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Evaluation of Oxygen Extraction Rate As A Guide Red Blood Cell Transfusion Strategy in Intensive Care Unit: A Protocol For A Prospective Observational Study

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Complete List of Authors:	Tüzen, Ahmet Salih; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation Aksun, Murat; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Intensive Care Medicine Sencan, Atilla; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Intensive Care Medicine Girgin, Senem; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation Gölboyu, Birzat Emre; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation Kırbaş, Gizem; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation Şanlı, Ozan; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation
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Evaluation of Oxygen Extraction Rate As A Guide Red Blood Cell Transfusion Strategy in Intensive Care Unit: A Protocol For A Prospective Observational Study

Author affiliations

Ahmet Salih Tüzen, M.D. (Corresponding author)
astuzen@icloud.com
+905353915577
Department of Anesthesiology and Reanimation, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Murat Aksun, M.D. Ph.D.
murataksun@yahoo.com
Department of Intensive Care Medicine, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Atilla Şencan, M.D.
atillasencan2000@yahoo.com
Department of Intensive Care Medicine, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Senem Girgin, M.D.
senemcan@gmail.com
Department of Anesthesiology and Reanimation, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Birzat Emre Gölboyu, M.D.
birzatemre@windowslive.com
Department of Anesthesiology and Reanimation, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Gizem Kırbaş, M.D.

gizembas35@gmail.com
Department of Anesthesiology and Reanimation, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Ozan Şanlı, M.D.
ozan_sanli@yahoo.com
Department of Anesthesiology and Reanimation, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir
Turkey

Author's initials: Tüzen AS., Aksun M., Şencan A., Girgin S., Gölboyu BE., Kırbaş G., Şanlı O.

Contributors

A.S.T. (Principal Investigator), M.A. and A.S. wrote the first draft of the protocol manuscript. A.S.T., M.A. and B.E.G. planned the conception and design of the study and the protocol. G.K., O.S. and S.G. contributed to the design and implementation of the protocol. All authors provided critical revisions to the manuscript before approving the final version.

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Competing interests

None declared.

Patient ant public involvement

Patients and/or the public were not involved in the , conduct, or reporting of this study or in plans for dissemination.

Patient consent for publication

Informed consent will be obtained from the patient's first-degree relatives.

Data availability statement

All data necessary to support the protocol are available upon reasonable request.

ORCID iDs

Ahmet Salih Tüzen: 0000-0001-9040-2262
Murat Aksun: 0000-0002-8308-3045
Atilla Şencan: 0000-0002-3765-9891
Senem Girgin: 0000-0003-0715-7695
Birzat Emre Gölboyu: 0000-0002-2011-2574
Gizem Kırbaş: 0000-0003-1178-5723
Ozan Şanlı: 0000-0001-8067-7908

ABSTRACT

Introduction

Restrictive and liberal transfusion approaches to hemoglobin targets have used when deciding on red blood cell transfusions in patients who do not have acute bleeding and have a hemodynamically stable course in the ICU. However, physiologic trigger points that evaluate tissue oxygenation when deciding on blood transfusion in patients have also been among the important topics of research in recent years. In this study, we will evaluate the O_2ER, which is an important indicator of the balance between oxygen delivery and consumption in tissues. Whether oxygen extraction rate can be used as a trigger for blood transfusion will be

determined by clinical outcomes in ICU patients. If physiologic transfusion targets are feasible, the risks of unnecessary transfusions can be avoided with individualized targets. Also, the decision to transfuse blood can be made without delay in patients requiring red blood cell transfusion.

Methods and analysis

We will perform a prospective, single-center, observational cohort study of 65 patients receiving red blood cell transfusions in the intensive care unit. Markers such as CaO_2, $CcvO_2$, O_2ER, AV-O_2 difference and NIRS will be measured before and 15 minutes after transfusion. We will investigate whether blood transfusion is really necessary and the frequency of transfusion-related events occur in patients with an O_2ER ratio less than 30% and equal to 30% or above. All patients will be followed up to 90 days after transfusion.

Ethics and dissemination

Ethics committee approval was obtained from Izmir Katip Celebi University Non-Interventional Clinical Studies Institutional Review Board. All patients must provide written informed consent prior to enrollment in the study. Results will be disseminated through publication in peer-reviewed journals and presentation at national or international conferences.

Trial registration number

NCT05798130

Keywords: INTENSIVE & CRITICAL CARE, Blood bank & transfusion medicine, Anaemia.

Strengths and Limitations Of This Study

1.This is the first study to investigate the feasibility of red blood cell transfusion with O_2ER in stable ICU patients outside perioperative care and acute bleeding.

2.Our study will determine the contribution of transfusion to the improvement of tissue oxygenation with O_2ER, which we will calculate separately both before and after transfusion.

3.Studies reporting the use of NIRS as a decision factor for blood transfusion to date have been performed in patients with acute head trauma, which is a significant disadvantage because NIRS is affected by hemodynamic changes and intracranial pressure changes. It is an important advantage that we exclude patients with acute head trauma in our study to evaluate the results of NIRS in terms of tissue oxygenation indication and actual blood transfusion results.

4.The main limitation of our study is that this single-center study will only be performed in a center where a restrictive blood transfusion strategy is applied. Therefore, the results of the study will be withheld from patients in whom liberal strategies are applied in the transfusion decision.

5. Because of the different methodology in our study, which will evaluate the O_2ER, AV-O_2 difference, NIRS results not only before but also after blood transfusion and compare them with the initial results, it may be difficult to compare and interpret the results of previous studies.

INTRODUCTION

Red blood cell transfusion in intensive care

Red blood cell transfusion(RBCT) is an important part of the treatment modality in about 50% of intensive care unit(ICU) patients [1]. In addition, unnecessary blood transfusions have been associated with an increase in various infectious, respiratory complications, prolonged ICU and hospital stay. This leads to a significant increase in mortality and care costs [2,3]. Because of these problems, clinicians have focused on minimizing a patient's exposure to red blood cell transfusion in the ICU.

Red blood cell transfusion thresholds

The decision of which red blood cell transfusion should be given to anemic patients in the intensive care unit has always been a critical issue. Clinicians have difficulties in making decisions about the potential risks of not transfusing in necessary patients and unnecessary blood transfusion in patients who do not need it. For this reason, intensivists often attempt to determine treatment modalities according to guideline recommendations, that are consistent with hemoglobin(Hb)-based restrictive (Hb < 7g/dl) or liberal (Hb < 10g/dl) transfusion approaches were established [4,5]. However, especially recently, liberal transfusion targets have been compared with restrictive strategies in several randomized controlled trials, and they have not been shown to differ in terms of clinical benefit [6]. The correct aim here is to know which patient does not need a transfusion and to protect this patient group from the risks of unnecessary transfusion.

Physiologically based approaches to guide blood transfusion

Hemoglobin is the main component responsible for oxygen transport in the body. Many studies in the literature have examined the critical hemoglobin level sufficient to transport oxygen to tissues [7-9]. Various methods have been investigated to determine this value, such as central venous oxygen pressure (CvO_2), AV-O_2 difference, and O_2ER, as well as tissue oxygenation and NIRS monitoring [10-12]. All methods used attempt to determine the oxygen demand of the tissue and whether this demand can be met with the current hemoglobin level. From this point of view, the minimum hemoglobin value that ensures tissue oxygenation represents the optimal threshold for red blood cell transfusion. When making an individual transfusion decision, patients can be evaluated not only by their hemoglobin concentration, but also by the balance between global oxygen delivery and consumption. It is of great benefit to these clinicians not to delay transfusion in necessary patients and to avoid transfusion-related complications in non-necessary patients.

