive and donors are becoming more scarce. In this vulnerable critically ill patient population with an enhanced risk for transfusion related complications, it is of utmost importance to only administer a RBC transfusion when the benefits outweigh the risks (16).

As both anemia and transfusion are associated with poor outcomes, observational studies cannot answer the question whether a restrictive Hb threshold is non-inferior to a liberal strategy (12,13,17). There is a need to define general thresholds to improve the efficiency of indications for RBC transfusion in ECMO. Since one of the most commonly used triggers for RBC transfusion is Hb concentration, this forms the basis for our study proposal to investigate whether it is non-inferior to maintain a restrictive transfusion threshold (intervention group: Hb 7 g/dL) compared to the current standard of 9 g/dL in patients on ECMO, independent of the mode.

Therefore, the research question is: is a restrictive threshold for RBC transfusion non-inferior to a liberal threshold in patients on ECMO regarding 90 day mortality?

2. OBJECTIVES

Primary Objective:

The primary objective of this trial is to study in a prospective randomized comparison whether a restrictive RBC transfusions strategy is non-inferior compared to a liberal strategy in patients on ECMO with respect to 90-day mortality.

Secondary Objective(s):

Transfusion practices:

- To evaluate the RBC transfusion practices (i.e. proportion of patients transfused, RBC volume infused per patient during ECMO and per transfusion event);
- To evaluate the indications for RBC transfusion, other than solely the Hb transfusion trigger;
- To evaluate differences in RBC transfusion practices between patients on VA ECMO and VV ECMO.

Patient outcomes:

- To evaluate the incidence of transfusion-related complications;
- To evaluate the duration of ECMO support;
- To evaluate duration of ICU and hospital stay;
- To evaluate complication rates and physical outcomes;
- Cost effectiveness including transfusion-related costs, productivity loss and medical consumption.

3. STUDY DESIGN

3.1 Study design

This is a non-inferiority, randomized controlled trial in patients receiving ECMO. Patients will be randomized directly after ECMO initiation in either the liberal (control group: Hb threshold ≥ 9 g/dL) or the restrictive (intervention group: Hb threshold ≥ 7 g/dL) transfusion regimen for RBC transfusion. The assigned transfusion regimen will continue until successful weaning (24 hours post-decannulation without indication for restart ECMO). After decannulation, the ICU's standard transfusion regimen will apply, which will mainly overlap with the restrictive arm. In case of initiation of a second ECMO run, patient will be re-allocated in their previous regimen. A global overview of the study is shown in Figure 1:

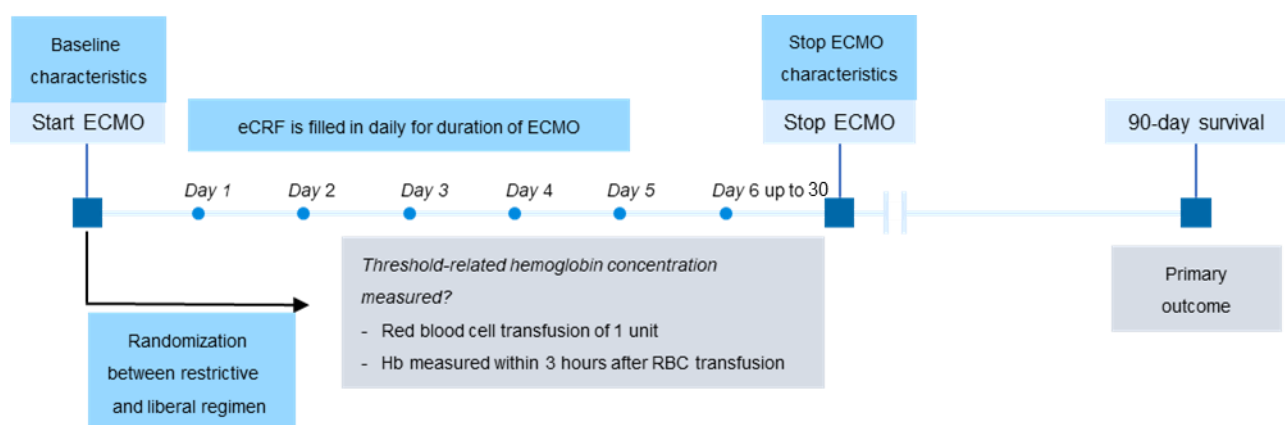


Figure 1. Overview of study measurements

The primary outcome, 90-day survival, will be evaluated at 90 days after ECMO initiation. After discharge, the patient, legal representative or patients' general practitioner will be contacted to evaluate survival status. For the secondary outcomes, patients will undergo daily measurements and data collection during ECMO. At 3, 6, 9 and 12-months, patients will be contacted by email or phone (after obtaining informed consent) and a questionnaire regarding health-related quality of life, medical consumption and productivity loss will be completed.

In our previous, observational study, approximately 90% of the patients receive RBC during ECMO (8). On the day that ECMO was initiated, 50% of the patients had a Hb equal to or lower than 9 g/dL. After ECMO initiation, the Hb level further decreased independently of the Hb threshold applied by the center. Therefore, it was decided to randomize patients to either the restrictive (7.0 g/dL) or liberal (9.0 g/dL) regimen directly after ECMO initiation.

3.2 Regimens

Patients allocated to the restrictive transfusion regimen group will receive one unit of RBC transfusion if their Hb is 7.0 g/dL or less during ECMO. Their target Hb range is defined as 7.1 – 9.0 g/dL. These thresholds are based on previous non-inferior trials in comparable patient populations in which VV ECMO (i.e. sepsis) and VA ECMO (i.e. cardiac surgery, acute myocardial infarction) are often applied (6–8).

Patients allocated to the liberal transfusion regimen will receive one unit of RBC transfusion if their Hb is 9.0 g/dL or less during ECMO. Their target Hb range is defined as 9.1 – 11.0 g/dL. These thresholds are based on what is currently used in ECMO.

3.3 Stratification

Randomization will be stratified by:

- Center;
- ECMO mode, divided by:
 - VV ECMO (or triple cannulation methods with primarily a pulmonary indication);
 - VA ECMO (or triple cannulation methods with primarily a cardiac indication or extracorporeal cardiopulmonary resuscitation [ECPR]).

3.4 Allocation assignment and duration

Assignment will take place in a 1:1 ratio, using a concealed centralized, Web-based system. Since this study regards a transfusion study, allocation cannot feasibly be blinded. The assigned transfusion regimen will continue until successful weaning (24 hours no ECMO post-cannulation). In case of initiation of a second ECMO run, patient will be re-allocated in their previous regimen. This does not apply to ICU-readmission without ECMO re-initiation.

3.5 Compliance

The Hb level is to be measured at least at the following intervals:

- i) daily from day 1 up to day 30, or until ECMO is successfully weaned, whatever comes first;
- ii) additionally, in case of transfusion of a RBC unit, within 3 hours after the transfusion was received.

3.6 Flowchart

An overview of the study flowchart is shown in Figure 2.

