PEER REVIEW HISTORY

ARTICLE DETAILS

Title (Provisional)

Oxygen extraction-guided transfusion strategy in critically ill patients: study protocol for a randomized, open-labelled, controlled trial

Authors

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VERSION 1 - REVIEW

Reviewer **1**

Name **Tüzen, Ahmet Salih**

Affiliation **Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation**

Date **10-Jul-2024**

COI **I do not have any conflict of interest.**

The strategy of using oxygenation-based physiological triggers regarding the RBCT decision is a very current and interesting issue. The methodology and study design created by the researchers, based on the valuable findings of their previous studies on similar subjects, are stimulating and guide future research in this area. Therefore, I congratulate all researchers who contributed to the article and the research.

Firstly, the researchers have provided a clear statement regarding the transfusion decisions for the control and intervention groups. Additionally, the study target and potential expectations were well explained based on previous findings, and an appropriate methodology was created. The sample size determination and power analysis are detailed and well-articulated, and the randomization protocol is clearly described. The basic data and analyses to be obtained from the study were planned appropriately in line with the expectations.

However, I have some questions that I am curious about:

If the same patient receives more than one transfusion at different times, will the patient be included in the study again, or will they be considered as a single case with multiple transfusions? Will patients be reported for recurrent transfusion needs?

Given the decreasing use of ScvO2 in contemporary intensive care medicine, there is a concern about the accuracy of calculations based on the assumption of SvO2 and ScvO2 compatibility. In this regard, do you believe that the correct central venous catheter location alone is sufficient for accurate O2ER calculation? Would you consider excluding patients with various potential confounding factors such as acute respiratory distress syndrome (ARDS), heart failure, and sepsis, which might create discrepancies between ScvO2 and SvO2?

These points, if addressed, could further strengthen the study's design and findings.

Best regards.

Reviewer	**2**
Name	**Carson, Jeffrey L**
Affiliation	**Rutgers Robert Wood Johnson Medical School**
Date	**31-Jul-2024**
COI	**None**

General Comments

This is an important are of investigation in the transfusion arena. Today we use hemoglobin triggers as the main guiding measure on whether to transfuse. This measure is highly likely to be an imperfect measure of oxygen delivery to the tissues. Thus, it is important to investigate if physiological triggers improves patient outcomes above hemoglobin triggers for transfusion.

It is extremely important that the investigators justify using acute kidney injury as their primary outcome. The explanation in this protocol should be expanded and the underlying data to substantiate using this outcome provided in more depth. In trials comparing liberal vs restrictive transfusion strategies no difference acute kidney has been identified.

Sample size section on page 12 needs further clarification. How can test for absolute risk reduction of 16%? Actually, looks like expecting a 10% absolute difference between 13% and 26%. This is a large effect and seems very optimistic.

The statistical principles section is largely unnecessary. Some basic concepts are described and are not needed in this publication.

DSMB charter overview should be described. How often will the data be looked at? Twice? What criteria will be used for stopping rules?

Specific Comments

Page 13. Recruitment strategies. The description is too general. Clearly, all the ICU attendings must be spoken to and a screening process of potential eligible patients be implemented. How this is done is key to finding potential cases. The statement of about informed consent (line32) can be deleted since described later.

Pge 13, line 50. Will block sizes be varied and what size will they be?

Page 15, line 10. What measures will be taken to maintain concealment. How will patients be randomized? Will coordinators log onto a website? These details are missing from the paper.

Page 14, line 28. Typo? Screefor

Study timeline: likely unrealistic

Page 15. Stats principles and trial populations. Just state what will be done.

Page 17. Baseline characteristics. Could create a table with the list of variables to be collected.

Page 18. Specify subgroups rather than state such as.

Discussion. Describe limitations of the trial.

VERSION 1 - AUTHOR RESPONSE

Reviewer: 1
Dr. Ahmet Salih Tüzen, Izmir Katip Celebi University Ataturk Training and Research Hospital
Comments to the Author:
The strategy of using oxygenation-based physiological triggers regarding the RBCT decision is a very current and interesting issue. The methodology and study design created by the researchers, based on the valuable findings of their previous studies on similar subjects, are stimulating and guide future research in this area. Therefore, I congratulate all researchers who contributed to the article and the research.
We thank the reviewer for his appreciation of our study protocol and our previous findings!