METHODS AND ANALYSIS

Study design

The present study is a prospective, single-center, observational cohort of 65 patients who received red blood cell transfusion in the intensive care unit. The study is conducted in the 23-bed capacity of the anesthesiology intensive care unit of İzmir Katip Celebi University Atatürk Training and Research Hospital. The study protocol conformed to the checklist of STROBE statements (Supplementary File 1). Ethics committee approval was obtained from Izmir Katip Celebi University Non-Interventional Clinical Studies Institutional Review Board. Written informed consent will be obtained from the first-degree relatives of all patients before enrollment in the study. The study is registered at Clinical-Trials.gov. (NCT05798130)

Study population

We will include patients aged ≥18 years old, who have been followed in the intensive care unit for at least 24 hours, who have a central venous catheter, and for whom the decision to transfuse red cells was made in the context of critical care protocols (Supplementary File 2). Patients who meet one of the following criteria will be excluded: (1) hemodynamically unstable patients; (2) patients with acute bleeding; (3) patients with hemorrhagic shock; (4) patients with acute traumatic brain injury, and (5) pregnant patients.

Patient recruitment

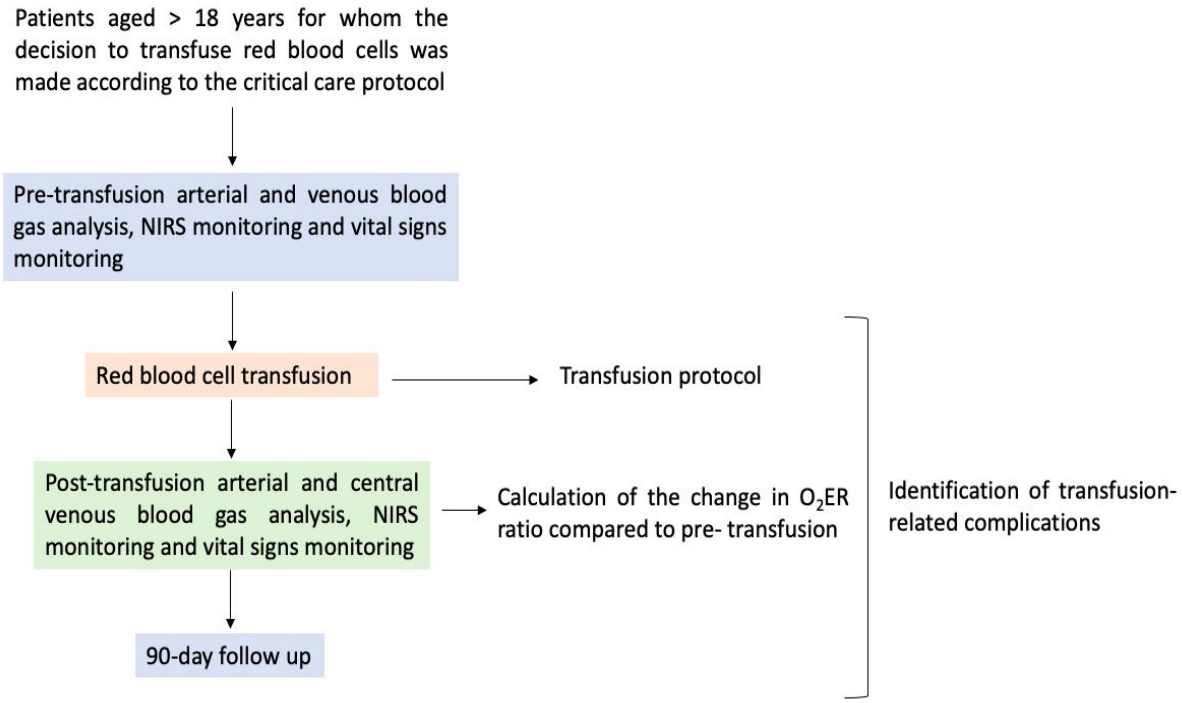
Research personnel who are not actively involved in the ICU team will screen ICU patients daily after the first visit to identify appropriate candidates. Potentially eligible patients for whom the critical care team has made a decision to transfuse blood as part of critical care protocols will be approached, and patients will be enrolled in the study by obtaining informed consent forms from their families. Written informed consent form will be obtained before the transfusion. Red blood cell transfusion will be performed in accordance with the intensive care transfusion protocol (Supplementary File 2). Patients' clinical data are recorded on case forms by the critical care team. In addition, the 90-day follow-up will be carried out by a research team that is not actively involved in the ICU. This independent team will make clinical observations, unaware of the patient's treatment decision.

Study procedures

Biomarkers' measurements

Arterial and central venous blood gas samples taken from the patient will be analyzed with the ABL800 Flex blood gas analyzer(Radiometer Medical ApS, Denmark). Both analyzes will be performed for each patient at two time points, immediately prior to blood transfusion and 15 minutes after completion of blood transfusion. In addition, routine blood drawn from patients on the day of transfusion is analyzed with the AU5800 Series Clinical Chemistry Analyzers (Beckman Coulter, USA), and the result of the hemogram is analyzed with the Sysmex XN-1000 (Sysmex Corporation, Japan). All patients will be closely followed for 90 days after transfusion. (Figure 1 – Study organigram). Complications and outcome definitions are assessed within the guidelines. (Supplementary File 3). Treatment will be at the discretion of the intensivist, taking into account the patient's risk factors and current guideline recommendations. Based on previous study recommendations, the cut-off for O_2ER will be 30%[13]. O_2ER will be calculated based on blood gas values and patients will be assigned to the appropriate group prior to statistical analysis.

Figure 1. Study organigram.



Follow-up

All patients enrolled in the study will be followed up by investigators during their hospital stay after the decision to undergo red blood cell transfusion. All patients included in the study will be followed by observers for up to 90 days during their stay in the intensive care unit or hospital (if discharged) for the occurrence of the following events: acute lung injury, acute renal failure, infections, acute myocardial infarction, delirium, thromboembolic events, stroke and death (Supplementary Material File 3). In addition, patients who are discharged home will be followed up by telephone for up to 90 days.

Outcomes

Primary outcome: Percentage change in patients' oxygen extraction rate before red blood cell transfusion and 15 minutes after transfusion.

Secondary outcome: Percentage change in NIRS values of patients before and after transfusion at the 15th minute and evaluation of complication rates such as acute lung injury, acute kidney failure, infections, thromboembolic events, also the number of days of ICU stay and hospital stay, mechanical ventilation and vasopressor-independent days, the presence of mortality at the 7th, 28th, and 90th day.

Variables

1.Variables before red blood cell transfusion: (a) age; (b) sex; (c) BMI; (d) comorbidities: hypertension, diabetes mellitus, congestive heart failure, COPD, past or current smoking, chronic renal failure, chronic liver failure, history of stroke, active malignancy; (e) reason for ICU admission: sepsis, respiratory failure, acute renal failure, trauma, cerebrovascular events, postoperative hospitalization; (f) number of days in ICU stay before transfusion; (g) number of vasopressor-dependent days before transfusion; (h) patient ventilation status; (i) number of ventilator-dependent days before transfusion; (j) hemoglobin from arterial and venous blood gas, pH, PaO_2, $PaCO_2$, SaO_2, base deficit, HCO_3, lactate values, PvO_2, $PvCO_2$, SvO_2; (k) right and left hemisphere NIRS values; (l) heart rate, systolic and diastolic blood pressure; (m) vasoactive inotropic score; (n) central venous pressure; (o) FiO_2; and (p) SAPS-II, APACHE-II,

SOFA scores; (r) hemoglobin, hematocrit, MCV, MPV, RDW, platelet values from the hemogram result; (s) INR and aPTT values; (t) BUN, creatinine, total bilirubin values; (u) CaO_2, $CcvO_2$, AV-O_2 difference, O_2ER, PaO_2/FiO_2 values.

