Peer Review File

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Reviewer A

Presented is a manuscript describing the protocol for the DARTS study. The protocol description is well written and the introduction and discussion offer strong justification for the importance of this study as well as presenting possible limitations. The trial is novel and the manuscript describes important aspects for consideration. Clarification around the central hypothesis and how "gold-standard" ARDS is being defined would significantly improve the manuscripts clarity. The authors have presented a novel bench-to-bedside study, which will garner significant interest from the ARDS community.

Response: We thank the reviewer for the thorough review. We have tried to address your comments as completely and detailed as possible.

Abstract

Line 58: It is confusing to use "suspected ARDS" from the remainder of the protocol it seems patients with primary clinician determined ARDS receive additional samples.

Response: Thank you for raising this point. We agree with the reviewer that the term 'suspected' can lead to confusion in this sentence. What is meant is a patient fulfilling the criteria of the Berlin Definition as checked by the researcher. In a later setting, the expert panel, consisting of three experts in the field, will reassess this criteria for this patient and decide if this patient indeed fulfilled the criteria of the Berlin Definition and should be classified as ARDS. So therefore, the term suspected was chosen, since at a later time point a final decision will be made about how to classify this patient. It does not mean primary clinician determined ARDS, since it is known that ARDS is missed in up to 50% of the cases by the clinician. However, if ARDS was clinically recognized this might be taken into account by the researcher. Because the term suspected is confusing in this context we adapted the text in the manuscript, so that it is clarified that it involves patients fulfilling the criteria as assessed by the researcher.

Page 3, line 59-60: *In patients fulfilling the criteria for ARDS, three additional breath samples will be taken to assess resolution.*

Background

Was this detection of any octane or was a specific threshold needed to accurately diagnosis ARDS in original MS studies?

Response: We are not entirely sure what is meant by the reviewer. We think it is meant if the POC breath test should be able to detect any ARDS or if already in advance was known that a certain threshold should be detected accurately. Based on the discovery and validation study in invasively ventilated patients with and without ARDS the concentration was expected to be in the 0.1-0.4 ppb range for patients without ARDS and between 0.2 and 2 ppb for patients with ARDS [1].

Line 104: This statement offers great biologic plausibility for the linkage of ARDS

pathophysiology and octane exhalation. The wording could be clearer to really highlight this linkage and the reasoning behind this clinical trial.
Response: Thank you for this suggestion. The text in the manuscript was adapted to highlight this linkage and to clarify the reasoning.
Page 5-6, line 103-106: *ARDS causes an increase in oleic acid in the circulation. At the same time, oxidative stress causes an increase in release of reactive oxygen species which leads to increased lipid peroxidation. Lipid peroxidation of oleic acid generates octane, making this a possible explanation for increased levels of octane In ARDS.*

Line 118: Mentioned in limitations, but is important to have published the data that the POC test to be used in this protocol is closely calibrated to the GCMS values that is used as preliminary data justification for this trial.
Response: We thank the reviewer for addressing this point. Currently the manuscript with data showing the accuracy and precision of the POC breath test is submitted and under review at a journal. We hope it will be published within the next couple of months.

Line 121-123: Clarity around if both the hypothesis and primary outcome is simply octane detection is required for POC testing ARDS diagnosis or if a certain threshold octane amount to accurately diagnose ARDS will be determined and test characteristics for this threshold will be published, would help clarify this last paragraph of the introduction.
Response: Thank you for raising this question. We will also search for a threshold with optimal sensitivity and specificity and test characteristics will be published.
This is clarified in the last paragraph of the introduction.
Page 6, line 121-125: *We hypothesize that the octane concentration in exhaled breath, as measured with a point-of-care breath test, can facilitate early diagnosis of ARDS in invasively ventilated ICU patients. We will assess the optimal threshold and report the diagnostic accuracy, as reflected by characteristics like the sensitivity and specificity.*

Methods:
The authors nicely describe the consenting procedures for this study.
Response: We thank the reviewer for this compliment.

Line 170: The authors should clarify how “is expected to be deceased within 24 hours” is being defined for the study.
Response: At the moment a patient fulfills the criteria for inclusion, the disease state of the patient will be considered. Since this study is performed in a patient population involving severely ill patients, sometimes in a very critical situation, it is relevant to take the life expectancy into account. If the researcher is concerned about the life expectancy being less than 24 hours, the patient will be discussed with the attending physician and the expectations will be discussed. It will be judged how ethical it is to include the patient at that moment, if the patients’

life expectancy is less than 24 hours we feel that it is not ethical to include a patient into the clinical trial, so the patient will be excluded.
We tried to clarify this in the manuscript.
Page 8, line 169-170: *have a life expectancy of less than 24 hours at the moment of inclusion,*

Line 188: What happens to patients that develop ARDS after the first 48 hours? The authors do highlight that most patients meet Berlin criteria early after intubation, but also use delay of CXR finding development and late diagnosis as justifications for how Octane Breath detection can improve upon current standard. Selection bias may be introduced against "late developers of ARDS" who may have different octane breath patterns.
Response: We agree with the reviewer that it might introduce selection bias against patients who later develop ARDS. However, we want to point out that we still have 2 initial measurements of these patients, only no follow up additional breath measurements. It is correct that on day 1 and day 2 the researcher will specifically assess if the patient fulfills the criteria of the Berlin Definition. In the follow up it will be assessed if the patient have developed ARDS later during the admission. So from the patients not receiving the additional measurement 3 to 5, we still have follow up data and we know if they developed ARDS later during their hospital admission. Therefore we think that it does not cause a selection bias for diagnosing ARDS, since we want to diagnose ARDS in the early course with the POC breath test, and the required data to assess this will be available.
We hope that this reassures the reviewer that the chosen methods are solid enough to not have a selection bias in diagnosing ARDS.
To clarify this point, the text in the manuscript was slightly adapted.
Page 9, line 191: *These three additional measurements are used to monitor the disease progress of the patients with ARDS.*

Classification of ARDS: The authors have defined three ways ARDS will be determined. It is unclear that this is necessary.
1) Which of these definitions will be used to define day 3 ARDS and further obtainment of follow-up breath tests
2) If different definitions are being used to determine who receives extra POC breath tests and what the "gold-standard ARDS" for use in the ROC analysis, sampling bias may develop as who has "ARDS" changes over time.
3) Additionally, the authors do not discuss if severity of ARDS will be considered. Will ARDS be considered en-bloc, such as if meet criteria for mild or higher, this is just ARDS, or will Mild/ Moderate and Severe ARDS be considered separately? Clarification of if individual thresholds for different severities will be generated or just for en bloc definition would also be helpful.
Response: Thank you for addressing this point. We understand the confusion of three methods used to determine if the patients have ARDS. Our rationale behind using three definitions is as follows:
The clinical ARDS is scored, to be able to analyze the clinical recognition. Since it is known

that only 34% of the patients is recognized at the moment the fulfill the criteria of the Berlin definition, the researcher ARDS was added [2]. The researcher systematically assesses if the patient fulfills the criteria of the Berlin Definition on day 1 and day 2, so that all patients with possible ARDS will receive the additional three measurements, to follow their disease progress. At last it is known that ARDS diagnosis is challenging, making the reliability moderate to substantial. To identify and classify the patients as best as possible, the expert panel was asked to assess each patient in retrospect.

To address your points:

1) Throughout the entire study duration the same team of researchers will assess if the patient fulfilled the criteria of the Berlin Definition on day 1 or day 2 and will then decide if the patient will undergo the additional assessments.
   The expert panel will review the patients in retrospect and will then assess if the patient fulfilled the criteria of the Berlin Definition.
2) As described above the same definition is being used throughout the study to determine if a patient receives the additional measurements.
3) ARDS severity is indeed not discussed, this will be a subgroup analysis and is not the primary research question.

These points have been clarified in the manuscript.
Page 11, line 205-210: *The second setting will be according to the researcher at inclusion and one day later, the researcher will use data of the 24 hours before assessment to score ARDS, this ARDS diagnosis is used to define if a day 3 assessment will be performed. The third setting will be assessment of ARDS by an expert panel in retrospect that will be blinded for the other clinical parameters.*
Page 11, line 214-215: *In a subgroup analysis the diagnostic accuracy for the different categories of severity will be analysed.*

The authors give a very thorough discussion of methods for VOC detection protocols which is helpful.
Response: Thank you for addressing this positive point.

Line 222: “clock” should be changed to stop-cock
Response: Thank you for paying attention. It is corrected in the manuscript.
Page 10, line 222: *way stop-cock to the…*

Study Outcomes: The authors could clarify if the primary outcome is to determine the concentration of exhaled Octane in POC testing that maximizes sensitivity and specificity or if the concentration cut point already exists and the diagnostic accuracy of that cut point is being tested?
Response: Thank you for this suggestion. The first is the case, we want to determine a concentration which shows the best diagnostic accuracy. This is clarified in the manuscript.

Page 13, line 293-296: *The primary endpoint of this study is the diagnostic accuracy of octane concentration in exhaled breath for ARDS defined by the panel of experts, depicted by the value which maximizes the specificity. The diagnostic accuracy will be described by the AUROCC and the optimal cut-off with sensitivity, specificity and likelihood ratios***.**

The authors nicely describe the secondary outcomes of interest.
Response: Thank you.

Line 330: The authors describe effect modification, but define this as mediation. These are separate statistical approaches and this should be clarified.
Response: Thank you for raising this point. The reviewer is correct, we indeed mean modification and not mediation. This is corrected in the manuscript.
Page 16, line 334-337: *Modification is defined as a statistically significant interaction term in the regression model. When modification is found, a stratified analysis will be performed within the subgroups.*

Discussion
The authors nicely describe the novelty of this study and its potential benefits to the field of ARDS.