Firstly, the researchers have provided a clear statement regarding the transfusion decisions for the control and intervention groups. Additionally, the study target and potential expectations were well explained based on previous findings, and an appropriate methodology was created. The sample size determination and power analysis are detailed and well-articulated, and the randomization protocol is clearly described. The basic data and analyses to be obtained from the study were planned appropriately in line with the expectations.

However, I have some questions that I am curious about:

If the same patient receives more than one transfusion at different times, will the patient be included in the study again, or will they be considered as a single case with multiple transfusions? Will patients be reported for recurrent transfusion needs?
We thank the reviewer for his comment. Patients requiring more than one transfusion will be considered as a single case with multiple transfusions. Each decision for transfusion (or not) will be according to the study group. Total number of RBC transfused during the ICU stay will be recorded for each patient. Now we clarified this issue in the methods, intervention section:

"If the same patient requires more than one transfusion at different time points, each decision to transfuse or not will be made according to the assigned study group. The total number of RBCTs will be recorded for each patient."

Given the decreasing use of $ScvO_2$ in contemporary intensive care medicine, there is a concern about the accuracy of calculations based on the assumption of SvO_2 and $ScvO_2$ compatibility. In this regard, do you believe that the correct central venous catheter location alone is sufficient for accurate O_2ER calculation? Would you consider excluding patients with various potential confounding factors such as acute respiratory distress syndrome (ARDS), heart failure, and sepsis, which might create discrepancies between $ScvO_2$ and SvO_2?
We agree with the reviewer that SvO_2 and $ScvO_2$ agreement may be impairment in some clinical conditions. However, measurement of SvO_2 requires placement of a pulmonary artery (PA) catheter, which remains controversial and outside routine care in participating centers. Furthermore, using only SvO_2 to accurate calculate O2ER would limit the generalizability of our protocol. We dealt with this issue in the limitations of the study.

Reviewer: 2
Dr. Jeffrey L Carson, Rutgers Robert Wood Johnson Medical School
Comments to the Author:
General Comments

This is an important are of investigation in the transfusion arena. Today we use haemoglobin triggers as the main guiding measure on whether to transfuse. This measure is highly likely to be an imperfect measure of oxygen delivery to the tissues. Thus, it is important to investigate if physiological triggers improve patient outcomes above haemoglobin triggers for transfusion.
We thank the reviewer for his appreciation of the aim of our protocol.

It is extremely important that the investigators justify using acute kidney injury as their primary outcome. The explanation in this protocol should be expanded and the underlying data to substantiate using this outcome provided in more depth. In trials

comparing liberal vs. restrictive transfusion strategies, no difference acute kidney has been identified.

We agree with the reviewer that our primary outcome deserve deeper explanation, giving that previous human trial did not find difference in occurrence of AKI. We added a related section in the discussion, where now you can find:

"Our choice to identify the occurrence of AKI as the primary outcome requires a clear explanation. First, given that renal oxygen consumption is particularly high [28], we hypothesized that avoiding excessive oxygen delivery/consumption mismatch with a targeted RBCT strategy may primarily protect the kidneys. RBCTs have been shown to significantly improve renal function and renal microvascular oxygenation in endotoxemic rats [29], and similar results have been observed in animal models of hemorrhagic shock [30]. On the other hand, previous trials comparing liberal versus restrictive RBCT strategies have not demonstrated a significant reduction in AKI incidence [31-32]. We speculate that many patients in the "liberal" transfusion arms of these trials may have had low or normal O2ER, meaning they did not derive substantial benefit from receiving RBCs.

Furthermore, our study is conducted in three distinct ICUs that treat medical, surgical, cardiothoracic, and neurological patients. While this diversity strengthens the potential generalizability of our results across different ICU settings, it necessitated identifying a common outcome, such as AKI, that applies to all these patient populations."

Sample size section on page 12 needs further clarification. How can test for absolute risk reduction of 16%? Actually, looks like expecting a 10% absolute difference between 13% and 26%. This is a large effect and seems very optimistic.

