Reviewer's report

Title: Effect of low tidal volume ventilation on lung function and inflammation in mice

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Reviewer: Ruud Veldhuizen

Reviewer's report:

In this paper by Hauber et al, the effect of a number of ventilation strategies is tested with specific focus on low tidal volume strategies. The outcomes involve lung function measurements, histology, polymorphonuclear leukocytes numbers and expression of inflammatory mediators. Although there is a need to better understand the effects of different ventilation strategies in mice, I have significant concerns with the current manuscript.

1) The purpose of this paper is not quite clear to me. Is there really a need to distinguish between tidal volumes of 5, 7 and 10ml/kg in the normal mouse? The rationale provided is that in humans 6ml/kg is optimal but that we don’t really know what is optimal in mice. However, this argument is not valid since the human data is based on the NIH study on ARDS patients whereas this study is performed on normal mice. The effect of tidal volume when ventilating a lung with underlying injury is markedly different than when ventilating a normal lung; in fact most hospitals will likely use tidal volumes in the range of 8-12ml/kg when ventilating patients without lung injury. The authors’ suggestion that 10ml/kg is considered protective ventilation in mice is only based on one study (or at least only one reference) which appears to use that tidal volume in conjunction with an underlying injury (ref 12).

2) Beyond the question of the rationale, the second related issue is whether or not this experimental design accurately tests differences between tidal volumes. All mice are ventilated with the same respiratory rate so there are be marked differences in minute ventilation which may be responsible for some of the effects observed. The time frame of ventilation is only 30 minutes, which considering most studies on VILI seems very short to observe significant differences based on small differences in tidal volume. In addition, the weight range of mice is reported to be between 25 and 35 gram. In my experience the weight above 25 gram is due to fat and not a proportional increase body size. This implies that the delivered tidal volume relative to the size of the lung may have varied considerably.

3) Supporting the above comments, and counteracting the authors’ conclusion that “Our data indicate that mechanical ventilation with a tidal volume of 10 ml/kg and addition of 2 cm H2O of PEEP in mice may be close to the protective ventilation in humans” is the fact that there are very few significant differences due to differences in tidal volume 5, 7 and 10ml/kg. As far as I can tell, only MIP-2 expression reaches a significant difference between 5 and 10ml/kg without
peep, all other values for all other outcomes are not different among the tidal volume groups. So overall, my interpretation of the data/experiment is that ventilation (surgery, anesthetic, flexivent manipulations for compliance measurements and changes in minute volume etc) are responsible for the majority of the observed effects and it is difficult to interpret the data in the context of differences in tidal volume as was the objective.

4) Complicating the evaluation of this paper is the poor quality of the figures. Regarding the figures, number 1 and 2 were not readable and figure 4 would have been improved by inclusion of the spontaneously breathing animals. I also don’t quite understand why compliance and resistance are expressed as a relative change, the flexivent assessment gives specific values for these measurements.

Minor comments:

1) The description of the results is often somewhat confusing; There are many comparisons that can be made: values at different time points compared to baseline values, changes over time, comparison between PEEP and no PEEP, and comparisons between the different tidal volumes. Making it very clear which comparison the authors are referring to in the paper would help the readability of the paper.

2) The minute volume between 100 and 200ul mentioned in the discussion is not correct. The authors presumable mean tidal volume, although I am not even sure if that calculation is accurate.

3) Were the lungs for histological analysis inflated during fixation?

4) Why were there such marked differences in n-values between different groups?

5) In the discussion the authors’ state “Moreover, the flexivent system can be used to apply nebulized fluids with a volume as low as 50 µL” …. I am not sure what the relevance of this sentence is in the context of this paper.

6) Typographical errors etc
   - no page numbers
   - Abstract “Oyxgen” “leukocyzes”
   - Protocols “endexpiratory”
   - Results “quantifaction”
   - Discussion “lungs of mice and men” …. mice and humans is probably better.
   - Discussion “areals”
   - Discussion “cytokine expression lower tidal volumes” …. Missing the words “in” and “groups” or “animals”.
   - Fig legend 1 “puls”

**Level of interest:** An article of insufficient interest to warrant publication in a
Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests