Author’s response to reviews

Title: Repeated open endotracheal suctioning causes gradual desaturation but does not exacerbate lung injury compared to closed endotracheal suctioning in a rabbit model of ARDS

Authors:

Hideaki Sakuramoto (gongehead@yahoo.co.jp)
Nobutake Shimojyo (nobu-shimojyo06@ob.md.tsukuba.ac.jp)
Subrina Jesmin (jsubrina@gmail.com)
Takeshi Unoki (iwhyh1029@gmail.com)
Junko Kamiyama (megmagmeggen@yahoo.co.jp)
Ken Miya (ken-miya@hotmail.co.jp)
Satoru Kawano (kawano_s@md.tsukuba.ac.jp)
Taro Mizutani (mizutani@md.tsukuba.ac.jp)

Version: 2 Date: 6 October 2013

Author’s response to reviews: see over
Responses to Associate Editor report

Comment: After having read their critiques and the manuscript myself, I regret to inform you that it cannot be accepted for publication in its present form. This decision is based on several issues, but the most relevant one is the lack of a control group. Ideally, a group with lung injury, but without open/closed suctioning, should have been included in the design. We would be willing to reconsider your manuscript if you are able to include such a control group in your study. In the Materials and Methods section, that group should be indicated as included a posteriori, i.e. clearly mentioning that animals did not undergo random assignment to groups. This limitation should be addressed in the Discussion section.

Response: Thank you for clearly stating the primary shortfall of our manuscript. Accordingly, we have repeated the whole experiment and added a control group with lung injury, but without endotracheal suctioning. The new results have been incorporated to the whole manuscript with Tables and Figures (please refer to revised manuscript with Tables and Figures). In addition, we have clearly indicated in the Method section that the newly added experimental animals did not undergo random assignment to groups (please see page 8, lines 15 – 18; page 9, lines1-2).

Responses to other editorial comments:

Comment: Abstract needs formatting according to journal style
Response: The abstract has been formatted in accordance with BMC anesthesiology formatting guidelines.
Responses to reviewers:
Reviewer: Dr. Peter Spieth

Overall Response: Thank you for your constructive comments to our manuscript.

Major concerns:
Comment 1: I believe that the model is not suitable to answer the research question. The saline lavage model is known to be highly recruitable and a PEEP level of 10 cm H2O is very high for rabbits considering their very elastic chest wall. The research question could have been answered much better using a large animal model.
Studies by Koh et al and Suh et al used a PEEP level of 10 cm H2O after measurement of lower inflection point in rabbit models and we followed their protocol in the present study. Undoubtedly, the rabbit model may be small to address our research question and other similar studies, as stated above (please refer to the revised discussion). In future studies, we intend to build on the current data by considering differences between small animals and clinical patients in elastic chest walls and size of respiratory tract relative to catheter diameter. For now, we are limited by finances and the logistical problems of our facilities. When these issues are resolved, we address the current research issue using a large animal model, such as pig through collaboration with other laboratories that can house animal models of such a size.

Comment 2: The observation period is may be too short. I’m fully aware that longer experiments are difficult, but the differences in interventions (open vs. closed suction...
maneuvers) need probably more time to develop biologically meaningful results. A more suitable time course would have been at least 24 or 48h. Of course I know that this is hard to realize in experimental research but in the end the study should reflect clinical standards and requirements.

Response: We completely agree with you that in the current study the observation period is short. Accordingly, we have added your opinion to the Discussion section of the revised manuscript (please see page 19, lines 1-5; page 22, lines 7-9; and page 24, lines 12-15). In addition, we have also included the data of the mRNA expression levels of molecules of interest to the revised version of the manuscript (please see revised manuscript and Figure 3). Further, we have considered your comments designing our next study, which will have a more prolonged time course in order to clarify the observed change for arterial desaturation and other data.

Comment 3: I think a healthy control group is missing to differentiate the effects of the suction maneuvers and the lung injury.

Response: We have accordingly repeated the whole experimental setting again with a healthy control group (HC) and the generated data were incorporated to the revised manuscript (please refer to the revised manuscript with revised tables and figures).

Comment 4: Please comment on the spontaneous breathing activity. You wrote that the animals were paralyzed. How is it then possible that you have respiratory rates ranging from 4-40 (page 10, last sentence)? In a well-controlled model this is far too much variation.

Response: All animals received a muscle relaxant. Therefore, all animal were paralyzed (non-spontaneous breathing). Our study protocol was a modification of a previous study (reference 15 in our manuscript; Hickling KG, Town IG, Epton M, Neill A, Tie A, Whitehead M, Graham P, Everest E, A’Court G, Darlow B et al: Pressure-limited ventilation with permissive hypercapnia and minimum PEEP in saline-lavaged rabbits allows progressive improvement in oxygenation, but does not avoid ventilator-induced lung injury. Intensive care medicine 1996, 22(12):1445-1452), where the mandatory respiratory rate was subsequently adjusted to maintain the \( \text{Pa}CO_2 \) in the range of 60-100 mm Hg, when possible, with a minimum rate of 4/min and maximum of 40/min. However, in our study the mandatory respiratory rate range was 15-26/min in pre-injury period and 30-40/min in post-injury period. We incorporated this fact to the revised Method and Discussion sections (please see page 10, lines 10-12 and page 23 lines 9-11).
**Comment 5:** 6h observation period is maybe too short for a response on the protein level. I suggest to check for mRNA expression of the same markers.

**Response:** We took your advice with great care and performed Real Time PCR for mRNA expression analysis. (please see Method: page 11, lines 15-18 and page 12 lines 1-9; Result: page 16, lines 3-5; Figure 3).