2.Variables after transfusion of red blood cells: (a) the amount of blood product used; (b) hemoglobin from arterial and venous blood gas, pH, PaO_2, $PaCO_2$, SaO_2, base deficit, HCO_3, lactate values, PvO_2, $PvCO_2$, SvO_2; (c) right and left hemisphere NIRS values; (d) heart rate, systolic and diastolic blood pressure; (e) vasoactive inotropic score; (f) the number of days in the ICU stay after transfusion; (g) the number of vasopressor-dependent days after transfusion; (h) number of ventilator-dependent days after transfusion; (I) central venous pressure ; (j) FiO_2; (k) SOFA day-5 score; (l) hemoglobin and hematocrit values for the first 5 days; (m) CaO_2, $CcvO_2$, AV-O_2 difference, O_2ER, PaO_2/FiO_2 values; (n) presence of events that may occur in the patient after transfusion: acute lung injury, acute renal failure, infections, thromboembolic events, acute myocardial infarction, and delirium.

Data collection and data management

We will record data from the hospital's electronic clinical records and clinical bedside assessment (admission, intensive care assessment, discharge to the ward, and discharge from the hospital). After red blood cell transfusion, patients will be followed up for 90 days and their clinical data will be collected. To maintain the quality and integrity of all data, an electronic case report form (eCRF) will be designed in a secure online database (REDCAP) to capture all necessary information for the protocol for each patient (www.projectredcap.org). All clinical records, source documents, follow-up protocols, and CRFs will be kept locked in the appropriate study files at the facility. Only members of the study team have access to protected health information. Upon completion of the study, the data requested by us for reasonable reasons may be made publicly available.

Statistical considerations

Sample size

The primary aim of the study was to determine the percentage change in O_2ER ratio with red blood cell transfusion. In the reference study[13], patients will be examined in 2 separate groups with oxygen extraction rates of <30% and ≥30%. There was a significant difference between the groups in the change in oxygen extraction rate after 15 minutes. The mean±standard deviation of the groups was -5.2±7.8 and 0.7±5.8, respectively, and p=0.004. In the power analysis performed, it was assumed that the type 1 error was 0.05 and the power of the study was 0.80, so it would be sufficient to reach 29 patients in both groups. A sample size of 65 subjects was planned, assuming dropout rate of 10%.

Statistical analysis

All variables will be reviewed to avoid inconsistencies, and a flowchart will be designed to explain dropouts from the study. Continuous variables will be presented as mean and SD, or median and IQR, and categorical variables as numbers and percentage (%). Prior to all analyzes, skewness- kurtosis tests, normality tests, and histogram graphs are used prior to all analyzes to determine if the data conform to the normal distribution. When determining differences in means between groups, the T-test for independent variables or the T-test for dependent variables is used for variables with normal distribution; the Mann-Whitney U-test or the Wilcoxon test is used for variables that do not conform to normal distribution. The chi-square test or the Fischer exact test is used to determine the differences between groups for categorical variables. The OR and 95% CI for all exposure factors will also be calculated. In the case of more than two time periods, the evaluation of time, groups, and joint effects will be performed using the two-way test ANOVA (analysis of variance) for repeated measures. Post-hoc analyzes will be performed using the Bonferonni test. The effect of independent variables on mortality will be assessed by logistic regression analysis. Kaplan-Meier survival curves will be analyzed with respect to the oxygen extraction rate predicting 28-day mortality in both groups. P values less than 0.05 will be considered statistically significant.

Study organisation

Our study was designed as an observational cohort, an oversight committee of investigators with considerable experience and knowledge in critical care medicine will be

established to ensure high data quality, consistency and accuracy of data entries in the eCRF, and protocol compliance and completeness.

Patient and public involvement

No patients were included in the proposal of the research question or outcome measures or in the design or conduct of the study. Management of complications that may occur after blood transfusion in patients observed in our study is performed by the intensive care team in consultation with the departments of thoracic diseases, infectious diseases, cardiology, and nephrology. There are no plans to share the results of the study with the study participants.

Ethics considerations

Ethical approval for this study, whose protocol ID was 2023-GOKAE-0103, was provided by Izmir Katip Celebi University Non-Interventional Clinical Studies Institutional Review Board. The study was designed in accordance with the Declaration of Helsinki, a statement of ethical principles for medical research. Research staff, adhering to good clinical practice, will obtain written informed consent for each patient who agrees to participate in the study. Only the investigators and ethics committees involved in the study will have access to the clinical data. All data obtained will be stored in a secure online database with a special identification number to maintain strict confidentiality.

DISCUSSIONS

Executive summary

Hemoglobin-based liberal or restrictive transfusion strategies are commonly used in decision-making blood transfusion in the intensive care unit. However, the available data on which patient benefits from blood transfusion and which patient is at risk for unnecessary transfusion-related complications, especially in stable patients without acute bleeding, are not yet convincing, which raises interest. For this reason, physiologically based transfusion

targets in blood transfusion decisions are the subject of research, particularly in perioperative medicine and cardiac surgery. This is particularly important for the patient population cared for in the intensive care unit, where the priority is to prevent complications. For this reason, in addition to hemoglobin-targeted transfusion approaches, the oxygen extraction rate, which also shows the physiological-based tissue oxygenation adequacy, can be taken into consideration when making the transfusion decision. We hypothesize that can better predict transfusion-related complications in this patient group. Our data provide a valuable overview to optimize transfusion decision-making and improve prognosis by preventing complications in patients receiving blood transfusions.

Our study in the context of previous research

This study is attracting attention because it is the first recent study evaluating tissue oxygenation-based blood transfusion decision making in patients without acute bleeding and hemodynamically stable follow-up in the intensive care unit. We believe that individualized strategies for transfusion decision-making based on adequate tissue oxygenation may improve the predictive value for transfusion-related complications compared with strategies based on hemoglobin levels, potentially improving the management and prognosis of these patients. In addition, determining the diagnostic thresholds of these biomarkers for transfusion-related risk stratification may not only help to reduce patient morbidity and mortality, but also reduce the length of hospital stay and thus the associated costs.

Implications for practice and research

The existence of a direct relationship between individualized, patient-based oxygen extraction rates and the decision to transfuse red blood cells and reduce transfusion-related complications may imply that physiologically based blood transfusion targets should be established and routinely incorporated into existing transfusion decision-making protocols.

Studys's strengths and limitations

Our study has several limitations. First, our single-center study will only be performed in a center where a restrictive blood transfusion strategy is applied. Therefore, the results of the study will be withheld from patients in whom liberal strategies are applied in the transfusion decision. Second, because of the different methodology in our study, which will evaluate the O_2ER, AV-O_2 difference, NIRS results not only before but also after blood transfusion and compare them with the initial results, it may be difficult to compare and interpret the results of previous studies.