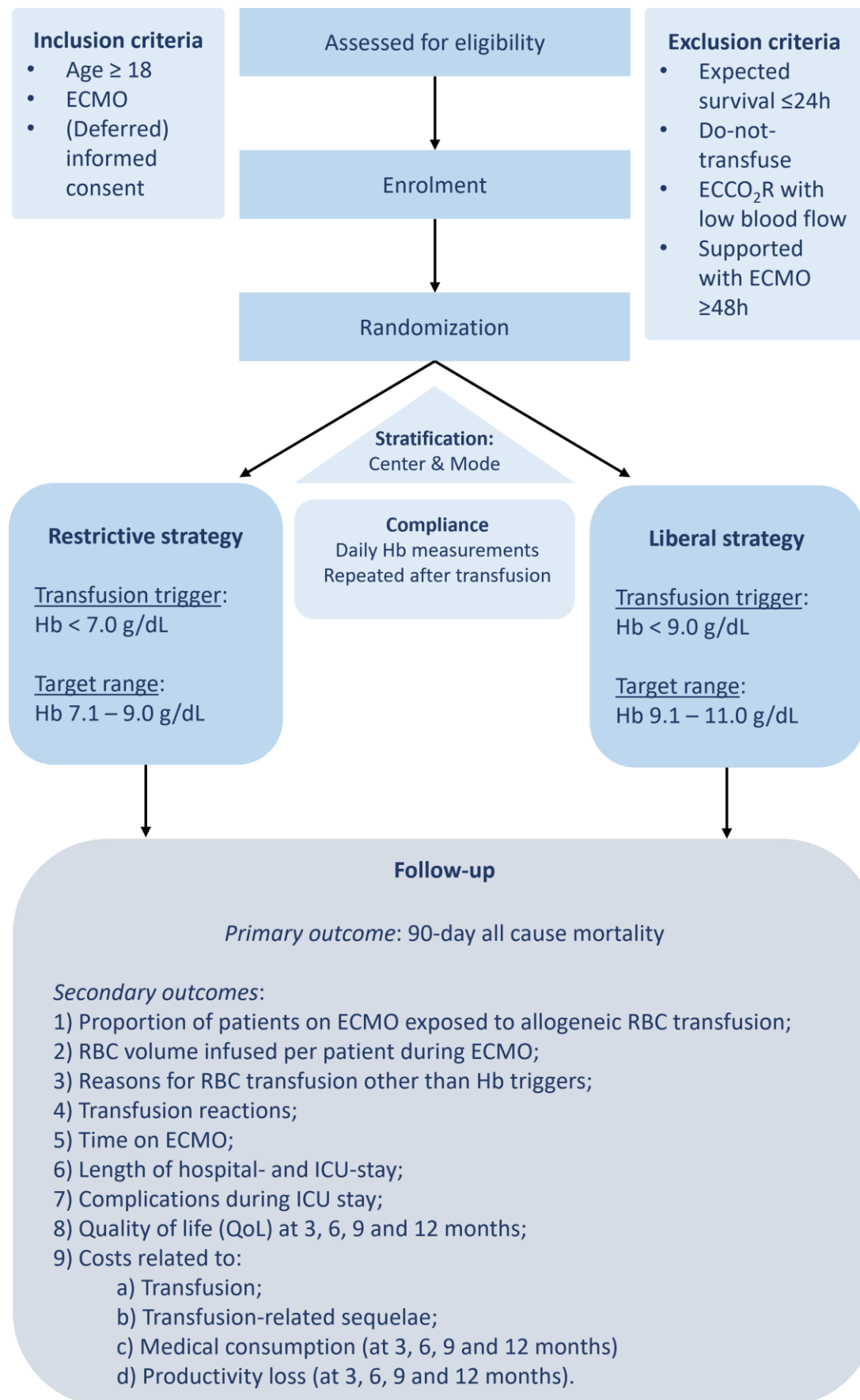


Figure 2. Study flowchart

4. STUDY POPULATION

4.1 Population (base)

Patients admitted to an adult ICU, either medical, surgical or mixed, receiving ECMO support, from which (deferred) informed consent (by proxy) is obtained.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Patient is aged 18 years or older;
- Is receiving ECMO;
- (Deferred) informed consent.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Not expected to survive for 24 hours when assessed;
- Inability to receive blood products;
- (Known) decline to blood transfusions (e.g., Jehovah's Witnesses);
- Extracorporeal carbon dioxide removal (ECCO₂R) using low blood flow devices or pumpless devices (i.e., MINILUNG®, PrismaLung+);
- Received ECMO over 48h before screening for eligibility.

4.4 Sample size calculation

The sample size is based on 55% versus 50% survival probability with experimental vs. control treatment, respectively, for an assumed survival probability difference of 5% (i.e., benefit from experimental treatment). The non-inferiority margin is set at -7.5% (i.e., harm from experimental treatment), defined as the clinically relevant cut-off based on expert opinion in the absence of fully translatable clinical trials. Using a non-inferiority design, if the true survival probabilities are 55% vs. 50% (experimental vs. control), then 500 patients (250 per arm) are required to exclude the -7.5% non-inferiority margin using a 97.5% one-sided confidence interval (or a 95% two-sided confidence interval) with 80% power (18). We expect based on previous experience a maximum drop out of 5% after initial enrollment of deferred consent. For this reason we target to enroll 526 patients.

The sample size is calculated under the assumption that the experimental treatment is performing better than standard care, based on recent results of non-inferiority trials investigating restrictive vs liberal RBC in non-ECMO critically ill patients in which a restrictive

transfusion resulted in lower mortality (12,15). The FDA has published in their “*Non-Inferiority Clinical Trials to Establish Effectiveness Guidance for Industry*” the following “*If, in reality, the test drug is somewhat more effective than the control, it will be easier to rule out any given NI margin than if the test drug is equivalent or slightly inferior to the control, a smaller sample size could be tested*”. Hence, it is unethical not to control for this as this will result in unnecessary increased exposure of patients in a trial, and increase of use of scarce resources.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Randomization will take place between a restrictive (intervention arm) and liberal (control arm) transfusion regimen for RBC transfusion in a 1:1 manner:

- The *restrictive strategy* will consist of a transfusion Hb threshold of 7.0 g/dL, with a target Hb range of 7.1 – 9.0 g/dL. These thresholds are based on previous non-inferior trials in the patient populations in which VV ECMO (comparable to sepsis) and VA ECMO (cardiac surgery, acute myocardial infarction) are often applied (12,13,15).
- The *liberal strategy* will consist of a transfusion Hb threshold of 9.0 g/dL, with a target Hb range of 9.1 – 11.0 g/dL. These Hb thresholds are based on thresholds that are currently used in ECMO (5).

When the appropriate Hb threshold is reached, patients in each group will have one unit of RBC administered at a time. Within 3 hours after the transfusion, a repeat Hb concentration will be measured. Each group will only be transfused when their Hb level drops below the transfusion threshold. In case of an outlier measurement, clinicians are advised to repeat the measurement. The RBC transfusion must take place within 4 hours when the Hb trigger was measured.

5.1.1 Duration of intervention

The assigned transfusion regimen will be applied starting from the moment of randomization, and will be continued until successful weaning (24 hours no ECMO post-cannulation). In case after primarily successful decannulation from ECMO, a second ECMO run is indicated, participants will receive transfusion based on the thresholds of their first assigned transfusion regimen. In case of ICU-readmission without ECMO re-initiation, this does not apply.

5.1.2 Measurement of hemoglobin

Any validated method for determining Hb (using co-oximetry, spectrophotometry) may be used for determining Hb levels for transfusion and measuring post-transfusion Hb levels. This includes, but not limited to: central laboratory Hb measurement, blood gas machine, point-of-care.

5.1.3 Threshold events & transfusion strategy adherence

All threshold events will be stated in the (electronic) case report form ([e]CRF). A *threshold event* is defined as an occurrence which starts when the Hb value measured is below the assigned regimen's Hb threshold, and ending either:

- 1) When an RBC transfusion is administered, or
- 2) A Hb value is recorded above the assigned threshold, e.g., in case of a repeated measurement after an outlier measurement.

Adherence to the transfusion threshold is considered to have occurred if:

- 1) An RBC transfusion is ordered within the stated time frame of 4 hours or;
- 2) An Hb value is recorded above the assigned threshold, e.g., in case of a repeated measurement after an outlier measurement.

Non-adherence to the transfusion threshold is considered to have occurred if:

- 1) An RBC transfusion is administered without a protocol-defined Hb threshold being met, or;
- 2) An RBC transfusion is not administered subsequent to a Hb threshold being met.

