Author’s response to reviews

Title: Lung virome in mechanically ventilated patients: a pilot study

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16th December 2019

Dear Professor Robin L. Cassady-Cain,

Editor of the BMC Pulmonary Medicine,

We would like to thank you for the opportunity to resubmit a revised version of our manuscript “Lung virome in mechanical ventilated patients: a pilot study” (PULM-D-19-00374). We are also very grateful to the reviewers for their relevant comments, which have contributed to a significant improvement in the quality of our manuscript.

Please find below our answers to the issues mentioned by the reviewers.
Sincerely yours,

Paulo Paixão

Editor and Reviewer Comments:

Kristine Wylie (Reviewer 1):

The authors describe a study aimed at characterizing viruses in the lower airway in adult patients who were mechanically ventilated. They compared patients without acute lower respiratory infection and those with acute lower respiratory infection. Importantly, both of these patient populations consist of subjects who are very ill, so it should be noted that the observations made in this study may not be generalizable to other groups.

Answer:

A full sentence, addressing this important point, was included in the chapter “Discussion”.

In the background, the authors make the case that the characterization of the human virome in healthy individuals may contribute to a better understanding of the associations with the virome with disease. While this is true, I would suggest changing the background to better fit the present study since no healthy individuals were evaluated.

Answer:

Changes were performed, according to this recommendation.

I would suggest clarifying the way that the groups are referred to throughout the text. The goal is to characterize the virome in those with infection and those without, and I found it a little confusing that those with infection were referred to as "controls" because it wasn't clear what specific hypothesis was being tested that made that group the control group. Alternatively, a more clear hypothesis regarding the presence of viruses in asymptomatic subjects on a ventilator could be stated and supported with the data.

Answer:

According to this recommendation, modifications were made throughout the text, deleting “control group”, and thus clarifying this point.
Conclusions: The authors say they can infer the presence of a lung viral community. I do not think this can be concluded because this could be contamination from ventilation or sample collection.

Answer:

Following this very important observation, we changed our conclusions.

Furthermore, they say that the viruses seem to replicate in this environment without causing symptomatic infection, but they have no data to support the these viruses were replicating.

Answer:

We fully agree with this comment and therefore the conclusions were modified.

A few sentences need to be clarified:

Line 58 in discussion - "This group obtained the best percentage of reads attributable to viral genomes, compared to similar studies." It is not clear what's meant by this or its implications..

Answer:

In fact the sentence was not clear, and therefore we modified it in this revised version.

Discussion next page line 10 - "common" respiratory viruses. Does this mean common among subjects in this study or common in the population?

Answer:

We changed to make it clear.

Line 5 - "reduced number of samples" should be "small number of samples".

Answer:

Corrected

Line 58, Background - the authors should provide a reference for the statement that the lung was previously considered sterile.

Answer:
Included

Next page, lines 1-10 - the authors describe studies that indicate that the microbiome of the lower respiratory tract may simply be explained by micro aspiration and would then reflect the composition of the upper respiratory tract. The authors should address whether this would be true in their study, and it seems very likely that both ventilation and sample collection would contribute to this type of contamination or seeding (depending on perspective) of the lower respiratory tract with viruses from the upper tract.

Answer:

Following this very important observation, we changed our conclusions.

There are typos in several words –

- "length" is written lenght in the text and in table 1. Answer: Corrected

-"analised" should be analyzed. Answer: Corrected

- BAL should be defined. Answer: It is on the first line of the Study design.

- Citomegalovirus should be cytomegalovirus. Answer: Corrected

- "sincytial" should be syncytial. Answer: Corrected

Marcus Panning (Reviewer 2):

Nazareth and colleagues aimed to describe the lung virome of ventilated patients with and without respiratory symptoms admitted to intensive care units in the Lisbon area.

They performed real-time PCR on mini-bronchoalveolar lavage samples for a number of viral respiratory pathogens and compared the detection rates among the two groups.

The study is of interest as it sheds light on the presence of viral respiratory pathogens which might be present in the lung. Technically, the study used a prospective-observational approach.

This brings me to the first point. The major issue of concern is that the inclusion criteria remain vaguely described. E. g., no information is given if patients assigned to the group without respiratory symptoms had respiratory infection shortly before admission to the ICU. In addition, it would be informative to know the definition of respiratory infection (i.e. influenza-like illness, fever etc.).

Answer:
This is now addressed both in the “Methods” and in the “Discussion”

To better appreciate the relevance of viral detections in lung specimens it might also be of interest to include throat/pharyngeal swab samples from the same patient.

Answer:

This is a very interesting suggestion, but we did not include it on our project.

Minor points:

Do the authors have information on influenza vaccination history?

Answer:

Answered on Study design

The authors aimed to describe the lung virome (title) but used a limited number of RT-PCR assays for respiratory viruses only. Although the authors mention this in the discussion I would suggest to delete the term virome from the manuscript/title and replace it by detection of respiratory viruses or alike.

Answer:

The title was changed “Respiratory viruses in mechanical ventilated patients: a pilot study “

In this respect, the objectives/conclusions need to be modified since only common respiratory viruses were detected and no attempts with an unbiased approach using Answer:

Changes were performed according to this recommendation

There are some typos scattered across the manuscript, e.g. Abstract, line 38 "respiratory sincytial virus", Mini-BAL procedure, line 48 "throught", Discussion, line 56 "analised"

Answer:

We thank the reviewer for this observations; corrections were performed.

Data analysis: Although mentioned, correlation of two parameters has not been shown in the manuscript. Please consider to delete the respective paragraph.

Answer:
This was a mistake. We followed the reviewer’s suggestion.

The authors conclude that respiratory viruses seem to replicate in the lung environment. Without using cell culture to isolate virus this conclusion seems rather far-fetched.

Answer:

We changed the texto according to this recommendation.