Our study also has some strenghts. First, to our knowledge, this is the first study to investigate the feasibility of red blood cell transfusion with O_2ER in stable ICU patients outside perioperative care and acute bleeding. Our study will determine the contribution of transfusion to the improvement of tissue oxygenation with O_2ER, which we will calculate separately both before and after transfusion. The development of markers such as O_2ER that indicate the adequacy of tissue oxygenation and the development of protocols that target the need for red blood cell transfusion may be very important in improving prognosis.

Studies reporting the use of NIRS as a decision factor for blood transfusion to date have been performed in patients with acute head trauma, which is a significant disadvantage because NIRS is affected by hemodynamic changes and intracranial pressure changes. It is an important advantage that we exclude patients with acute head trauma in our study to evaluate the results of NIRS in terms of tissue oxygenation indication and actual blood transfusion results.

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Supplementary File 1

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page: 1,3)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page: 3-4)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page: 5-6)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page: 5-13)
Methods		
Study design	4	Present key elements of study design early in the paper (Page: 6)

Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page: 7)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (Page: 7)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page: 9-10)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page: 8-10)
Bias	9	Describe any efforts to address potential sources of bias (Page: 11-12)
Study size	10	Explain how the study size was arrived at (Page: 11)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page: 9-10)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page: 11)
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed (Page: 11)
		(*d*) If applicable, explain how loss to follow-up was addressed (Page: 9-11)
		(*e*) Describe any sensitivity analyses (Page: 11)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page: 13)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page: 12-13)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page: 12-13)
Other information		

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page: 2)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Supplementary File 2

The decision to transfuse is made by the intensivist as part of the local critical care protocol. This protocol consists of policy that include:

- Hb target of 7-8 g/dL
- A clinical judgment that transfusion is appropriate, in addition to the Hb concentration
- Hydration with 150 mL of normal saline prior to transfusion
- The infusion is administered at a rate of 2 ml/min for the first 15 minutes. If vital signs are normal at the 15th minute, transfusion is complete after 40 minutes.
- Administer single unit transfusion, clinical assessment, then use 2nd unit strategy (if 2 units transfusion is decided)
- Red cell suspensions with leukocyte filters are used for all red cell transfusions.
- Red cell packets transfused during the study period will be stored at ± 2°C for an average of up to 40 days.

Supplementary File 3

Complication and Outcome definitions:

1.Acute lung injury: Acute onset(< 7days), PaO2/FiO2< 300mmHg, diffuse-bilateral infiltrates on CXR, No signs of hydrostatic pulmonary edema(CVP≤ 15 mmHg), no other risk factor for ALI.

2. Acute kidney injury: One of the following: (1) ≤7 days, (2) Creatinine ≥1.5 times baseline (or increase of ≥0.3 mg/dL within any 48 h period), and (3) Urine volüme<0.5 ml/kg for ≥6h.

3.Nasocomial infections: Any positive result from blood, sputum, or urine cultures (requiring antibiotic use) within 48 hours of a blood transfusion or 7 days after discharge from the intensive care unit.

4.Acute myocardial infarction: One of the following: (1) a typical rise of troponin, a typical fall of a raised troponin, or a rapid rise and fall of CK-MB; (2) ischaemic symptoms (eg, chest, epigastric, arm, wrist, or jaw discomfort, or shortness of breath); (3) ECG changes indicative of ischaemia

5.Delirium: Impaired (i.e., decreased clarity of environmental awareness), cognitive changes (e.g., memory deficit, disorientation, language impairment, perceptual impairment), symptoms develop over a short period of time (usually hours to days) and tend to fluctuate throughout the day, and medical history, physical examination demonstrating that the complaints are due to an underlying organic general medical cause

6.Thromboembolic events: Additional diagnostic tests are performed in patients with high D-dimer levels: Serial bedside ultrasound, including the entire proximal and lower extremities will be performed to diagnose DVT, and computed tomography pulmonary angiography (CTPA) will be performed for PE.

7.Stroke: A new focal neurological deficit thought to be vascular in origin with signs and symptoms lasting more than 24h.

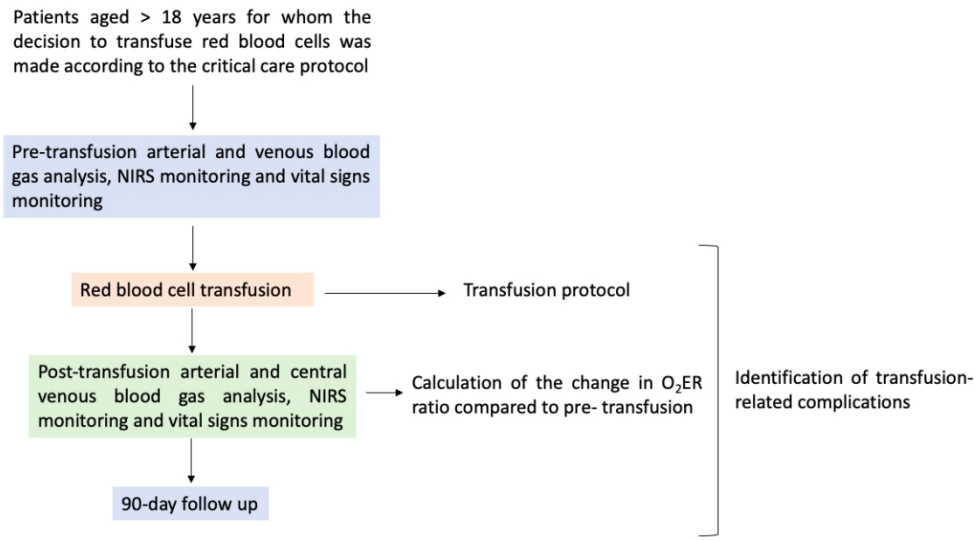

Figure 1

419x237mm (72 x 72 DPI)

Supplementary File 1

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page: 1,3)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page: 3-4)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page: 5-6)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page: 5-13)
Methods		
Study design	4	Present key elements of study design early in the paper (Page: 6)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page: 7)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (Page: 7)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page: 9-10)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page: 8-10)
Bias	9	Describe any efforts to address potential sources of bias (Page: 11-12)
Study size	10	Explain how the study size was arrived at (Page: 11)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page: 9-10)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page: 11)
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed (Page: 11)
		(*d*) If applicable, explain how loss to follow-up was addressed (Page: 9-11)
		(*e*) Describe any sensitivity analyses (Page: 11)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included

		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page: 13)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page: 12-13)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page: 12-13)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page: 2)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Supplementary File 2

The decision to transfuse is made by the intensivist as part of the local critical care protocol. This protocol consists of policy that include:

- Hb target of 7-8 g/dL
- A clinical judgment that transfusion is appropriate, in addition to the Hb concentration
- Hydration with 150 mL of normal saline prior to transfusion
- The infusion is administered at a rate of 2 ml/min for the first 15 minutes. If vital signs are normal at the 15th minute, transfusion is complete after 40 minutes.
- Administer single unit transfusion, clinical assessment, then use 2nd unit strategy (if 2 units transfusion is decided)
- Red cell suspensions with leukocyte filters are used for all red cell transfusions.
- Red cell packets transfused during the study period will be stored at ± 2°C for an average of up to 40 days.

Supplementary File 3

Complication and Outcome definitions:

1.Acute lung injury: Acute onset(< 7days), PaO2/FiO2< 300mmHg, diffuse-bilateral infiltrates on CXR, No signs of hydrostatic pulmonary edema(CVP≤ 15 mmHg), no other risk factor for ALI.

