Peer Review File

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Reviewer A

Comment 1. The authors state that patients had PEEP increased by 3 cmH2O and decreased by 3 cmH2O from baseline. Looking at eTable 1, it appears some patients only had PEEP changed by 2 cmH2O, and some by 4 cmH2O. For example, patient 2 was increased from PEEP 14 to PEEP 18. Patient 3 was only increased from PEEP 12 to PEEP 14. If these values are incorrect, they should be corrected. If they are not correct, the authors should address the ranges of PEEP changes that were made in the manuscript.

Reply 1: Thank you very much, and we are very sorry that it was not clearly described in the manuscript. First, we just wanted to evaluate the transmission of airway pressure, the ARDS status of the lung, such as compliance, resistance, consolidation, heterogeneity, et al was not intended to disrupt. Recruitment maneuver and extremely low PEEP that might cause the reinflation or collapse of lung was not chosen to avoid any modification of the lung status. But in order to test our hypothesis, the PEEP levels should be changed significantly to cause Pes changes. 24 to 37 percent of airway pressure was transmitted to pleural space as previously reported (**Chest 1985, 85(5):653-658;** PMID: 3902386). As a 30% transmission of 3 cmH2O PEEP could cause 1cmH2O change of Pes, PEEP level was designed to change with an average of 3 cmH2O. hemodynamics of certain patients were severely affected when high airway pressure was applied clinically, as a result the PEEP level was changed 2 to 5cmH2O, with the median of 3cmH2O. The manuscript (including eTable1) has been revised accordingly.

Changes in the text:

1) Abstract-Methods: "PEEP was increased and decreased for 3cmH2O" was changed into "PEEP level was decreased and increased subsequently (with an average change of 3 cmH2O)"
2) Methods: 3) Respiratory Mechanics measurements "PEEP was increased (PEEP+3cmH2O) and decreased (PEEP-3cmH2O) for 3cmH2O from the baseline PEEP (PEEPbaseline)" was changed to "In accordance with the study aim, the Pes response to PEEP changes was evaluated, and it was previously reported that 24 to 37 percent of airway pressure was transmitted to pleural space. Besides, hemodynamics could be severely affected when high airway pressure was applied, and the ARDS Status of the lung, such as compliance, resistance, consolidation, heterogeneity, et al, was not intended to disrupt, so recruitment maneuvers (RM) and extremely low PEEP that might cause the reinflation or collapse of lung was not chosen. As a 30% transmission of 3 cmH2O PEEP could cause 1cmH2O change of Pes, the PEEP level was designed to change with an average of 3cmH2O."
3) in the Result Section we added the PEEP changes in the second paragraph in the revised. manuscript, which reads

"PEEP level was designed to change with an average of 3 cmH2O. however, hemodynamics of certain patients was severely affected when high airway pressure was applied clinically, as a result the PEEP level was changed 2 to 5cmH2O, with the median of 3cmH2O".

Comment 2. The PEEP dependent and independent groups were defined by the changes in Pes were more or less than 30% of changes in PEEP. Was 30% chosen apriori? If so, why 30%? Or is there a physiologic rationale for this choice. Additionally, the 30% should be added to the eFigure 4 flowchart.

Reply 2: Many thanks for highlighting this import issue. Airway pressure transmission was rarely studied. Jardin et al. found that 24 to 37 percent of airway pressure was transmitted to pleural space and the transmission was influenced by the lung stiffness because of the damping effect (**Chest 1985, 85(5):653-658;** PMID: 3902386). Since then, no more relevant studies were identified, so we chose the 30% of transmission empirically. As suggested by the Reviewer, the 30% has been added to the eFigure 4 flowchart.

