Author's response to reviews

Title: High tidal volume mechanical ventilation-induced lung injury in rats is greater after acid instillation than after sepsis-induced acute lung injury, but does not increase systemic inflammation: an experimental study.

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Version: 4 Date: 13 July 2011

Author's response to reviews: see over
Dear Dr. Harris,

It is a pleasure to submit our revised manuscript entitled “High tidal volume mechanical ventilation-induced lung injury in rats is greater after acid instillation than after sepsis-induced acute lung injury, but does not increase systemic inflammation: an experimental study.” by Jan Willem Kuiper, Frans B Plötz, AB Johan Groeneveld, Jack J Haitsma, Serge Jothy, Rosanna Vaschetto, Haibo Zhang and Arthur S Slutsky.

We have attempted to answer the reviewers comments. In particular the comments of reviewer 2 concerning the study design and data interpretation. We therefore comment on the sham group, the IL-6 data and added arterial blood gas data. We made changes to the manuscript according to the suggestions of reviewer 1.

The changes are marked red in the revised version. On separate pages we have addressed the comments point by point in more detail as requested.

We would like to take the opportunity to thank the reviewers for reviewing our manuscript. We hope that the aforementioned changes have improved the manuscript in accordance to the recommendations.
I certify that the revised manuscript, or part of it, has not been published nor is currently under consideration for publication by any other journal. The co-authors have read the revised manuscript and approved its submission to BMC Anesthesiology.

Sincerely,

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REPLY

“High tidal volume mechanical ventilation-induced lung injury in rats is greater after acid instillation than after sepsis-induced acute lung injury, but does not increase systemic inflammation: an experimental study.” by Jan Willem Kuiper, Frans B Plötz, AB Johan Groeneveld, Jack J Haitsma, Serge Jothy, Rosanna Vaschetto, Haibo Zhang and Arthur S Slutsky. We thank the reviewers for their valuable comments.

Reviewer number: 1

**Major compulsory revision:**
The paragraph concerning studies of the systemic inflammatory response in different models should be eliminated.

*We eliminated the paragraph and added the suggested reference. We made more clear what this study adds in comparison to the suggested reference in the discussion in the last paragraph on page 13. We clearly feel that our ventilator setting are more clinically relevant. We also noted that we analysed more mediators than TNF-α alone.*

**Discretionary revisions:**
The chosen animal models (intra-tracheal acid instillation and cecal ligation and puncture) are well established but vary in the degree of lung damage. Although the findings are consistent the paper is solid a paragraph should be added to the discussion, discussing the choice of models and their limitations.

*We added a paragraph in the discussion (second paragraph of the discussion, page 11) outlining the arguments for choosing the abovementioned models. Acid instillation followed by mechanical ventilation has been described to be a good model for clinically relevant situations. CLP is widely used, especially to mimic septic endothelial insults and organ damage. We also elaborated on the limitations of these models.*

The timing of different cytokines involved in the immune response is variable and the pattern of secretion also varies with type of injury model, intensity of the stimulus, duration and so on. Other mediators could have yielded different results. The sepsis model produced less systemic response, suggesting that this was a mild stimulus.
Mediator release during mechanical ventilation has been topic of debate, in this study this is further complicated by the additional models (acid instillation and sepsis). The intrinsic dynamics of different cytokines and severity of the sepsis model are certainly factors to take into account. In the last paragraph of the introduction on page 4 we motivate our choice of mediators. In the paragraph on the effects of the models and mechanical ventilation we added the suggested reference (first paragraph on page 14). Also, in the limitations paragraph, the differences in severity of the models is highlighted (the second paragraph on page 14).

**Reviewer number:** 2

**Major comments:**

The effects of mechanical ventilation per se on inducing lung injury were not investigated in this study. The study would be more complete if two groups (low and high tidal volume ventilation) of sham instrumentated animals were included.

*As mentioned in the discussion, mechanical ventilation with a high tidal volume of 15 ml/kg does not injure normal rat lungs. We therefore did not needed to study the effects of mechanical ventilation per se to draw the conclusions that the effects of mechanical ventilation on lung injury and mediator release differed between direct and indirect lung injury.*

Data regarding lung inflammation and arterial blood gas data are lacking. The data would be more complete if these data were included.

*Specific mediator measurements in, for example, bronchoalveolar lavage have not been performed, but would have added to the data. However, a quantitative morphometric analysis of the lungs has been performed. This also included the number of alveolar polymorphonuclear leukocytes and macrophages (materials and methods section on page 8 and 9). Arterial blood gas data (pH and PaCO\(_2\)) are readily available and have been added to table 1 on page 26, PaO\(_2\)/FiO\(_2\) data are shown in figure 1.*

The authors frequently withdrew arterial blood for analysis during the experiment. This may have caused significant blood loss, and therefore, induce hemorrhagic shock. The
authors should justify the their decision for frequent blood drawing during the experiment and report how they control the effects of this manipulation.

Before sacrifice a maximum of 7 blood gas analysis were performed for each rat. The blood gas analyser we used, only requires 80 microliter per sample. Obviously a little more was withdrawn from the rats, maximum approximately 100 microliters. For the whole experiment before sacrifice, we estimate that maximally 1 ml was taken from each rat with an estimated circulating volume of over 20 ml. All withdrawn volumes were equally compensated with normal saline. We don not think that this results in hemorrhagic shock since no drop in blood pressure has been observed following withdrawal of blood. In the materials and methods section (second paragraph on page 9) we added information about the blood withdrawals and compensation.

Data presentation and analysis require major revision. The reported difference between IL-6 plasma concentrations after acid instillation and during sepsis are not supported by the data reported in table 2. In addition there appears to be a difference in IL-6 levels between LVt and HVt after acid instillation. The authors should report the result of the statistical analysis and the significance.

We apologize for the misunderstanding. We used univariate analysis of variance and generalized estimating equations for longitudinal data. First, we analysed the effects of both models (acid instillation and sepsis), by combining all data of each model. Subsequently, we analysed the effects of the different MV strategies. Lastly, we determined interaction to show model dependent effects of MV. We added extra information to the statistical analysis paragraph on page 9 to clarify the reported statistics. As shown by a lack of interaction between model and MV for IL-6, the difference between LVt and HVt IL-6 levels are not statistically significant. We report this more clearly in the results section on mediators on page 10.

Abundant data have indicated that high tidal volume mechanical ventilation could aggravate lung injury in septic animals. Data in this study seem to indicate otherwise, as shown by the lack of difference between the effects on lung injury of LVt and HVt ventilation or sepsis alone.
The reviewer is certainly right that high tidal volume ventilation can cause significant lung injury during sepsis. This is clearly shown in the suggested studies. However, these and other studies apply tidal volumes that are much higher than the tidal volumes we use. For example, Nin et al. and Hu et al. used tidal volumes of 35 ml/kg and 28 ml/kg respectively. Yang et al. found increased lung injury with a tidal volume of 20 ml/kg, similar to the study by Herrera we referred to. Herrera et al. also reported from their preliminary studies that tidal volumes of less than 20 ml/kg do not result in easily identifiable lung injury during sepsis. We made the abovementioned more clear in the discussion in the first paragraph on page 13.

**Minor comments:**

The symbols of figure two were not labelled.

*The labels are explained in the legend of figure 2.*