We thank the reviewer for his comment. We will test for absolute risk reduction of 13%, e.g. from 26% to 13%; even if it may seem a large clinical effect, we selected these values according to our previous observational study results. We agree that a closer difference may still be clinically significant; we dealt with this issue in limitation, where you can now find:

"Finally, we aimed for an absolute risk reduction of 13% in AKI incidence, based on our previous observational study [11]. Although this threshold was selected for statistical power, smaller differences in AKI incidence might still be clinically significant but undetectable within the scope of our trial."

The statistical principles section is largely unnecessary. Some basic concepts are described and are not needed in this publication.

We agree with the reviewer that some statistical principles can be moved from the main manuscript. Now we added the complete statistical analysis plan in supplement, leaving only main concept in the manuscript. Now you can find in the manuscript:

"Study design specification is shown in Appendix 1."

DSMB charter overview should be described. How often will the data be looked at? Twice? What criteria will be used for stopping rules?

We want to thank the reviewer for the comment. A full specification has been included in the Appendix. Further, you can now find in the manuscript:

"Data Safety Monitoring Board (DSMB): An independent Data Safety Monitoring Board will oversee the safety of study participants and review safety data at regular intervals. The DSMB will provide recommendations regarding the continuation, modification, or termination of the study based on safety considerations. Additional information regarding DSMB are given in Appendix 1."

And in Appendix 1

The DSMB (Data Safety Monitoring Board) for the OXY-TRIP trial will conduct regular safety and efficacy reviews of the trial data. Specifically, the data will be reviewed twice during the study: once at the interim analysis point, which is scheduled to occur after approximately 50% of the target enrolment has been reached (e.g. 162 patients), and once at the end of the trial. These reviews will focus on the primary and secondary outcomes, safety data, and any adverse events reported.

The criteria for stopping the trial will follow the guidelines of a Group Sequential Design (GSD) using an O'Brien-Fleming boundary for interim analysis. The stopping rules are defined based on the following considerations:

The trial may be stopped early for efficacy if the interim analysis shows a statistically significant difference in the primary outcome (AKI rate) between the treatment groups, exceeding the predefined significance level. For the interim analysis, a stringent significance level of 0.0026 (two-sided) will be used, with an overall alpha of 0.05 to account for multiple testing and control the Type I error rate. No formal early stopping rule for futility reasons has been provided.

The DSMB may consider stopping the trial if there is evidence of harm or an unacceptable safety profile in the individualized intervention group compared to the control group. This includes, but is not limited to, higher-than-expected rates of adverse events.

Specific Comments

Page 13. Recruitment strategies. The description is too general. Clearly, all the ICU attendings must be spoken to and a screening process of potential eligible patients be implemented. How this is done is key to finding potential cases. The statement of about informed consent (line32) can be deleted since described later.

We agree with the reviewer's concern; more details about the recruitment process have been included.

“All ICU attending physicians will receive a dedicated training session to familiarize them with the study protocol and eligibility criteria, ensuring they are well-prepared to identify potential participants. During the trial, a systematic screening process will be implemented at least twice daily. ICU staff will review patient records and discuss potential eligible patients during routine rounds, assessing the inclusion criteria. This approach ensures consistent identification of patients who meet the study criteria.

A screening log will be maintained at each site to document all patients considered for the study, including those excluded, with the reasons for their exclusion. Additionally, regular weekly meetings will be held with ICU staff to review the screening process, address any challenges, and provide updates on recruitment progress. These meetings will help maintain staff engagement and promptly address any barriers to recruitment.”

Pge 13, line 50. Will block sizes be varied and what size will they be?

In our study, we have decided to use fixed block sizes of 4 for randomization. We chose this approach to maintain a consistent balance between the treatment groups throughout the recruitment period. By using a fixed block size, we ensure an equal allocation of participants to each group, minimizing the potential for imbalances that could affect the study's validity.