In case of two (or more) units of RBC parallel administered or immediately consecutively, without measuring the Hb value between units, and if the first Hb value measured after the RBC transfusion of these multiple units is still lower than the transfusion trigger, then the administration of each unit (within the transfusion event) will be considered adherent.

5.1.4 Temporary protocol suspensions

Transfusion is allowed to be administered at any time in the following documented instances:

- 1) Any (suspicion of) massive bleeding which may result in a life-threatening bleeding, either overt bleeding, bleeding resulting in shock presumably due to blood loss occurring after randomization or in case of an important decrease in Hb level and no time to wait for repeated Hb measurement indicating suspected massive bleeding. This is based upon the clinical judgement by the researcher or clinical staff.
- 2) Indication for surgical intervention, other than changes in positioning of the cannula or ECMO decannulation.

After such an event is over, protocol will be resumed immediately, and RBC transfusions administered during this temporary suspension time frame will not be considered a breach of protocol or non-adherent. However, all transfusions given in this time frame, will be recorded

in the eCRF including the reason for temporary protocol suspension. In case of (suspected) massive overt active bleeding, protocol can temporarily be suspended for 24 hours or until (surgical) hemostasis, whatever comes first. In case protocol is suspended outside of these predefined borders, protocol will be resumed and all RBC transfusions administered outside of the assigned strategy, will be considered non-adherent.

5.1.5 Other products used in bleeding problems

Other blood derived products and coagulation factors are allowed to be administered, independent of the assigned Hb transfusion threshold for RBC. All transfusions should be given in line with published guidelines and generally accepted practice. All types of blood derived products and coagulation factors will be collected in the eCRF, including amount and indication. Blood derived products and coagulation factors will consist of:

- Platelet transfusion, either pooled 5-donor platelets, apheresis or single buffy coat platelets;
- Plasma transfusion, either pooled plasma or fresh frozen plasma;
- Fibrinogen;
- Cryoprecipitate;
- Prothrombin complex concentrate;
- Tranexamic acid;
- Desmopressin;
- AT III;
- INN-eptacog alfa (activated) (NovoSeven ®);
- Protamine.

5.2 Use of co-intervention (if applicable)

Not applicable

5.3 Escape medication (if applicable)

Not applicable

6. INVESTIGATIONAL PRODUCT

Not applicable.

6.1 Name and description of investigational product(s)

Not applicable.

6.2 Summary of findings from non-clinical studies

Not applicable.

6.3 Summary of findings from clinical studies

Not applicable.

6.4 Summary of known and potential risks and benefits

Not applicable.

6.5 Description and justification of route of administration and dosage

Not applicable.

6.6 Dosages, dosage modifications and method of administration

Not applicable.

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable.

6.8 Drug accountability

Not applicable.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

All RBC units will be manufactured, screened and stored according to the national regulations of each participating center (19). In addition, RBC units are Conformité Européenne (CE)-marked and are routinely used in clinical practice, also in the setting of ECMO. This is all part of standard care in this critically ill population.

7.2 Summary of findings from non-clinical studies

Not applicable.

7.3 Summary of findings from clinical studies

Not applicable.

7.4 Summary of known and potential risks and benefits

The main indication for RBC transfusion in critically ill patients is anemia. Anemia can be induced by different patient-, disease- and iatrogenic factors, including: chronic disease, shortened RBC circulatory life span and diminished RBC production, e.g., due to inflammation, hemolysis and hemorrhage (20–22). As main purpose of RBC is to provide oxygen delivery, anemia can result in the requirement of compensatory responses, placing an extra burden on critically ill patients (23). Moreover, although anemia has been associated with mortality in critical illness, the same accounts for blood transfusion (24,25), emphasizing its importance to only transfuse when necessary.

7.5 Description and justification of route of administration and dosage

RBC will be transfused following local hospital protocols. All RBC units will be manufactured, screened and stored according to the national regulations of each participating center (19). Clinicians will be informed on the assigned transfusion regimen and corresponding Hb transfusion trigger, and will be responsible for ordering RBC units when necessary.

7.6 Dosages, dosage modifications and method of administration

No distinctions from local hospital protocols will be made with regards to the size and method of administration of RBC units. To correct for possible differences in RBC units between the participating centers, total volume per unit transfused will be registered in the eCRF as well as the transfusion thresholds and yield.

7.7 Preparation and labelling of Non Investigational Medicinal Product

All RBC units will be manufactured, screened and stored according to the national regulations of each participating center (19).

7.8 Drug accountability

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary outcome parameter is 90-day all-cause mortality.

8.1.2 Secondary study parameters/endpoints (if applicable)

Secondary study parameters will include:

- To evaluate the transfusion practices:
 - Proportion of patients on ECMO exposed to allogeneic RBC transfusion;
 - RBC volume infused per patient during ECMO and day after decannulation;
 - RBC volume infused per transfusion event during ECMO and day after decannulation;
 - Adherence versus non-adherence.
 - Number of adherence and non-adherence events;
 - Daily, prior- and post-transfusion Hb levels.
- To evaluate the reasons for RBC transfusion, other than solely Hb as transfusion trigger;
- Survival time/days till death (e.g., mortality at 30 days and 1 year);
- The duration of ECMO support, ICU and hospital stay;
- Ventilator free days;
- Patient outcomes including mortality and complications:
 - In-hospital mortality;
 - Temporary protocol suspensions (according section 5.1.4)
 - Complications during ICU stay, including:
 - Neurological: i.e.,
 - Ischemic stroke: verified by CT- or MRI-scan;
 - Intracranial bleeding: verified by CT- or MRI-scan;
 - Cardiac: i.e.,
 - New acute myocardial ischemia, defined as: 1) acute myocardial infarction with or without ST-elevation, with 2) elevated biomarkers of myocardial injury.
 - Cardiac arrhythmias, consisting of:
 - Atrial fibrillation or atrial flutter; or

- Ventricular fibrillation or ventricular tachycardia. For which treatment, either consisting of medication, defibrillation or pacemaker indication, was indicated and initiated.
- Hemorrhagic
 - A.o. site and degree of severity: major bleeding is defined as being fatal OR in a critical area (e.g., intracranial, intraspinal, or intraocular) OR requiring intervention (coiling or surgery) OR transfusion of ≥ 3 packed cells < 24hours.
- Abdominal: i.e.,
 - Intestinal ischemia: verified by endoscopy or open surgery.
 - Intra-abdominal hypertension (IAH): intra-abdominal pressure >12 mmHg
 - Abdominal compartment syndrome: pathologic state caused by an acute increase in IAP >20–25 mmHg, presence of adverse effects on end-organ function, and abdominal decompression has beneficial effects.
- Renal: i.e.,
 - Acute kidney injury (AKI), defined as: Increase in serum creatinine by ≥ 26.5 $\mu\text{mol/L}$ within 48 hours; or increase in serum creatinine to ≥ 1.5 times baseline, which is known or presumed to have occurred within the last 7 days (26).
 - Renal replacement therapy (RRT): as defined by the initiation of either continuous veno-venous hemofiltration or other forms of hemodialysis which were not yet indicated based on the patient's medical history.
- Infection: i.e.,
 - Defined as culture (i.e. blood, respiratory tract) proven new infection during ECMO, or new initiation of treatment due to high suspicion of infection (i.e. fever, leukocytosis, increased inflammatory laboratory parameters, newly developed tachycardia, clinical signs for inflammation such as calor, tumor, rubor, excrete).
- Peripheral: i.e.,
 - Acute peripheral limb ischemia, defined as: 1) clinical signs and 2) need of open/percutaneous vascular intervention, amputation or initiation or increased antithrombotic treatment, other than

possibly cannula-related in case of partial arterial cannula-induced obstruction.