2. Acute kidney injury: One of the following: (1) ≤7 days, (2) Creatinine ≥1.5 times baseline (or increase of ≥0.3 mg/dL within any 48 h period), and (3) Urine volüme<0.5 ml/kg for ≥6h.

3.Nasocomial infections: Any positive result from blood, sputum, or urine cultures (requiring antibiotic use) within 48 hours of a blood transfusion or 7 days after discharge from the intensive care unit.

4.Acute myocardial infarction: One of the following: (1) a typical rise of troponin, a typical fall of a raised troponin, or a rapid rise and fall of CK-MB; (2) ischaemic symptoms (eg, chest, epigastric, arm, wrist, or jaw discomfort, or shortness of breath); (3) ECG changes indicative of ischaemia

5.Delirium: Impaired (i.e., decreased clarity of environmental awareness), cognitive changes (e.g., memory deficit, disorientation, language impairment, perceptual impairment), symptoms develop over a short period of time (usually hours to days) and tend to fluctuate throughout the day, and medical history, physical examination demonstrating that the complaints are due to an underlying organic general medical cause

6.Thromboembolic events: Additional diagnostic tests are performed in patients with high D-dimer levels: Serial bedside ultrasound, including the entire proximal and lower extremities will be performed to diagnose DVT, and computed tomography pulmonary angiography (CTPA) will be performed for PE.

7.Stroke: A new focal neurological deficit thought to be vascular in origin with signs and symptoms lasting more than 24h.

BMJ Open

Assessment of Oxygen Extraction Rate Changes Following Red Blood Cell Transfusion in the Intensive Care Unit: A Protocol for a Prospective Observational Non-Interventional Study

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Complete List of Authors:	Tüzen, Ahmet Salih; Izmir Katip Celebi University, Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation Aksun, Murat; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Intensive Care Medicine Sencan, Atilla; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Intensive Care Medicine Girgin, Senem; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation Gölboyu, Birzat Emre; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation Kırbaş, Gizem; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation Şanlı, Ozan; Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation
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Assessment of Oxygen Extraction Rate Changes Following Red Blood Cell Transfusion in the Intensive Care Unit: A Protocol for a Prospective Observational Non-Interventional Study

Author affiliations

Ahmet Salih Tüzen, M.D. (Corresponding author)
astuzen@icloud.com
+905353915577
Department of Anesthesiology and Reanimation, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Murat Aksun, M.D. Ph.D.
murataksun@yahoo.com
Department of Intensive Care Medicine, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Atilla Şencan, M.D.
atillasencan2000@yahoo.com
Department of Intensive Care Medicine, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Senem Girgin, M.D.
senemcan@gmail.com
Department of Anesthesiology and Reanimation, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Birzat Emre Gölboyu, M.D.
birzatemre@windowslive.com
Department of Anesthesiology and Reanimation, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Gizem Kırbaş, M.D.

gizembas35@gmail.com
Department of Anesthesiology and Reanimation, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

Ozan Şanlı, M.D.
ozan_sanli@yahoo.com
Department of Anesthesiology and Reanimation, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir
Turkey

Author's initials: Tüzen AS., Aksun M., Şencan A., Girgin S., Gölboyu BE., Kırbaş G., Şanlı O.

ORCID iDs

Ahmet Salih Tüzen: 0000-0001-9040-2262
Murat Aksun: 0000-0002-8308-3045
Atilla Şencan: 0000-0002-3765-9891
Senem Girgin: 0000-0003-0715-7695
Birzat Emre Gölboyu: 0000-0002-2011-2574
Gizem Kırbaş: 0000-0003-1178-5723
Ozan Şanlı: 0000-0001-8067-7908

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ABSTRACT

Introduction

Hemoglobin transfusion thresholds have been utilized in the intensive care unit (ICU) to guide red blood cell transfusion (RBCT) decisions. Recent research has also focused on physiological indicators of tissue oxygenation as trigger points for blood transfusion. This study aims to

assess the oxygen extraction rate (O2ER) as a critical indicator of the oxygen delivery-consumption balance in tissues and investigate its potential as a reliable trigger for blood transfusion in ICU patients by analyzing clinical outcomes. The utilization of physiological indicators may expedite the decision-making process for RBCT in patients requiring immediate intervention, while simultaneously minimizing the risks associated with unnecessary transfusions.

Methods and analysis

This prospective, single-center, observational cohort study will include 65 ICU patients undergoing RBCT. We will evaluate essential markers such as arterial oxygen content (CaO_2), central venous oxygen content ($CcvO_2$), arteriovenous oxygen difference (AV-O_2 difference), O_2ER, and near-infrared spectroscopy (NIRS) before and 15 minutes after transfusion. The primary outcome is the percentage increase in O_2ER between the two groups relative to the initial O_2ER level. Secondary outcomes will assess complications and patient outcomes in relation to baseline O_2ER. A 90-day comprehensive follow-up period will be implemented for all enrolled patients.

Ethics and dissemination

This study has obtained ethics committee approval from the Izmir Katip Celebi University Non-Interventional Clinical Studies Institutional Review Board. Written informed consent will be obtained from all patients before their enrollment in the study. The findings will be disseminated through publication in peer-reviewed journals and presentation at national or international conferences.

Trial registration number

NCT05798130

Keywords: red blood cell transfusion, oxygen consumption, intensive care unit.

Strengths and Limitations Of This Study

1. Our study will assess the impact of transfusion on tissue oxygenation by calculating O_2ER both before and after transfusion, aiming to determine its contribution to improvement in critical care patients.

2. In this study, we will exclude patients with acute head trauma to evaluate the correlation between NIRS measurements as an indicator of tissue oxygenation and the actual blood transfusion outcomes.

3. The primary limitation of our study is its single-center design, which may limit the generalizability of the results to patients managed in other centers implementing different protocols or practices.

4. The unique methodology of our study, which involves the assessment of O_2ER, $AV\text{-}O_2$ difference, and NIRS results before and after blood transfusion, may present challenges in directly comparing and interpreting findings from previous studies.

List of abbreviations

$AV\text{-}O_2$: Arteriovenous oxygen, CaO_2: Arterial oxygen content, $CcvO_2$: Central venous oxygen content, ICU: Intensive care unit, NIRS: Near infrared spectroscopy, O_2ER: Oxygen extraction ratio, RBCT: Red blood cell transfusion.

INTRODUCTION

Red blood cell transfusion in intensive care

Red blood cell transfusion (RBCT) plays a crucial role in the treatment of approximately 50% of intensive care unit (ICU) patients [1]. However, the administration of unnecessary blood transfusions has been linked to elevated risks of infectious and respiratory complications, prolonged ICU and hospital stays, and ultimately, increased mortality and healthcare expenses

[2,3]. Clinicians have prioritized efforts to reduce patients' exposure to red blood cell transfusions in the ICU.

Red blood cell transfusion thresholds

The decision regarding RBCT for anemic patients in the ICU presents a significant challenge for clinicians. Balancing the potential risks of not transfusing when necessary and the risks of unnecessary transfusion is complex. As a result, intensivists often rely on guideline recommendations and established transfusion approaches [4,5]. However, recent randomized controlled trials comparing hemoglobin-based transfusion strategies have shown no significant differences in clinical benefits [6]. The primary objective is to identify patients who do not require transfusion and mitigate the potential risks associated with unnecessary blood transfusions.

