Author’s response to reviews

Title: Soluble receptor for advanced glycation end products as an indicator of pulmonary vascular injury after cardiac surgery

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Author’s response to reviews: see over
Dear Editor,

Please find enclosed our revised manuscript entitled “Soluble receptor for advanced glycation end products as an indicator of pulmonary vascular injury after cardiac surgery”.

We thank the reviewers for their fruitful comments - we believe they have improved our manuscript significantly.

We revised our manuscript accordingly, highlighting all changes in the revised manuscript. A point–by–point reply to all comments is provided in an additional file. Of note, we added two figures as supplemental files to the manuscript.

We look forward to your response.

Pieter Roel Tuinman, corresponding author
Reviewer 1.

Abstract
- The authors investigated the relationship of plasma sRAGE levels and the development of lung vascular injury after cardiac surgery, so they could not conclude that the lung vascular injury after cardiac surgery was mediated by sRAGE.

We agree. However, we do not conclude that lung vascular injury is mediated by sRAGE, but we state that the data suggest that injury is partly mediated by sRAGE. We think our conclusion is carefully chosen and does not overestimate the results. See also last comment of this reviewer that the conclusion is 'relatively weak'.

- is keyword “TRALI” necessary? And it is not shown in the list of abbreviations.

We choose the keyword TRALI, since we investigated the effects of transfusion on sRAGE, a marker of lung injury. However, since we did not use the word TRALI in the manuscript, we deleted the keyword TRALI from the list.

Methods:
- The section is somewhat disorganized and can be more organized.

We used subheadings which are commonly used in medical journals to organize this section. We agree with the reviewer that some definitions need to be more precise (see next comment), and we elaborated on the patient data collection.

- Some definitions need to be more precise.

We agree with the reviewer and added details about potential risk factors to the manuscript, also see next comments.

- The details of the cardiothoracic surgery/anesthesia procedures, and the assays procedure can be in an addendum but the details regarding the potential risk factors for ALI score and biomarker need to be in more detail.

We think it is of interest for the reader to read about the anesthesia procedures, including among others transfusion strategy, mechanical ventilation strategy and use of possible anti-inflammatory medications, because these factors might influence lung injury after cardiac surgery. We therefore suggest that this section remains in the manuscript, but if the reviewer disagrees, the section can be added as supplemental file.

We added to the manuscript: ….. “including diabetes, smoking and alcohol abuse. Some known risk factors for ALI, such as pneumonia, trauma and sepsis were not taken into account as the patients included were elective surgery patients. For this reason, presence of such a risk factor was a reason to cancel surgery for these patients.”

We are unsure in what way the details regarding ‘biomarker’ should be more specific. We described the details of the sRAGE assay and provided a reference.

- The statistical review needs to be more precise.

We added to the statistical review: “Categorical data were analyzed with the chi-square test”. “To evaluate independent causal factors for an increase in PLI, a binary
logistic regression analysis, using the Enter method, was applied. Statistically and/or clinically relevant factors were added to the model. The model was evaluated using Hosmer-Lemeshow goodness-of-fit test. Furthermore we deleted (as suggested by reviewer 2): “Kruskal-Wallis test and ANOVA, since only two data sets were compared.

Results:
- There are many details which need to be better organized. According to the suggestion of the reviewer, we added results from logistic regression analysis to the results and included graphs of the relation between sRAGE and PLI. We hope that this adds to the clarity of the presentation of the results.
- Graphs can be used for sections such as the relationship. We agree with the reviewer and a scatter plot as well as a ROC curve of the relation between sRAGE and PLI were added as supplemental files.
- Since these data are exploratory, the authors need to perform a logistic regression to adjust the confounder factors with the outcome elevated PLI; and the parameters presenting potential risk factors for ALI, such as operation time should be built into the model. This is an important remark by the reviewer. A logistic regression analysis was performed and added to the manuscript. To the text was added:” For exploration of confounding, a logistic regression analysis was performed. Besides sRAGE, operation-time, EuroSCORE and amount of blood products were added to the model. Analysis showed that sRAGE and operation time were risk factors for a strongly increased PLI (Table II). Of note, an OR of 1.005 for sRAGE applies to each increment of 1 pg/ml. Thereby, a patient with an sRAGE level of 190 pg/mL (which is the median increase in sRAGE in this population) has an OR of 2.59 (95% CI 1.47-3.71) for an increased PLI.

Discussion:
- The conclusion in relatively weak and nonspecific. We are unsure how to interpret this comment by the reviewer considering his/her first comment: “The authors investigated the relationship of plasma sRAGE levels and the development of lung vascular injury after cardiac surgery, so they could not conclude that the lung vascular injury after cardiac surgery was mediated by sRAGE.”