- Transfusion of other blood derived products or coagulation factors;
- Transfusion-related costs;
- Quality of life: The 5-level EQ-5D version (EQ-5D-5L) at 3, 6, 9 and 12 months;
- Medical consumption: iMTA Medical Consumption Questionnaire (iMCQ) at 3, 6, 9 and 12 months;
- Productivity costs: iMTA Productivity Cost Questionnaire (iPCQ) at 3, 6, 9 and 12 months.

8.1.3 Other study parameters (if applicable)

Other study parameters include:

- Patient demographics, such as age, sex and comorbidities (e.g., diabetes mellitus, myocardial infarction);
- ECMO-duration;
- ECMO-characteristics (i.e. blood flow, rotations per minute [RPM]) ;
- Duration of supportive therapies: i.e. invasive mechanical ventilation, renal replacement therapy;
- Severe adverse reactions to transfusion:
 - Anaphylactic/allergic reactions after transfusion (occurrence within 6 hours of transfusion) – mucocutaneous signs and symptoms such as laryngeal edema, hypotension, rash or nausea.
 - Severe hemolytic complications after transfusion (occurrence within 24 hours of transfusion) – hemoglobinemia, hemoglobinuria
 - TRALI (27) after transfusion, defined as:
 - Acute or worsening hypoxemia ($P/F \leq 300$ or $SpO_2 < 90\%$ on room air) AND
 - Onset during or within 6 hours of transfusion AND
 - acute or worsening pulmonary infiltrates on frontal chest X-ray OR clinical signs of overt pulmonary edema AND
 - No evidence of left atrial hypertension (LAH), or if LAH is present, it is judged to not be the main contributor to the hypoxemia.
 - TACO (28) after transfusion, defined as (*required / #additional):
 - Onset during or up to 12 hours after transfusion* AND
 - Evidence of acute or worsening respiratory distress*, AND/OR
 - Evidence of acute or worsening pulmonary edema based on*:

- Clinical physical examination, AND/OR;
 - Radiographic chest imaging and/or other non-invasive assessment of cardiac function.
- Development of cardiovascular system changes not explained by the patient's underlying medical condition, including development of tachycardia, hypertension, jugular venous distension, enlarged cardiac silhouette, and/or peripheral edema[#].
 - Evidence of fluid overload including any of the following: a positive fluid balance, clinical improvement following diuresis[#].
 - Supportive result of a relevant biomarker, e.g., an increase of B-type natriuretic peptide levels (BNP or NT-pro BNP) above the age group-specific reference range and greater than 1.5 times the pre-transfusion value[#].

8.2 Randomization, blinding and treatment allocation

Block-randomization will be stratified per center and per ECMO mode (either “VV ECMO or triple cannulation with primary pulmonary indication” or “VA ECMO or triple cannulation mode with primary cardiac or circulatory indication or ECPR”). Randomization will be performed if the subject meets the eligibility criteria and will be processed centrally by means of a web-based system that will provide the randomization treatment arm (Hb threshold 7.0 or 9.0 g/dL). The online system is constructed and validated for randomization and data management and has an audit trail. Local investigators of all participating centers will be provided a login to can sign in and randomize their patients. As the design is open label, no indications for breaking the randomization code are provided in the protocol. Randomization is communicated with the local principal investigator of each participating hospital who further carries out the necessary arrangements.

8.3 Study procedures

8.3.1 Extracorporeal membrane oxygenation [standard medical treatment]

ECMO will be provided according to local guidelines and each participating center will use local ECMO machines. All different types and modes of ECMO will be accepted during participation in this study. Currently, two main modes of ECMO are provided: VV and VA ECMO. However, all other used modes are eligible during participation (excluding ECCO₂R with low blood flow devices such as PrismaLung+ device on PrisMax).

- VV ECMO is indicated in case of severe respiratory failure refractory to other therapies (28). ECMO is considered a ‘last resort’ therapy. Patients are generally receiving invasive mechanical ventilation and are always unconscious, due to severe respiratory failure or due to sedatives. After the decision for VV ECMO is made, the patient is directly cannulated. Cannulation can be performed percutaneously or surgically. Percutaneous cannulation is performed ultrasound-guided by a trained physician. Type of cannulation (i.e. surgical vs. percutaneous) and location of cannulation (i.e. ICU, operation theatre) are dependent of the center’s standard practices.
- VA ECMO is indicated in case of reversible severe cardiac, cardio-circulatory or cardio-respiratory failure refractory to other therapies. Main indications for VA ECMO include post-cardiotomy (“*failure to wean*” cardiopulmonary bypass), cardiogenic shock, myocarditis, or massive pulmonary embolism (29). The decision for VA ECMO is often made within minutes in an hyper-acute setting, where no other options can be offered to improve the acute setting and critical patient condition. The patient is always unconscious, receiving invasive mechanical ventilation and high doses of medication (e.g., neuromuscular blockers, narcotics, and vasoactive medication). Site (i.e. peripheral vs. central), type of cannulation (i.e. surgical vs. percutaneous) and location of cannulation (i.e. operation theatre, on-scene, cath room, emergency room or ICU itself, are dependent of the center’s standard practices.
- In addition to the above mentioned bi-cannula modes, either VA or VV ECMO can be extended to a triple cannulation mode such as V-VA, V-AV, VV-A or VV-V to serve the purpose of expanding the capabilities of the ECMO circuit. Triple cannulation modes enable improved oxygenation, and circulatory support, depending on the specific needs of each patient.
- Lastly, a specific indication of VA ECMO is Extracorporeal Cardiopulmonary Resuscitation (ECPR). ECPR is used to provide circulatory support in patients in whom conventional cardiopulmonary resuscitation is not successful in achieving sustained return of spontaneous circulation. In case of out-of-hospital cardiac arrest (OHCA), cannulation takes place by a cardiothoracic surgeon, (intervention) cardiologist, intensivist, or anesthesiologist. For each participating center, local guidelines and indications are allowed as well as local available ECMO machines.

To summarize, ECMO is a last resort therapy which is provided in case of potentially reversible cardiac and/or respiratory failure refractory to conventional therapies. Due to the severity of disease, the patient is unconscious at the time of defining the indication. Treating physicians

often have to make the call before consulting the legal representative for the purpose of patient survival.

When a patient participates in the trial, one of transfusion threshold strategy (liberal or restrictive) will be initiated within a maximum of 12 hours after randomization. Deferred consent for the trial will be obtained from the patients' representative. See below.

8.3.2 Blood sampling [standard measurements]

All patients on ECMO have either one or two arterial lines as part of standard care. Standard protocols (e.g., time interval) may differ per participating center, including the following blood samplings to collect during the study:

- 1) Arterial blood gas (ABG) every 2-6 hours, which in case of reaching equilibrium over time will be decreased to every 6 hours. This ABG includes (but not limited to [differ per hospital]): arterial pH, paCO_2 , PaO_2 , bicarbonate level, lactate, hemoglobin, glucose, sodium (Na^+), potassium (K^+).
- 2) Every 12-24 hours, samples will be taken, included but not limited to measure Hb (if not already measured during ABG analysis), platelet count, fibrinogen, central venous oxygen saturation (ScvO_2).
- 3) Lastly, every 24 hours will be assessed:
 - a. Standard: Magnesium, Chloride, phosphate, creatinine level, leucocyte count, D-dimer, hepatic function panel, albumin, C-reactive protein (CRP) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP). Coagulation levels including: prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), Activated clotting time (ACT) or Anti-Xa if applicable.
 - b. If indicated: hemolysis parameters, including: haptoglobin, LDH or free hemoglobin. Thromboelastometry e.g., ROTEM®.