We did not explicitly state the block size in the manuscript to prevent potential bias or predictability in the randomization process, which could occur if site investigators or study staff were aware of the block sizes. Maintaining the confidentiality of the block size helps ensure that the randomization process remains robust and prevents any potential manipulation. Not declaring the block size in the protocol is useful to ensure compliance with the regulatory requirements of the FDA and EMA(European Medicines Agency, s.d.). The guidelines on statistical principles for conducting clinical trials(Food and Drug Administration, s.d.), on page 13, state: "Details of the randomization that facilitate predictability (e.g., block length) should not be contained in the trial protocol."

Page 15, line 10. What measures will be taken to maintain concealment. How will patients be randomized? Will coordinators log onto a website? These details are missing from the paper.

We agree with the reviewer's indication. We will include these details in the revised version of the manuscript to clarify the randomization procedure.

Patients will be randomized using a centralized, computer-based system via the REDCap [24] platform, ensuring allocation concealment and unpredictability. Study coordinators at each site will access the REDCap interface to randomize patients. The system is designed to prevent any prior knowledge of allocation by generating the randomization result only after patient eligibility has been confirmed and consent obtained. These measures are implemented to preserve the integrity of the randomization process and prevent selection bias.

Page 14, line 28. Typo? Screefor
This has been corrected, accordingly.

Study timeline: likely unrealistic
The timeline of the study was ideated according to the number of anaemic patients usually enrolled in the ICUs involved. By the way, we admit that we cannot ensure a complete enrolment during the anticipated study period. In case of unsatisfactory sampling, the study end will be postponed until the completion. We added this issue in the methods:

“In case of insufficient recruitment, the study end will be postponed until the completion of the sample size.”

Page 15. Stats principles and trial populations. Just state what will be done.
In the new version of the manuscript you’ll be able to find a shorted version of stats principles with only relevant points left in the main text. Now you can find in statistical principles

“Study design specification is shown in Appendix 1.”

Page 17. Baseline characteristics. Could create a table with the list of variables to be collected.

We added figure of the daily variables collected and a Table with the baseline characteristic. Now you can find Figure S1 and Table S1 as supplement! In the methods you can now find:

“Baseline characteristics collected are shown in Table S1.”

Page 18. Specify subgroups rather than state such as.
The reviewer is right as we were not clear about the pre-planned sub-group. Now you can find in additional analyses:

“Subgroup Analyses: Subgroup analyses will be performed using logistic regression models to explore potential treatment effects within specific patient subpopulations based on relevant characteristics, i.e.: AKI stage>1, CRRT use, and the presence of comorbidities (history of heart disease, vascular surgery, oncologic disease, septic shock at study enrolment).”

Discussion. Describe limitations of the trial.

We added the limitation section according to the reviewer’s suggestions. Now you can find in limitation:

“Limitations

Our protocol has some limitations. Firstly, although the outcome assessors are blinded to the study group, this is an open-label trial for the treating physicians. However, this limitation is common in most transfusion-threshold trials [33]. Additionally, concerns may arise regarding the accuracy of O2ER calculation, which assumes compatibility between SvO2 and ScvO2 [34]. While SvO2 measurement requires the placement of a pulmonary artery (PA) catheter, the risk-benefit ratio of this intervention remains uncertain. Using SvO2 to calculate O2ER more accurately would limit the protocol's generalizability, and routine PA catheterization for each anemic patient raises ethical concerns. Finally, we aimed for an absolute risk reduction of 13% in AKI incidence, based on our previous observational study [11]. Although this threshold was selected for statistical power, smaller differences in AKI incidence might still be clinically significant but undetectable within the scope of our trial."

VERSION 2 - REVIEW

Reviewer	**1**
Name	**Tüzen, Ahmet Salih**
Affiliation	**Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Anesthesiology and Reanimation**
Date	**13-Oct-2024**
COI	

Dear Authors,

Thank you for your revisions and for addressing the concerns raised in the initial review. I commend the care and attention you have put into refining your manuscript based on the feedback provided. Your detailed corrections have strengthened the manuscript, particularly in areas where the initial concerns were raised. I appreciate the depth of your responses, which have clarified key points and enhanced the overall quality of the study. I have no further suggestions or contributions to offer.

I believe that your research addresses a significant gap in the existing knowledge and will make a valuable contribution to the field. I wish you success in your future endeavors and look forward to observing the impact of your work within the academic community.