The statement that sRAGE is elevated in cardiac surgery is clear and specific and supported by the data. The statement that sRAGE indicates pulmonary vascular permeability is also specific and we think this is supported by the data. The statement that sRAGE is not affected by blood transfusion is also a specific statement.

- The discussion sections need to be more cohesive.

The discussion opens with a sentence with the main findings: ‘This study suggests that sRAGE levels, a biomarker of pulmonary tissue damage, are elevated in plasma after cardiac surgery and may serve as an indicator of pulmonary vascular injury,
independent of blood product transfusion’. This sentences comprises 2 findings, which are then discussed separately:

1) sRAGE is indicative of ALI after cardiac surgery. This is related to previous research findings on the increase of sRAGE in ALI after cardiac surgery and expanded with our finding that sRAGE correlates with PLI, which has not been done before.

2) the relation between transfusion and sRAGE is discussed. We did not find a relation whereas others did. Explanations for disparate findings are given.

We then discuss the relevance of the findings. We added a sentence to the discussion to indicate that this paragraph is about implications of findings. We close with a final paragraph on the limitations of the study.

We believe that this is a structured approach.

Reviewer 2

Introduction
- only specify between as a possible cause of ALI in cardiac surgery also the mechanical ventilation.

As suggested by the reviewer, mechanical ventilation was added as a risk factor for ALI in cardiac surgery to the text of the introduction.

Methods
- why table I is presented as a supplementary file?

We apologize for this error. We corrected our mistake and added Table 1 to the main manuscript.

- dichotomize the left ventricular ejection fraction as preserved or reduced using a cut-off value of 50% (bad, moderate or good do not sound at all!)

We changed the left ventricular ejection fraction to preserved or reduced as suggested by the reviewer. This was added to the manuscript: “Left ventricular function was categorized as preserved (ejection fraction (EF) > 45%) or reduced (EF ≤ 45%)”

- supply a more punctual timing for sRAGE, PLI and BALF…not just before and after but 1,2 or 3 hours before and 1, 2 or 3 hour (±SD)

Unfortunately, this is not possible since we did not record exact timing of PLI, BALF or blood sampling. We accounted for variation in sampling by limiting the sampling time to 3 hours postoperatively. We do not think that variation in timing of sampling importantly influenced results.

- Figure 3 is not necessary. It is enough to give data into the Results

We deleted figure 3 from the manuscript and added the figure as supplemental file.

Statistics
- why ANOVA? You have only two data sets to compare

We are sorry to have accidently added ANOVA as a method. This was not used as the reviewer is right that only two data sets were compared.” This is deleted.

- I think you forgot to write how you compare all categorical variables (I think with chi-square method or fisher)
That is correct and added to the manuscript: “Categorical data were analyzed with the chi-square test”.

See comment above

- to correlate two variables with a linear (normal) distribution is enough to use the Pearson method rather than the Spearman one

An asymmetrical distribution was analyzed and accordingly the Spearman method was chosen.

Discussion

- An emerging marker of alveolar-capillary unit “health” is represented by surfactant protein type B. With respect to sRAGE, SPB owns a more “simple” underlying release-mechanism (leakage in the bloodstream because of an alveolar-capillary damage/stretch). Indeed SPB was found to increase in chronic alveolar-capillary damage (i.e. chronic heart failure: Magri D, et al. Circulating plasma surfactant protein type B as biological marker of alveolar-capillary barrier damage in chronic heart failure. Circ Heart Fail. 2009;2(3):175-80) as well acutely during major surgery (your actual reference 12). As Authors acknowledged, sRAGE has certainly a conflicting meaning because it could be marker and/or part of lung injury. Supporting this potential bias, an increase in SRAGE circulating values was also observed in a recent study conducted in patients undergoing vascular but not cardiac surgery (Agostoni PG et al. Kinetics of plasma SPB and RAGE during mechanical ventilation in patients undergoing major vascular surgery. Respir Physiol Neurobiol. 2011 Sep 15;178(2):256-60). Because I can imagine that Authors cannot supply data about SPB for the current study, I would like they stress (in the Discussion or, at least, in the limitation) the lack of data on this novel biomarker in order to investigate the alveolar-capillary unit.

We agree. We added to the text in the limitation section: “Furthermore, a comparison of different biomarkers (for example surfactant protein B, which leaks in the bloodstream because of alveolar-capillary damage) [28], would have strengthened our data.”