As part of this study, Hb levels will be measured at the following time points, as shown in the figure 4 below:

- Once daily;
- In case of RBC transfusion, repeated measurement **within 3 hours** after the RBC transfusion has been given, to check whether the Hb target range has been reached.

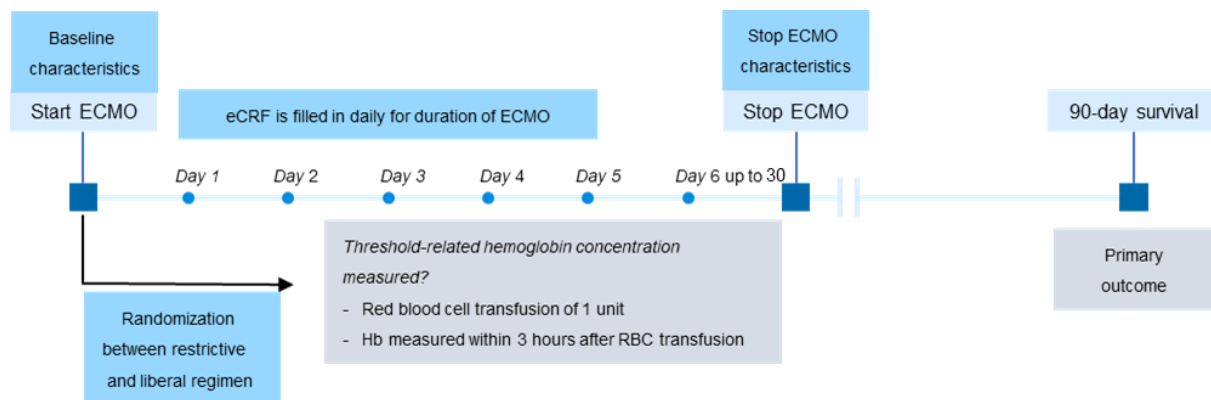


Figure 3. Hb measurements (derived from Figure 1.)

8.3.3 Follow-up of subjects

Patients included in the study who survived the hospital admission will be contacted by the researchers after 12 months and eventually after 3 months:

- After 3 months, the researchers will collect medical data regarding the patient current status. This will be done through the electronic patient record, their general practitioner, or, in the case of death, the Central Agency for Statistics (to inquire about the cause of death). This is necessary to examine the relationship with the study. If the mentioned options are incomplete, the researcher may contact the patient by email or phone.
- At 3, 6, 9, and 12 months, the researcher will contact the patient either by phone or through an automated email, including a QoL questionnaire (EQ-5D-5L, iMCQ and iPCQ) from the eCRF (if allowed in the participating country) for follow-up. During these interactions, patients will be asked about their overall well-being and any limitations they may experience in daily functioning. Each conversation or questionnaire completion will take approximately 10 minutes. In total, the researcher will contact each patient up to four times.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.5 Replacement of individual subjects after withdrawal

Patients who are randomized but excluded from the analyses due to a failure to obtain consent or withdrawal of consent (per section 11.2) do not count toward the sample size of evaluable

patients. Patients will be recruited according the randomization process until the required sample size of patients with evaluable data is reached.

8.6 Follow-up of subjects withdrawn from treatment

Patients withdrawn from treatment will not be subjected to follow-up.

8.7 Premature termination of the study

The study can be ended prematurely by the steering committee based on recommendations of the DSMB. The following criteria are defined as ground for the Safety Committee to decide whether the trial has to be terminated prematurely: A proven superiority of a restrictive regimen over the liberal regimen or a proven inferiority (and thus harm) of the restrictive regimen over the liberal regimen. This will be determined during the first DSMB meeting.

The safety Committee reports the recommendations to the Principal Investigator in writing and in verbal. The Principal Investigator is further responsible for the dissemination. In case of prematurely termination of the study, this will be informed to the METC, sponsor and local principal investigators of the study as soon as possible.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, considered related to the RBC transfusion regimen. These adverse events collected or observed by the investigator or his staff will be recorded:

- Clinical significant and relevant abnormal results (see below, 9.2.2).
- New conditions detected or diagnosed after enrollment in the study, considered related to the RBC transfusion regimen.
- AEs are limited to events that are known to result from RBC transfusion or that might reasonably occur as a consequence of RBC transfusion such as, but not limited to febrile non-hemolytic transfusion reactions (FNHTR).

9.2.2 Special considerations for assessment of AE and non-reportable findings in the ICU setting

In clinical intensive care studies, it is common to observe deviations in laboratory values and temporary changes, for example in arterial blood pressure. The determination of whether an abnormal laboratory finding or other abnormal assessment is clinically significant and constitutes an AE will be made by the investigator using their medical and scientific judgment (i.e., is the events related to the RBC transfusion regimen). Reporting of an AE or SAE will not be necessary for the mere presence of "progressive disease" or "progression of disease".

9.2.3 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that:

- results in death;
- is life threatening (at the time of the event);

- requires a significant prolongation of the current inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention, but could have been based upon appropriate judgement by the investigator.

In the current study severe hemorrhagic complications (e.g., severe bleeding, hemorrhagic stroke), transfusion related complications and mortality are considered as the main serious adverse events. An elective hospital admission will not be considered as a serious adverse event.

It is important to mention that clinical research involving critically ill patients illustrates several concerns with the existing system for monitoring adverse events. Currently, morbidity and mortality rates are high among patients in the ICU; in our study population receiving ECMO, mortality rates exceed 50% and the majority will suffer from multiple complications, such as hemorrhage and acute kidney injury. Critical illness itself often reflects a series of established or acquired complications that evolve, resolve or persist. Therefore, whether enrolled in a trial or not, ICU patients are particularly likely to experience clinical events that fall within the definition of a serious adverse event. These events include but are not limited to death and nosocomial infections. Therefore, if the foregoing definition is strictly applied, a high proportion of ICU patients may experience a serious adverse event. Every year, we will send a list of all SAEs referable to the study parameters.

We propose the following solutions for more rational reporting of (S)AEs in this study:

- We have labelled adverse events as secondary outcomes in this trial and will label mortality as primary outcome in the finite RCT.
- Adverse events defined and reported as study outcomes are not also labelled and reported as SAEs.
- Both AE and SAEs are limited to (serious) events that are known to result from the given transfusion threshold, RBC transfusion or that might reasonably occur as a consequence of RBC transfusion.
- SAEs are reported during the RCT until the day ICU discharge. Mortality will be assessed at 90-days and at 12-months after ECMO initiation.
- Periodic reports (every 12 months) of SAEs will be reported through the web portal Research Portal to the accredited METC that approved the protocol and the safety committee.

- Since case fatality in the patient population under study is known to be around 50%, line listing of deaths will be performed, with reporting once per twelve months (same moment as the DSMB interim analysis and meeting). This reporting will be the responsibility of the study coordinator, the primary investigator and independent reviewer.
- As SAEs in the patient population under study is known to be around 60-70% line listing of SAE and case fatality per treatment allocation will be reported in the annual safety report.

9.2.4 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable

9.2.5 Procedures for recording, reporting, causality and follow-up

All (S)AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

Recording:

- The investigator is responsible for reviewing all relevant documentation, such as hospital progress notes, laboratory reports, and diagnostic reports, related to an (S)AE.
- Using signs, symptoms, and other clinical information, the investigator will attempt to establish a diagnosis of the event (if possible).
- All relevant information will be recorded in the eCRF by the investigator.

Reporting:

- SAEs outlined under Section 9.2.3 must be reported within 24 hours of becoming aware through the eCRF by investigators and other site personnel.
- The eCRF will serve as the primary mechanism for reporting SAEs to the safety vendor.
- Follow-up information on SAEs must also be reported within 24 hours of becoming aware.