Physiologically based approaches to guide blood transfusion

Hemoglobin, as the primary component responsible for oxygen transport in the body, perform a crucial role in tissue oxygenation. Extensive research has been conducted to determine the critical hemoglobin level necessary for adequate oxygen delivery to tissues [7-9]. Various methods, including arterial oxygen content (CaO_2), central venous oxygen content ($CcvO_2$), arteriovenous oxygen ($AV\text{-}O_2$) difference, oxygen extraction ratio (O_2ER), and tissue oxygenation monitoring through near infrared spectroscopy (NIRS), have been investigated to assess this value [10-12]. These methods aim to evaluate tissue oxygen demand and assess whether the current hemoglobin level is sufficient to meet that demand. Thus, the minimum hemoglobin value required for optimal tissue oxygenation represents the threshold for RBCT. When making individualized transfusion decisions, clinicians can assess patients not only based on their hemoglobin concentration but also by considering the balance between global oxygen delivery and consumption. Prompt transfusion in necessary cases and the avoidance of transfusion-related complications in patients who do not require it are of significant benefit to clinicians.

METHODS AND ANALYSIS

Study design

This prospective, single-center, observational cohort study will enroll 65 ICU patients who received red blood cell transfusions. Based on a previous similar study [13], we will compare O2ER results before and 15 minutes after the completion of transfusion in this study. Key markers, including CaO_2, $CcvO_2$, O_2ER, AV-O_2 difference, and NIRS, will be evaluated two time points. The primary outcome will calculate the percentage increase in O_2ER between the two groups relative to the initial O_2ER level. Secondary outcomes will include the assessment of complications and patient outcomes in relation to the baseline O_2ER. A comprehensive follow-up period of 90 days will be conducted for all enrolled patients. The study adheres to the STROBE guidelines (Supplementary File 1) and has obtained approval from the Izmir Katip Celebi University Non-Interventional Clinical Studies Institutional Review Board, ensuring compliance with ethical considerations. Written informed consent will be obtained from the first-degree relatives of all patients prior to enrollment. The study is registered at ClinicalTrials.gov with the identifier NCT05798130.

Study population

The inclusion criteria for this study are as follows: patients aged 18 years or older, who have been admitted to the ICU and have been under observation for a minimum of 24 hours, have a central venous catheter in place, and have received RBCT as per critical care protocols (Supplementary File 2). Patients who meet any of the following exclusion criteria will not be included in the study: (1) hemodynamically unstable patients (receiving inotropes and vasopressors); (2) patients with acute bleeding; (3) patients with hemorrhagic shock; (4) patients with acute traumatic brain injury; and (5) pregnant patients.

Patient recruitment

The screening of potential participants for this study will be performed by research personnel who are not affiliated with the ICU team. Patients who meet the inclusion criteria and for whom the critical care team has already made a decision to perform a RBCT as part of the

critical care protocols will be approached for enrollment in the study. Informed consent forms will be obtained from the patients' families before transfusion. The RBCT procedure will follow the critical care transfusion protocol outlined in Supplementary File 2. The critical care team will record patients' clinical data on case forms. Additionally, a 90-day follow-up will be conducted by a separate research team not directly involved in the ICU, ensuring an unbiased assessment of patient outcomes without knowledge of the treatment decision. Patient enrollment commenced on April 4, 2023, initiating data collection. The process of patient recruitment is currently ongoing. All data analyses will be performed upon the completion of patient enrollment.

Study procedures

Biomarkers' measurements

Arterial and central venous blood gas samples obtained from each patient will be analyzed using the ABL800 Flex blood gas analyzer (Radiometer Medical ApS, Denmark). These analyses will be conducted at two time points for each patient: immediately before the blood transfusion and 15 minutes after the completion of the transfusion. Routine blood samples collected from patients on the day of transfusion will also be analyzed using the AU5800 Series Clinical Chemistry Analyzers (Beckman Coulter, USA) for standard blood chemistry parameters, while the hemogram results will be analyzed using the Sysmex XN-1000 (Sysmex Corporation, Japan) for complete blood count parameters. The patients will be closely monitored for a period of 90 days following the transfusion. The flow chart, as depicted in Figure 1, illustrates the flow and organization of the study. Complications and outcome definitions will be assessed in accordance with established guidelines, which are provided in Supplementary File 3. The treatment approach will be determined at the discretion of the intensivist, considering the patient's risk factors and current guideline recommendations. Based on recommendations from previous studies, an O_2ER cut-off value of 30% will be utilized [11,13].

Follow-up

All patients who are enrolled in the study will be closely monitored by investigators throughout their hospital stay following the decision to undergo red blood cell transfusion. Observers will be responsible for tracking the occurrence of specific events during the patients' stay in the ICU or hospital (if discharged) for a period of up to 90 days. These events include acute lung injury, transfusion associated circulatory overload, acute renal failure, infections, acute myocardial infarction, delirium, thromboembolic events, stroke, and death, as outlined in Supplementary File 3. Additionally, patients who are discharged home will be followed up via telephone for up to 90 days to ensure ongoing monitoring and assessment.

Outcomes

The primary outcome of this study is the percentage change in patients' O_2ER before undergoing RBCT compared to 15 minutes after the transfusion. This measurement will provide valuable information on the impact of transfusion on oxygen utilization in the body. The secondary outcome includes various complication rates such as acute lung injury, acute kidney failure, infections, and thromboembolic events. The number of days stay in the ICU and hospital, as well as the duration of mechanical ventilation and vasopressor-independent days, will also be assessed. Furthermore, the presence of mortality at the 7th, 28th, and 90th day will be documented to examine the overall impact of transfusion on patient outcomes. These outcomes will collectively contribute to a comprehensive evaluation of the effects of RBCT on oxygen utilization, tissue oxygenation, and clinical outcomes in the study population.

Variables

1.Variables before red blood cell transfusion: (a) age; (b) sex; (c) BMI; (d) comorbidities: hypertension, diabetes mellitus, congestive heart failure, chronic obstructive pulmonary disease, past or current smoking, chronic renal failure, chronic liver failure, history of stroke, active malignancy; (e) reason for ICU admission: sepsis, respiratory failure, acute renal failure, trauma, cerebrovascular events, postoperative hospitalization; (f) number of days in ICU stay before transfusion; (g) number of vasopressor-dependent days before transfusion; (h) patient ventilation status; (i) number of ventilator-dependent days before transfusion; (j) hemoglobin

from arterial and venous blood gas, pH, PaO_2, $PaCO_2$, SaO_2, base deficit, HCO_3, lactate values, PvO_2, $PvCO_2$, SvO_2; (k) right and left hemisphere NIRS values; (l) heart rate, systolic and diastolic blood pressure; (m) vasoactive inotropic score; (n) central venous pressure; (o) FiO_2; and (p) SAPS-II, APACHE-II, SOFA scores; (r) hemoglobin, hematocrit, MCV, MPV, RDW, platelet values from the hemogram result; (s) INR and aPTT values; (t) BUN, creatinine, total bilirubin values; (u) CaO_2, $CcvO_2$, AV-O_2 difference, O_2ER, PaO_2/FiO_2 values.

2.Variables after transfusion of red blood cells: (a) the amount of blood product used; (b) hemoglobin from arterial and venous blood gas, pH, PaO_2, $PaCO_2$, SaO_2, base deficit, HCO_3, lactate values, PvO_2, $PvCO_2$, SvO_2; (c) right and left hemisphere NIRS values; (d) heart rate, systolic and diastolic blood pressure; (e) vasoactive inotropic score; (f) the number of days in the ICU stay after transfusion; (g) the number of vasopressor-dependent days after transfusion; (h) number of ventilator-dependent days after transfusion; (I) central venous pressure ; (j) FiO_2; (k) SOFA day-5 score; (l) hemoglobin and hematocrit values for the first 5 days; (m) CaO_2, $CcvO_2$, AV-O_2 difference, O_2ER, PaO_2/FiO_2 values; (n) presence of events that may occur in the patient after transfusion: acute lung injury, transfusion associated circulatory overload, acute renal failure, infections, thromboembolic events, acute myocardial infarction, and delirium.