**Comment 6:** You disclosed in your limitations the lack of compliance or FRC measurements. Indeed this is a very big problem and makes the interpretation of your data.

**Response:** Thank you for the pertinent comment. We have these limitations in compliance measurements, because of technical and financial issues, as stated earlier. We do agree that this point requires clarification, and please refer to the revised Limitations (please see page 23, lines 17-18 and page 24, lines 1-5).

**Minor Revisions:**

**Comment 1:** page 5 and following: please change shearing force to atelectrauma.

**Response:** In accordance with the Reviewer’s comment, we have changed shearing force to atelectrauma (please see page 4, lines 10 and 13).

**Comment 2:** page 5, last sentence: It’s not MOV that is associated with biotrauma, biotrauma leads to MOV. Please revise this part and clarify the connection among baro-, volu-, atelec- and biotrauma.

**Response:** The issue has been clarified in the revised Introduction (please see page 4, lines 7-18).

**Comment 3:** page 7, 1st sentence: what do you mean by "..in the fall of end-expiratory .." ? difficult. Do you mean "in the case"?

**Response:** The necessary correction has been made to the Introduction (please see page 5, line 18 - page 6, lines 1-2).

**Comment 4:** page 9, last sentence: please change "rocked" to another more scientific description.

**Response:** In accordance with the Reviewer’s comment, we have changed description to “Rotated” (please see page9, line 11).
Comment 5: Did you really packed your samples on dry ice? The standard is to snap freeze them in liquid nitrogen, that´s important for some of the mediators you measured.
Response: We used liquid nitrogen and samples were frozen immediately in liquid nitrogen after tissue harvest (please see page 11, line 7).

Comment 6: page 26, please change numbering from 1-3 instead of three time 1).
Response: We changed numbering from 1) - 3) (Please see page 26, lines 2-9)

Comment 7: Please let the manuscript check for grammar and style by a native speaker or professional language editing service.
Response: A native English speaker with extensive experiences in biomedical research checked the revised manuscript.

Reviewer: Dr. Marcus J Schultz

Overall Response: The reviewer acknowledges that the current report expands the knowledge in the field investigated.

Major comments
Comment 1: Introduction – page 7: The authors defined research questions, but the hypothesis is lacking. A clear hypothesis could help to translate the research questions into a prediction of expected outcomes. Could the authors provide a clear hypothesis?
Response: Thank for such a crucial comment and accordingly we have developed a clear research hypothesis in the revised Introduction section (please see page 6, lines 4-18).

Comment 2: Methods # page 10: After induction of lung injury the fraction of inspired oxygen was set at 100%. This could result in resorption atelectasis and as such add to development of lung injury. Also, this is not easily translated into clinical practice. The authors should discuss this in the Discussion–section.
Response: We have incorporated the issue in the revised Discussion as study limitation (please see page 23, lines 11-17).

Comment 3: Results # page 13: Gas exchange in this study has been reported as PaO2, which is not sufficient: usually the PaO2 to FiO2 ratio (P/F) is used. Unless the fraction
of inspired oxygen was set at 100% during the whole experiment.

**Response:** In accordance with the Reviewer’s comment, we have changed the description “the PaO2 to FiO2 ratio (P/F)” (please refer to the revised manuscript with figures and tables).

**Comment 4:** Methods – The aim of this study was to assess the effects of repeated de-recruitment due to suctioning in a model for ARDS; PaO2 levels (and thus P/F) were far higher than those in patients with ARDS. Then, were the animals suffering from lung injury at all, or at least as severe as with ARDS? The authors should discuss this in the Discussion–section.

**Response:** We do agree with the reviewer and this issue has been discussed in the revised Discussion (please see page 21, lines 2 - 9).

**Comment 5:** Discussion – The authors advocate the use of closed endotracheal suction in patients with acute lung injury, however the authors should also mention the finding of previous reports that closed endotracheal suction seems less efficient in terms of secretion removal compared to open endotracheal suctioning (Lasocki Anesthesiology 2005).

**Response:** Thank you for raising such crucial point and this issue has been added to the revised Discussion (please see page 19, lines 7-9). In addition to your pointed our reference, we also added another one reference in the revised manuscript (please see newly added reference number 32 in our revised manuscript).

**Comment 6:** Discussion – In addition, previous studies show both OS and CS to cause derecruitment. A recruitment maneuver immediately following endotracheal suctioning could be effective in counteracting the deterioration in PaO2 and lung volume. The authors could discuss prevention of arterial desaturation by performing recruitment maneuvers.

**Response:** We addressed the raised concern in the revised Discussion (please see page 19, lines 10-18 and newly added references number 32 and 33 in our revised manuscript).

**Comment 7:** General – Overall the manuscript has English grammar and syntax issues.

**Response:** The English was checked by a native speaker with extensive experiences in biomedical research.
**Minor comments**

**Comment 1:** Title – The title should describe better what was found in this study.

**Response:** In accordance with the Reviewer’s comment, we have modified the title as follows: “Repeated open endotracheal suctioning causes gradual desaturation but does not exacerbate lung injury compared to closed endotracheal suctioning in a rabbit model of ARDS” (Please refer to revised manuscript)

**Comment 2:** Introduction – page 6: “During these interventions, patients may be…” It is not clear which interventions are referred to.

**Response:** Appropriate changes have been made to the revised Introduction (please see page 5, lines 6 - 9).

**Comment 3:** Introduction – page 6: The authors wrote: “The reports suggest that OS induces alveolar de-recruitment” and refer to Wolf CCM 2007. Wolf however discussed endotracheal suctioning without disconnection from the ventilator, i.e., closed suctioning.

**Response:** Changes were made accordingly in the revised Introduction (please see page 5, line 15 and page 6, lines 2 - 4).