Causality:

- The investigator has a duty to evaluate the connection between the treatment and every instance of an (S)AE.
- The investigator will employ clinical judgment to determine the relationship.

Follow-up:

- SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.3 Data Safety Monitoring Board (DSMB)

For this trial, The DSMB will be composed of 3 individuals, one of which will be the chairman.

- The DSMB will first meet by teleconference before the first patient is enrolled; the first meeting will be scheduled after 50% of total included patients, or after twelve months, whichever comes first.
- Subsequent to this meeting the DSMB will meet virtually every twelve months;
- All unexpected (serious) adverse events will be reported to the DSMB;
- The DSMB will review the overall status of the program: number of patients enrolled overall and in each center, adherence to the protocol overall and by each center, adverse events overall and by each center;
- The DSMB may be composed of the following individuals: Gavin Murphy (gjm19@leicester.ac.uk), Jaap Jan Zwaginga (j.j.zwaginga@lumc.nl) and Marcel Dijkgraaf (m.g.dijkgraaf@amsterdamumc.nl).

The advice(s) of the DSMB will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

10. STATISTICAL ANALYSIS

Baseline characteristics will be summarized using simple descriptive statistics. Normal distributed continuous variables will be presented as mean (standard deviation); non-normal as median (interquartile range [IQR]). Categorical data will be presented as numbers (n) and fractions (%). Distribution of data will be assessed by visual inspection of histograms and Q-Q normality tests. All statistical analyses will be described in full detail in a statistical analysis plan (SAP). Analysis will be performed using R in the Rstudio interface. (The R Foundation, Lucent Technologies, Inc., Murray Hill, NJ, USA, www.r-project.org).

10.1 Primary study parameter(s)

The primary outcome will be analyzed for all randomized participants in an intention-to-treat and per-protocol analysis. In non-inferiority trials, it is recommended to perform both intention-to-treat and per-protocol analyses. In case both methods produce the same result, non-inferiority can be concluded. The primary outcome will be analyzed as a binary variable (alive versus deceased at 90-days), expressed in a relative risk estimate and absolute risk increase, with the associated 95% upper confidence limit. Non-inferiority is demonstrated if this interval does not exceed the non-inferiority limit of 7.5% difference in favor of a liberal transfusion threshold.

For the intention-to-treat population, all patients will be included except for whom consent is withdrawn. No assumptions will be made regarding the pattern of missing data. First, missing data will be described per variable. Second, the pattern of the missing data will be evaluated and pre-described scenarios for handling the missing data will be performed. In case of data missing completely at random (MCAR) or missing at random (MAR), a tipping point analysis will be performed using multiple imputation. In a tipping point analysis, missing data are imputed over a range of possible scenarios for treatment effect (e.g. best-worse and worst-best scenario). The 'best-worst' case scenario assumes that all patients lost to follow-up in the restrictive threshold group have had a beneficial outcome, and those with missing outcomes in the liberal (control) group have harmful outcome. In the worst-best case scenario, these are the other way around.

In addition to the intention-to-treat analysis, a per-protocol and as-treated analysis will be performed. The per-protocol analysis will consist of patients in whom the allocated protocol was adhered to. The as-treated analysis will consist of all patients who received RBC transfusion according the specific threshold arms, regardless of allocation. The per-protocol and as-treated analysis will be described and further explained in the SAP.

The primary outcome will not be adjusted for the stratification variables (center and ECMO type), as some centers are expected to include only a very small number of patients. This leads to inferential problems in the statistical adjustment procedure, substantially complicating the interpretation of the results (30). If the lower confidence limit is very close the non-inferiority margin at the final analysis, additional analyses will be performed (using mixed-effects modeling) to assess the influence of site stratification adjustment on the final outcome.

10.2 Secondary study parameter(s)

For all secondary outcomes, either Chi square or Mann-Whitney-U tests will be performed. All tests of statistical significance will be two-sided with a type I error risk of 5%. Methods to correct for multiplicity in these secondary outcomes will be described in the SAP. Lastly, predefined subgroups analysis will be performed on sex (male versus female), type of ECMO mode (VV versus VA), cannulation-mode (surgical versus percutaneous), renal failure present (yes versus no) and ECPR (ECPR versus non-ECPR within VA ECMO) as sensitivity analysis. We will present the results in forest plots. Kaplan-Meier mortality curves will be used to describe mortality rates and length of ICU- and hospital-stay. A Cox regression will be performed comparing the days-until-event (death) between the two groups. Additionally, QoL outcomes for each domain from the EQ-5D-5L survey will be compared between randomization groups for each quarter during the 12 months of follow-up. Furthermore, EQ-5D-5L data will be analyzed over time using generalized linear mixed models.

10.3 Other study parameters

Variables will be expressed as numbers and frequencies, means and standard deviations (SD), or medians and interquartile ranges (IQR) whenever appropriate. Differences between groups in continuous variables will be analyzed with Student's t-test or, if continuous data is not normally distributed, the Mann-Whitney U test will be used. Categorical variables will be compared with the Chi-squared test or Fisher's exact test, as appropriate.

10.4 Interim analysis

During the first DSMB meeting, it was decided not to establish stopping criteria during interim analysis. There are multiple factors that led to this decision:

1. Carrying out an interim analysis would lead to alpha wasting, which would require an increase in sample size or using a smaller alpha for the final analysis. Alternatively, the use of more rigorous statistical tests during the interim analysis would keep the risk of a false-positive

conclusion under 5%. However, this approach may pose challenges in interpreting the results in a clinical setting.

2. It is not anticipated to perform an interim analysis to produce results that would lead to the termination or modification of the study due to either harm or proven superiority of the intervention group. This is mainly due to the heterogeneity of the patient population, high mortality rates, and significant differences in mortality between VV and VA ECMO patients. Consequently, the likelihood of obtaining an outcome from the interim analysis that could indicate harm or superiority of the intervention arm is small. Even if such an outcome were to occur, it would still be difficult to draw reliable conclusions due to the small sample size and heterogeneity of the patient population.

In light of these considerations, the decision was made that the DSMB will perform an extensive review of the data either around the midpoint of patient inclusion or 12 months after the commencement of patient inclusion.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (as approved on the 64th WMA General Assembly, Fortaleza, Brazil, October 2013, retrieved on September 19th via: <https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>), and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts.

11.2 Recruitment and consent

11.2.1 Recruitment

In view of the high incidence of anemia and possible anemia-related morbidity on one hand, and transfusion-related morbidity and mortality on the other hand, it is important to start the assignment as soon as possible. The study intervention regards an emergency intervention that has to be applied without delay and fulfills the ethical requirement of clinical equipoise. The study participant can benefit from the intervention, but up to now there is a state of honest, professional disagreement in the community of expert practitioners as to the preferred treatment (liberal or restrictive threshold). Some centers in the Netherlands and Belgium transfuse RBC with thresholds levels similar aimed for in this study and others do not. Furthermore, the eligible patients have an extremely high risk of dying (the indication for ECMO

is a mortality probability of more than 80% with conservative therapy) and the legal representatives will therefore be in a disturbed mental state complicating an immediate informed decision.

Consequently, we will employ a "deferred consent" approach, similar to previous studies conducted in this population and we appeal to the emergency procedure for consent in medical research as stated in article 6, paragraph 4 of the WMO, for reasons as explained below.

Following confirmation of eligibility, patients will be randomized directly into the study. The relatives will be promptly informed of the randomization by a trained intensive care doctor and/or medical researcher. They will be asked for consent to continue the treatment according to the study protocol. Once the patient has sufficiently recovered and is capable of making a decision, they will be approached to provide informed consent regarding their participation in the study. We propose to include and sample each patient in the ICU with an indication for VA or VV ECMO who meets the inclusion criteria. Eligible patients are recruited as soon as possible by their attending physician following initiation of ECMO treatment (but within 48h after ECMO initiation), given that the majority of transfusion events occur during the initial stages following the initiation of ECMO.