The protocol, nicely highlighted in this discussion, strives to characterize ARDS in multiple modalities, including lung ultrasound, breath metabolomics (both GCMS and POC) and serum biomarkers.
Response: These compliments are much appreciated.

The hypothesis stated in the conclusion is clearer than the one found in the introduction section. The authors should consider harmonizing the different ways the primary hypothesis is stated throughout the manuscript.
Response: We thank you for giving us this option. We agree with the reviewer that the hypothesis written in the conclusion is clearer. Therefore the hypothesis in the introduction was replaced with the one written in the conclusion.
Page 6, line 121-123: *We hypothesize that the octane concentration in exhaled breath, as measured with a point-of-care breath test, can facilitate early diagnosis of ARDS in invasively ventilated ICU patients.*

Figure 1
The figure is helpful for clarifying study procedures. The caption could clarify that a patient can meet criteria for ARDS at any time in first 2 days and proceed to the ARDS “yes” arm, as it was stated in the manuscript text.
Response: We agree with the reviewer that this would be helpful in interpreting the figure. A

clarification was added.
Figure 1, figure description: *The researcher assesses if the patient fulfils the ARDS criteria at any time during the first two days. If the researcher diagnoses the patient with ARDS, the patient will undergo assessment 3 to 5, as indicated with the arrow "yes",*

Figure 2
If using this multi-definitional approach should define, both in text and figure, which definition is used to determine progression to day 3 and beyond POC testing and which will be used as "gold-standard" for POC breath octane level ROC analysis.
Response: Thank you for paying attention. It is added to the figure description.
Figure 2, figure description: *The diagnosis of ARDS by the researcher is used to decide if additional measurements as shown in figure 1 will be performed.*

Reviewer B
Hagens and colleagues present a clearly written account of a well-designed study with the potential to significantly improve clinical practice in ARDS, including both diagnosis and monitoring of this common and serious condition. I am certain that this trial when published will be of interest to many critical care physicians.
Response: We thank the reviewer for the compliments and the useful comments. We feel the revised version of the manuscript has improved due to these changes.

My main comment would be that the current protocol does refer to COVID-19 anywhere, even though this new condition is probably the most common cause of ARDS globally yet is widely recognised as behaving very differently to 'classical' ARDS in many ways. I wonder how the triallists will be accounting for this – is COVID diagnosis being recorded? Is COVID induced ARDS being considered as a subgroup in the analysis?
Response: We thank the reviewer for raising this important question. It is recorded if a patient was diagnosed with COVID-19. We will perform subgroup analysis with COVID induced ARDS.

Also is anything known about how infection with COVID-19 (either with or without resulting ARDS) itself affects exhaled levels of octane? Increasingly COVID-19 is being recognised as an oxidative stress as well as a prothrombotic condition, so I would expect octane (and other exhaled stress marker) levels to be higher in these patients even if they do not meet the Berlin definition for ARDS and I am not clear how this will be accounted for in the current analysis plan?
Response: There is one pilot study which assessed the breath of 40 patients with ARDS, of whom 28 had COVID-19 [3]. They found methylpent-2-enal, 2,4-octadiene 1-chloroheptane, and nonanal in exhaled breath may identify ARDS in patients with COVID-19.
So there is no knowledge about how COVID-19 affects levels of exhaled octane specifically. Therefore we will include this into the post-hoc analysis.

There are also some minor typos that could be corrected on page 8:
- End of line 154 should read “In cases where the representative…” to make grammatical sense
- Line 156, this line would make more tense and fit better with the tenses used elsewhere in this parapraph is ‘could’ is replaced with ‘can’
- Line 158 – this sentence does also not make sense currently. Should this read ‘In cases where the representative gave consent…’?
Response: Thank you for correcting and bringing up these typos. We all corrected them in the manuscript.
Page 8, line 154 – 159: *In cases where the representative does not give consent, the collected data will be destroyed. When the representative cannot be reached, and the patient does not regain capacity, the patient will be excluded and the collected data and all biological samples will be destroyed. In cases where the representative gave consent, the patient will have the possibility of an opt-out procedure.*

Reviewer C
ARDS is easy to be diagnosed by chest radiography, history and arterial blood gas. It seems that there is unnecessary to diagnose ARDS by bedside exhaled breath octane for clinical practice. However, it is an interesting issue whether exhaled breath octane could early predict ARDS development in patients without ARDS.
Response: We thank the reviewer for reviewing our manuscript.

References

[1] L. D. J. Bos *et al.*, “Exhaled breath metabolomics as a noninvasive diagnostic tool for acute respiratory distress syndrome,” *Eur. Respir. J.*, vol. 44, no. 1, pp. 188–197, 2014.
[2] G. Bellani *et al.*, “Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries,” *JAMA - J. Am. Med. Assoc.*, vol. 315, no. 8, pp. 788–800, 2016.
[3] S. Grassin-Delyle *et al.*, “Metabolomics of exhaled breath in critically ill COVID-19 patients: A pilot study,” *EBioMedicine*, vol. 63, Jan. 2021.