Data collection and data management

Data collection for the study will involve recording information from the hospital's electronic clinical records and conducting clinical bedside assessments at various stages of patient care (admission, intensive care assessment, discharge to the ward, and hospital discharge). Throughout the 90-day follow-up period after red blood cell transfusion, patients' clinical data will be collected and documented. To ensure data quality and integrity, an electronic case report form (eCRF) will be developed using a secure online database called REDCap (Research Electronic Data Capture) at www.projectredcap.org. This platform will enable the systematic collection of all necessary information specified in the study protocol for each patient. All relevant clinical records, source documents, follow-up protocols, and completed case report forms will be securely stored and locked in study files at the facility. Access to protected health information will be restricted to authorized members of the study team to ensure

confidentiality. Upon completion of the study, certain data may be made publicly available upon reasonable request. The specifics of which data will be shared and under what conditions will be determined and communicated by the researchers responsible for the study.

Statistical considerations

Sample size

The primary objective of the study is to assess the percentage change in the O_2ER following RBCT. The study will compare two groups of patients based on their initial O_2ER levels: one group with $O_2ER < 30\%$ and the other with O2ER ≥ 30%. In a reference study [13], significant differences were observed between the two groups in terms of the change in O_2ER after 15 minutes. The mean ± standard deviation values for the groups were -5.2 ± 7.8 and 0.7 ± 5.8, respectively, with a p-value of 0.004. To determine the sample size for the current study, a power analysis was conducted assuming a type 1 error of 0.05 and a study power of 0.80. Based on these calculations, it was determined that a total of 29 patients in each group would be sufficient. Taking into account a potential dropout rate of 10%, a sample size of 65 subjects was planned for the study.

Statistical analysis

Prior to conducting the analyses, tests for skewness, kurtosis, normality, and histogram graphs will be performed to assess if the data follows a normal distribution. For variables that follow a normal distribution, independent samples T-test or paired samples T-test will be used to determine differences in means between groups or within groups, respectively. For variables that do not follow a normal distribution, the Mann-Whitney U-test or Wilcoxon test will be employed. Continuous variables will be presented as mean and standard deviation (SD) or median and interquartile range (IQR), while categorical variables will be presented as numbers and percentages (%). To assess differences between groups for categorical variables, the chi-square test or Fischer's exact test will be used. In cases where there are more than two time periods, the two-way analysis of variance (ANOVA) for repeated measures will be used to

evaluate the effects of time, groups, and their interactions. Post-hoc analyses will be conducted using the Bonferroni test. Logistic regression analysis will be employed to assess the effect of independent variables on mortality. A significance level of $p < 0.05$ will be used to determine statistical significance.

Study organisation

To ensure the high quality and integrity of the study data, an oversight committee consisting of experienced investigators in critical care medicine will be established. The committee's role will be to oversee the study, monitor data quality, ensure consistency and accuracy of data entries in the electronic case report form (eCRF), and ensure compliance with the study protocol. The oversight committee will actively review the data collected, identify any potential inconsistencies or errors, and work closely with the study team to address any issues that arise. They will also provide guidance and support to ensure that the study protocol is followed correctly and that data collection is complete.

Patient and public involvement

Patients were not involved in the selection of outcome measures, study design, or study implementation. The intensive care team, in collaboration with the departments of thoracic diseases, infectious diseases, cardiology, and nephrology, will be responsible for managing any complications that may arise following blood transfusion in the observed patients. There are no plans to share the results of the study with the study participants.

Ethics considerations

The study protocol, with the identification number 0085 and the approval date of 02.23.2023, has obtained ethical approval from the Institutional Review Board for Non-Interventional Clinical Studies at Izmir Katip Celebi University. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, which governs medical research involving human subjects. Prior to enrollment, the research team, following good clinical practice guidelines, will obtain written informed consent from each patient who expresses

willingness to participate in the study. The clinical data collected will be accessible only to the investigators and the ethics committees involved in the study, ensuring confidentiality. To maintain strict confidentiality, all data will be securely stored in an online database with unique identification numbers assigned to each participant.

DISCUSSIONS

Executive summary

Hemoglobin-based transfusion strategies are commonly employed in determining blood transfusions for ICU patients. However, the existing evidence regarding which patients truly benefit from transfusion and who may experience avoidable complications remains inconclusive, particularly for stable patients without acute bleeding. Addressing this knowledge gap has generated considerable interest, emphasizing the importance of research focusing on physiologically based transfusion targets, particularly in perioperative and critical care medicine. This is of particular significance in ICU patients, where preventing complications takes precedence. In addition to hemoglobin-targeted transfusion approaches, O_2ER as an indicator of tissue oxygenation adequacy can be considered when making transfusion decisions. We believe that incorporating O_2ER as a predictive factor can enhance our ability to anticipate transfusion-related complications in this patient population. Our study aims to offer a comprehensive overview, optimizing transfusion decision-making and enhancing patient outcomes by mitigating complications associated with blood transfusions.

Our study in the context of previous research

This study is anticipated to draw significant attention as it represents a pioneering investigation into tissue oxygenation-based blood transfusion decision-making among hemodynamically stable patients without acute bleeding in the ICU. We hypothesize that adopting individualized transfusion strategies guided by assessments of tissue oxygenation adequacy may offer superior predictive value for transfusion-related complications compared to approaches solely based on hemoglobin levels. These personalized strategies have the potential to enhance patient management and prognosis in this specific population.

Moreover, establishing diagnostic thresholds of these biomarkers for risk stratification related to transfusion can not only reduce patient morbidity and mortality but also lead to shorter hospital stays, thereby mitigating associated costs. By emphasizing the significance of adequate tissue oxygenation in transfusion decision-making, this study aims to provide valuable insights that can positively impact patient outcomes and optimize resource utilization in clinical settings.

Implications for practice and research

This study aims to explore the association between personalized oxygen extraction rates and the decision-making process for red blood cell transfusions, highlighting the significance of integrating physiologically-based transfusion targets with current protocols. By adopting a patient-centered approach that considers individual physiological characteristics and oxygenation requirements, clinicians can make well-informed and optimized transfusion decisions. This has the potential to enhance patient outcomes, decrease transfusion-related complications, and improve overall transfusion practices in clinical settings.

Studys's strengths and limitations

Our study has several acknowledged limitations. Firstly, it is a single-center study conducted in a facility following a restrictive blood transfusion strategy, potentially limiting the generalizability of our results to patients managed under different transfusion approaches. Secondly, our unique methodology, may hinder direct comparisons with previous studies using different approaches. Despite these limitations, our study boasts notable strengths. To our knowledge, it is the first investigation exploring the O_2ER as a transfusion indicator in stable ICU patients outside of perioperative care and acute bleeding scenarios. A distinct advantage of our study is the exclusion of patients with acute head trauma, enabling us to evaluate NIRS in terms of tissue oxygenation and its correlation with actual blood transfusion outcomes in different clinical conditions.