11.2.2 Consent procedure

All patients on ECMO are, without exception, not able to give informed consent. Persons who may take the role of legal representative in accordance with the Medical Treatment Agreement Act (WGBO) are: a predefined representative, husband or wife, registered partner or other life partner, a parent or child, brother or sister, and incidentally a curator appointed by a judge. However, the legal representatives are frequently absent at the moment their beloved ones are admitted to or when ECMO is initiated in the operating room (OR), or the ICU. Obtaining informed consent from a legal representative usually takes time, even by an experienced research team, as consent requires sufficient time to read and consider the provided written information (31). As ECMO is already a difficult treatment to explain and understand for relatives, time to read and consider this written information is essential, as well ethical as methodological.

Moreover, the experience of ICU patients enrolled under deferred consent is mainly positive. For example, an investigation of the contentment of participants that were included using deferred consent in the 'Normoglycemia in Intensive Care-Survival Using Glucose algorithm Regulation' ('NICE-SUGAR') trial, showed that a majority of the patients were happy with the

decision made by the representative (93%) and would have granted consent if asked (96%) (32).

Patients will therefore be enrolled under a deferred consent procedure, where informed consent from the patient or a legal representative must be obtained as soon as possible in the Netherlands. This duration, allowed for obtaining consent, is subject to national regulations and may differ when compared to Dutch sites. As such, for Belgian sites, (oral) informed consent has to be obtained within a maximum of 5 (working) days. The rationale for the deferred consent procedure is the low attributable risk of the interventions, the time-limited nature of the intervention and fact that both treatment and control interventions are common practice in different centers. The consent procedure will be performed according to the following procedures:

11.2.2.1 Legal representative

Given that patients are incapacitated and legally incompetent during the randomization process and the implementation of the randomized regimen assignment, legal representatives will be approached to obtain consent as soon as they become available. The legal representatives will be notified about the patient's enrollment in the trial.

If the legal representative(s) provide informed consent, treatment based on the assigned allocation will proceed. In the event that the legal representative(s) require additional time for consideration, treatment based on the assigned allocation will continue until a decision regarding consent is made by the legal representative(s). The assent will be documented in the records.

In the event that the legal representative(s) express objection to treatment based on the assigned allocation and consequently do not sign the written informed consent form, patients will be treated according to standard practice. This entails that treatment will proceed according to the predetermined thresholds established by each participating hospital.

If the patient has died prior to informing the legal representative or receiving their written informed consent after verbal given consent of the legal representative, the study data will be used. The rationale for the latter is that the legal representatives have no independent right on inspection or say on of therapeutic or study data (CCMO: "De nabestaanden hebben geen zelfstandig recht op inzage van de tijdens de behandeling en het onderzoek verkregen gegevens en hebben daar ook geen zeggenschap over. Van toestemming voor het gebruik

van de data door de nabestaanden kan daarom ook geen sprake zijn"). Furthermore, possible refusal may cause selection bias and this is ethically unwanted (CCMO: "het introduceren van selectiebias door het moeten vragen van toestemming aan de nabestaanden, mocht daar een grond voor zijn, ethisch niet wenselijk is").

11.2.2.2. Patient

Once patients have recovered, they will be informed of their enrollment in the study and that their legal representative has been notified of their participation and has agreed to continue the study procedures. They will also be told that information about their clinical progress is needed for up to 90 days, with data collection continuing for up to 12 months. Patients will be invited to join an email conversation or phone call at the 3-month mark to assess the 90-day mortality outcome, if necessary. Additionally, a phone call or automated survey will be conducted at 3, 6, 9, and 12 months to complete a questionnaire on quality of life, productivity loss, and medical consumption.

Patients will be asked to provide their consent by signing a written informed consent form, allowing us to use the collected data and agreeing to participate in a survey (via telephone call or email) about their quality of life, productivity loss, and medical consumption. They will also consent to the collection of follow-up clinical information from hospital and general practitioner records after their hospital discharge.

If a patient refuses to give consent, no additional information will be collected and the patient will be excluded from the analyses. Patients can choose to participate in the study up to the collection of the primary endpoint without taking part in the surveys conducted up to 12 months. It is also important to note that patients have the right to discontinue clinical follow-up after hospital discharge at any time without giving a specific reason.

11.3 Objection by minors or incapacitated subjects (if applicable)

Because of the nature of the study population, all subjects are incapacitated adults at the time of informed consent for which reason the method of deferred consent is used in all cases. Minors are not recruited.

11.4 Benefits and risks assessment, group relatedness

Main indication for RBC transfusion in critically ill patients is anemia. Anemia can be induced by different patient-, disease- and iatrogenic factors, including: chronic disease, shortened

RBC circulatory life span and diminished RBC production, e.g., due to inflammation, hemolysis and hemorrhage (20–22). As main purpose of RBC is to provide oxygen delivery, anemia can result in the requirement of compensatory responses, placing an extra burden on critically ill patients (23). Moreover, although anemia has been associated with mortality in critical illness, the same accounts for blood transfusion (24,25), emphasizing its importance to only transfuse when necessary to reduce transfusion associated complications.

Potential risk factors after blood transfusion (but not limited to):

- Allergic reactions;
- Fever;
- Acute immune hemolytic reaction;
- TRALI;
- TACO;
- Graft-versus-host disease;
- Blood-borne infections.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives (if applicable)

Not applicable.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Data will be encoded and handled confidentially. To ensure data security and to protect the subject's privacy, data on individual subjects will be encoded according to a subject identification code list. The key to the code will be safeguarded by both the executive investigator(s) and the coordinating investigators. Only local investigators will have access to the key of this code. Local investigators will have access to the source data at any time. Data will be collected and stored in CastorEDC. Data will be stored for 15 years. The study will be reported to the "privacy functionary" of the Amsterdam University Medical Centre, location Amsterdam Medical Centre. All handling of personal data will comply with the European GDPR act and the '*Reuse of care data for the purpose of research*' standard of the Amsterdam UMC. More details on handling and storage of the data can be found in the Data Protection Impact Analysis and Data Management Plan.

12.2 Monitoring and Quality Assurance

Besides ECMO, this study is evaluated as having a low/moderate risk. Collected data in each participating center will be monitored by an independent monitor (i.e., quality officer) from the Amsterdam UMC according Good Clinical Practice (GCP). In some (random) included patients the following issues will be monitored:

- Initiation visits at all sites if possible;
- Documented informed consent;
- Documented delivery or non-delivery in the eCRF of the intervention according to the protocol compared with source data being patients' hospital records;
- The coordinating center will continuously monitor that all eCRFs are fulfilled according to the protocol.

Additional monitoring visit will be made to selected sites if the steering committee finds this necessary based on monitoring findings. The monitoring plan will be enclosed in the trial master file.

12.3 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit, defined as 90-day survival status. The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

We are free to make a publication and have no restrictions made by a sponsor. Data will be published anonymously. Our goal is to publish all results, regardless of the outcome, within 12 months after the completion of the study.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

This paragraph is not applicable since the RBC transfusions are registered products within the indication and not used in combination with other products.

a. Level of knowledge about mechanism of action

Not applicable.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

Not applicable.

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

Not applicable.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Not applicable.

e. Analysis of potential effect

Not applicable.

f. Pharmacokinetic considerations

Not applicable.

g. Study population

Not applicable.

h. Interaction with other products

Not applicable.

i. Predictability of effect

Not applicable.

j. Can effects be managed?

Not applicable.

13.2 Synthesis

Not applicable.

14. REFERENCES

1. Gattinoni L, Carlesso E, Langer T. Clinical review: Extracorporeal membrane oxygenation. *Crit Care*. 2011;15(6).
2. Extracorporeal Life Support Organization. Extracorporeal Life Support Organization ECLS Registry Report Overall Outcomes. 2019;2019(c):11408. Available from: <https://www.else.org/Registry/Statistics/InternationalSummary.aspx>
3. Makdisi G, Wang IW. Extra Corporeal Membrane Oxygenation (ECMO) review of a lifesaving technology. *J Thorac Dis*. 2015;7(7):E166–76.
4. Vlaar AP, Oczkowski S, de Bruin S, Wijnberge M, Antonelli M, Aubron C, et al. Transfusion strategies in non-bleeding critically ill adults: a clinical practice guideline from the European Society of Intensive Care Medicine. *Intensive Care Med*. 2020;
5. De Bruin S, Scheeren TWL, Bakker J, Van Bruggen R, Vlaar APJ, Antonelli M, et al. Transfusion practice in the non-bleeding critically ill: An international online survey-the TRACE survey. *Crit Care*. 2019;23(1):1–8.
6. Martucci G, Grasselli G, Tanaka K, Tuzzolino F, Panarello G, Schmidt M, et al. Hemoglobin trigger and approach to red blood cell transfusions during veno-venous extracorporeal membrane oxygenation: the international TRAIN-ECMO survey. *Perfus (United Kingdom)*. 2019;34(1_suppl):39–48.
7. Aubron C, DePuydt J, Belon F, Bailey M, Schmidt M, Sheldrake J, et al. Predictive factors of bleeding events in adults undergoing extracorporeal membrane oxygenation. *Ann Intensive Care*. 2016;6(1).
8. Raasveld SJ, Karami M, van den Bergh WM, Lansink-Hartgring AO, van der Velde F, Maas JJ, et al. RBC Transfusion in Venovenous Extracorporeal Membrane Oxygenation: A Multicenter Cohort Study. *Crit Care Med*. 2022;50(2):224–34.
9. Vincent JL, Baron J-F, Reinhart K, Gattinoni L, Thijs L, Webb A, et al. Anemia and blood transfusion in critically ill patients. *J Am Med Assoc*. 2002;288(12):1499–507.
10. Brogan T V., Lequier L, Lorusso R, MacLaren G, Peek G. Extracorporeal Life Support : The ELSO Red Book. 2017. 831 p.
11. Bosboom JJ, Klanderman RB, Zijp M, Hollmann MW, Veelo DP, Binnekade JM, et al. Incidence, risk factors, and outcome of transfusion-associated circulatory overload in a mixed intensive care unit population: a nested case-control study. *Transfusion*. 2018;58(2):498–506.
12. Hebert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, et al. A Multicenter, Randomized, Controlled Clinical Trial of Transfusion Requirements in Critical Care. *N Engl J Med*. 1999;162(1):280–280.
13. Mazer CD, Whitlock RP, Fergusson DA, Hall J, Belley-Cote E, Connolly K, et al.

- Restrictive or Liberal Red-Cell Transfusion for Cardiac Surgery. *N Engl J Med*. 2017;377(22):2133–44.
14. Murphy GJ, Pike K, Rogers CA, Wordsworth S, Stokes EA, Angelini GD, et al. Liberal or restrictive transfusion after cardiac surgery. *N Engl J Med*. 2015;372(11):997–1008.
 15. Ducrocq G, Gonzalez-Juanatey JR, Puymirat E, Lemesle G, Cachanado M, Durand-Zaleski I, et al. Effect of a Restrictive vs Liberal Blood Transfusion Strategy on Major Cardiovascular Events among Patients with Acute Myocardial Infarction and Anemia: The REALITY Randomized Clinical Trial. *JAMA - J Am Med Assoc*. 2021;325(6):552–60.
 16. Kracalik I, Mowla S, Basavaraju SV, Sapiiano MRP. Transfusion-related adverse reactions: Data from the National Healthcare Safety Network Hemovigilance Module - United States, 2013-2018. *Transfusion*. 2021;61(5):1424-34.
 17. Holst LB, Wetterslev J, Johansson P, Wernerman J, Aneman A, A.B G, et al. Transfusion requirements in septic shock (TRISS) trial: Protocol of an ongoing scandinavian multicenter, randomised clinical trial of the efficacy and safety of red blood cell transfusion in patients with septic shock. *Trials*. 2013;14(150):1–12.
 18. Blackwelder WC. “Proving the null hypothesis” in clinical trials. *Control Clin Trials* [Internet]. 1982 Dec 1 [cited 2019 Sep 12];3(4):345–53. Available from: <https://www.sciencedirect.com/science/article/pii/0197245682900241?via%3Dihub>
 19. Sanquin S. Bloedwijzer. 2019;(september 2018).
 20. Hayden SJ, Albert TJ, Watkins TR, Swenson ER. Anemia in critical illness: Insights into etiology, consequences, and management. *Am J Respir Crit Care Med*. 2012;185(10):1049–57.
 21. Ji C, Sj S, Roubinian N, Da F, Triulzi D, Doree C, et al. Transfusion thresholds and other strategies for guiding allogeneic red blood cell transfusion (Review) SUMMARY OF FINDINGS FOR THE MAIN COMPARISON. *Cochrane Database Syst Rev*. 2016;(10).
 22. Docherty AB, Turgeon AF, Walsh TS. Best practice in critical care: anaemia in acute and critical illness. *Transfus Med*. 2018;28(2):181–9.
 23. Spinelli E, Bartlett RH. Anemia and transfusion in critical care: Physiology and management. *J Intensive Care Med*. 2014;31(5):295–306.
 24. Gong MN, Thompson BT, Williams P, Pothier L, Boyce PD, Christiani DC. Clinical predictors of and mortality in acute respiratory distress syndrome: Potential role of red cell transfusion. *Crit Care Med*. 2005;33(6):1191–8.
 25. Corwin HL, Gettinger A, Pearl RG, Fink MP, Levy MM, Abraham E, et al. The CRIT Study: Anemia and blood transfusion in the critically ill - Current clinical practice in the United States. *Crit Care Med*. 2004;32(1):39–52.

26. International Society of Nephrology. Chapter 5.4: Vascular access for renal replacement therapy in AKI. KDIGO Clin Pract Guidel Acute Kidney Inj. 2012;2(1):3.
27. Vlaar AP, Pearl T, Fung M, Looney MR, Juffermans NP, Bux J, et al. A consensus redefinition of transfusion-related acute lung injury.pdf. Transfusion. 2019;59(7):2465–76.
28. Wiersum-Osselton JC, Whitaker B, Grey S, Land K, Perez G, Rajbhandary S, et al. Revised international surveillance case definition of transfusion-associated circulatory overload: a classification agreement validation study. Lancet Haematol [Internet]. 2019;6(7):e350–8. Available from: [http://dx.doi.org/10.1016/S2352-3026\(19\)30080-8](http://dx.doi.org/10.1016/S2352-3026(19)30080-8)
29. Makdisi G, Wang I-W. Extra Corporeal Membrane Oxygenation (ECMO) review of a lifesaving technology. J Thorac Dis. 2015;7(7):E166-76.
30. APJ Vlaar, M Witzénrath, P van Paassen, et al. Anti-C5a antibody (vilobelimab) therapy for critically ill, invasively mechanically ventilated patients with COVID-19 (PANAMO): a multicentre, double-blind, randomised, placebo-controlled, phase 3 trial Lancet Respir Med, 10 (2022), pp. 1137-1146.
31. Burns KEA, Zubrinich C, Tan W, Raptis S, Xiong W, Smith O, 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(11):1212–8.
32. Potter JE, McKinley S, Delaney A. Research participants' opinions of delayed consent for a randomised controlled trial of glucose control in intensive care. Intensive Care Med. 2013;39(3):472–80.