Contributorship statement

A.S.T. (Principal Investigator), M.A. and A.S. wrote the first draft of the protocol manuscript. A.S.T., M.A. and B.E.G. planned the conception and design of the study and the protocol. G.K., O.S. and S.G. contributed to the design and implementation of the protocol. All authors provided critical revisions to the manuscript before approving the final version.

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None.

Funding

None.

Competing interests

None declared.

Patient consent for publication

Informed consent will be obtained from the patient's first-degree relatives.

Data availability statement

All data necessary to support the protocol are available upon reasonable request.

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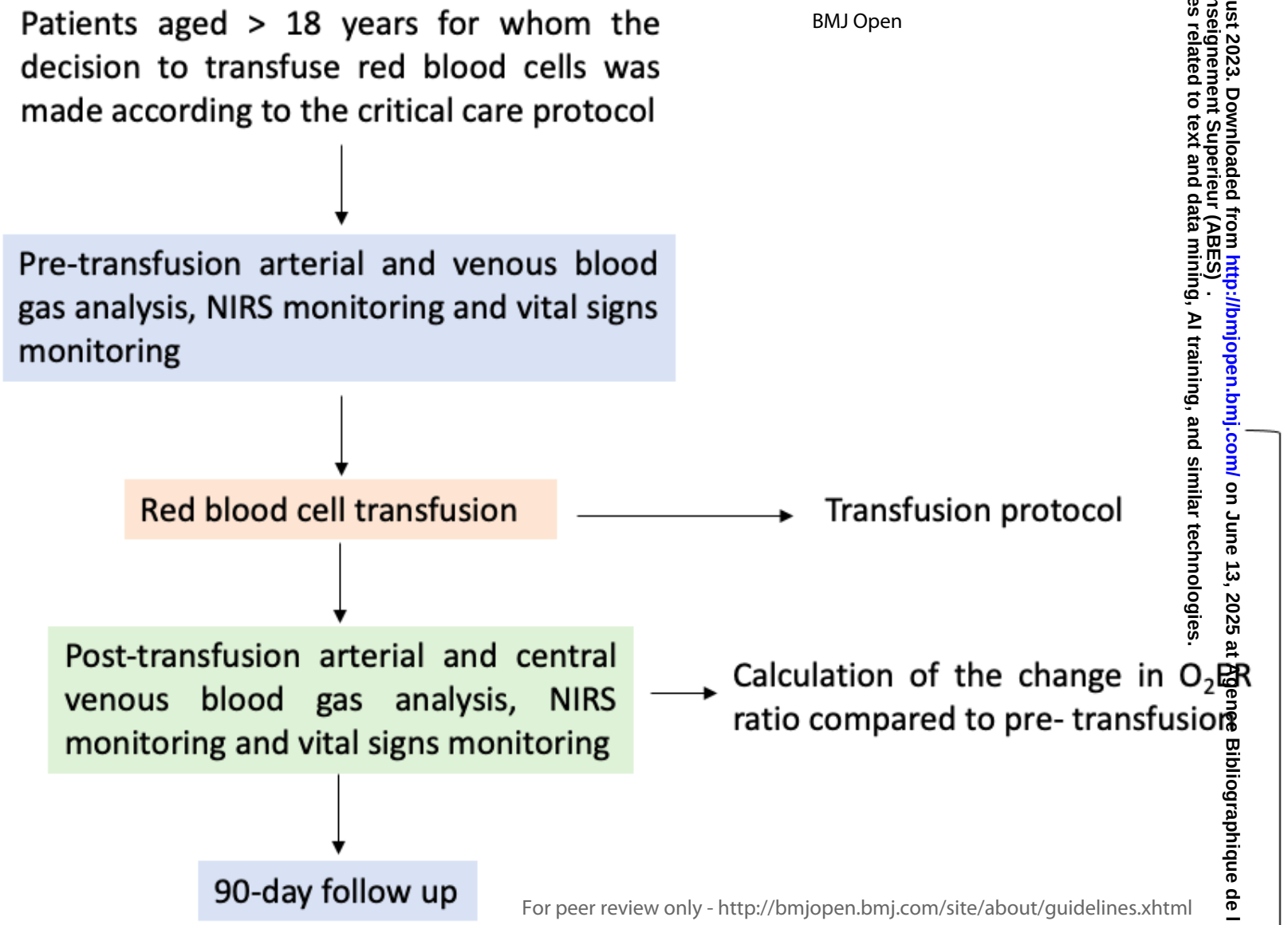
Figure Legend:

Figure 1 - Flow chart.



Supplementary File 1

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page: 1-3)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page: 3-4)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page: 4-5)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page: 4-5)
Methods		
Study design	4	Present key elements of study design early in the paper (Page: 6)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page: 7)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (Page: 6)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page: 8-9)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page: 7-10)
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at (Page: 10)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page: 8-9)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page: 10-11)
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses (Page: 10-11)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included

		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page: 13)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page: 12-13)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page: 12-13)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page: 14)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Supplementary File 2

The decision to transfuse is made by the intensivist as part of the local critical care protocol. This protocol consists of policy that include:

- Hb target of 7-8 g/dL
- A clinical judgment that transfusion is appropriate, in addition to the Hb concentration
- Hydration with 150 mL of normal saline prior to transfusion
- The infusion is administered at a rate of 2 ml/min for the first 15 minutes. If vital signs are normal at the 15th minute, transfusion is complete after 40 minutes.
- Administer single unit transfusion, clinical assessment, then use 2nd unit strategy (if 2 units transfusion is decided)
- Red cell suspensions with leukocyte filters are used for all red cell transfusions.
- Red cell packets transfused during the study period will be stored at ± 2°C for an average of up to 40 days.

Supplementary File 3

Complication and Outcome definitions:

1.Acute lung injury: Acute onset(< 7days), PaO2/FiO2< 300mmHg, diffuse-bilateral infiltrates on CXR, No signs of hydrostatic pulmonary edema(CVP≤ 15 mmHg), no other risk factor for ALI.

2.Transfusion associated circulatory overload: Acute respiratory distress, tachycardia, elevated blood pressure, acute or deteriorating pulmonary edema, and positive fluid balance within 6 hours post-transfusion.

3. Acute kidney injury: One of the following: (1) ≤7 days, (2) Creatinine ≥1.5 times baseline (or increase of ≥0.3 mg/dL within any 48 h period), and (3) Urine volüme<0.5 ml/kg for ≥6h.

4.Nasocomial infections: Any positive result from blood, sputum, or urine cultures (requiring antibiotic use) within 48 hours of a blood transfusion or 7 days after discharge from the intensive care unit.

5.Acute myocardial infarction: One of the following: (1) a typical rise of troponin, a typical fall of a raised troponin, or a rapid rise and fall of CK-MB; (2) ischaemic symptoms (eg, chest, epigastric, arm, wrist, or jaw discomfort, or shortness of breath); (3) ECG changes indicative of ischaemia

6.Delirium: Impaired (i.e., decreased clarity of environmental awareness), cognitive changes (e.g., memory deficit, disorientation, language impairment, perceptual impairment), symptoms develop over a short period of time (usually hours to days) and tend to fluctuate throughout the day, and medical history, physical examination demonstrating that the complaints are due to an underlying organic general medical cause

7.Thromboembolic events: Additional diagnostic tests are performed in patients with high D-dimer levels: Serial bedside ultrasound, including the entire proximal and lower extremities will be performed to diagnose DVT, and computed tomography pulmonary angiography (CTPA) will be performed for PE.

8.Stroke: A new focal neurological deficit thought to be vascular in origin with signs and symptoms lasting more than 24h.