Changes in the text:

we have modified our text as advised:

a. Methods: 3) Respiratory Mechanics measurements: “In accordance with the study aim, the Pes response to PEEP changes was evaluated, and it was previously reported that 24 to 37 percent of airway pressure was transmitted to pleural space. Besides, hemodynamics could be severely affected when high airway pressure was applied, and the ARDS Status of the lung, such as compliance, resistance, consolidation, heterogeneity, et al, was not intended to disrupt, so recruitment maneuvers (RM) and extremely low PEEP that might cause the reinflation or collapse of lung was not chosen. As a 30% transmission of 3 cmH2O PEEP could cause 1cmH2O change of Pes, the PEEP level was designed to change with an average of 3cmH2O.”

b. eFigure 4:

1) “PEEP-dependent Type (Δpes at end-expiratory occlusion≥ΔPEEP) (N=12)” to “PEEP-dependent Type (Δpes at end-expiratory occlusion≥30% ΔPEEP) (N=12)”

2) “PEEP-independent Type (Δpes at end-expiratory occlusion<ΔPEEP) (N=6)” to “PEEP-independent Type (Δpes at end-expiratory occlusion<30% ΔPEEP) (N=6)”

Comment 3. The authors do a nice job of addressing the clinical context of their findings in the discussion; however, I think they can provide a more nuanced discussion of regional differences in pleural pressure with changes in PEEP, and where esophageal manometry is accurate. PMID 29323931 provides an elegant physiologic study showing the pleural pressure gradient between non-dependent and dependent lung regions and how these pressures are affected by changes in PEEP. Esophageal manometry correlates well to dependent lung regions, but dramatically underestimates transpulmonary pressures in non-dependent lung regions, and these inspiratory pressures, where pressure is more distributed to non-dependent regions rather than some distribution to the pleura in the “PEEP-independent” will increase risk for regions of overdistension. I think such a nuanced discussion will add to the overall claims that the author is making. Additionally, it can be discussed in one or two sentences that these issues may be overcome in the prone position (PMID 33406012).

Reply 3: Thank you very much for your valuable comments. After thoroughly reading the literatures. You provided, a more nuanced discussion was rephrased. Your review comments added a lot to the article and serve as the finishing touch. Thanks again for your excellent work, and we really appreciate it.

Changes in the text:

The fifth paragraph of discussion section was rephrased as

“Although measuring Pes might be of great interest to identify the optimal PEEP required to prevent alveolar collapse, with the aim for an end-expiratory P_L of 0, it could always be obtained with an incremental PEEP titration, but patients were put at high risk of hemodynamic instability and lung overdistension, especially in the nondependent regions. Yoshida et al. showed the pleural pressure gradient between non-dependent and dependent regions in lung-injured pigs and human cadavers **(PMID 29323931)**. Esophageal pressure was found to correlate well with pleural pressure in dependent lung regions. In the PEEP-independent patients, Pes did not change with PEEP changed, as the pressure is more distributed to the pleura in non-dependent regions. So, PEEP increase in these patients would significantly increase the risk of overdistension. Due to the heterogeneity of ARDS lung, it was impossible to obtain full lung recruitment without overdistension simply through airway pressure titration. This could also be the reasons why EPVent 2 Trial failed to demonstrate the superiority of esophageal pressure guided PEEP strategy. The differences in P_L among lung regions may introduce pendelluft, which can be monitored at the bedside with electrical impedance tomography (EIT) (PMID: 35246748). Previous study showed that PEEP can decrease the level of pendelluft in spontaneously breathing subjects . It was still unclear whether PEEP could alter the regional P_L(PMID: 27002273). Further study could combine Pes and EIT to explore the correlation between the presented Pes phenotypes and pendelluft. Prone position reduces the dorsal-ventral pleural pressure gradient and homogenization was much less dependent on PEEP levels in prone than supine position (PMID 33406012). The different Pes responses to PEEP change could be perfectly overcome by prone position and Pes could be much more “PEEP-dependent” during proning.”

Comment 4. Overall, the writing can be significantly improved as there are many grammatical. errors throughout the manuscript. There are some areas where the phrasing or sentence structure is awkward, or words are incorrect. Some examples are provided below.

a. Line 269: I presume the authors are trying to say the following: "Although measuring Pes. might be of great interest to identify the optimal PEEP required to prevent alveolar collapse, with the aim of an end-expiratory PL of 0 performed by incremental PEEP titration, patients are at high risk for inducing hemodynamic instability and lung overdistension, especially in non-dependent regions."

b. Line 275: should read "…failed to demonstrate the superiority of esophageal pressure-guided PEEP strategy"

c. Line 280: should read "…better sever the clinician…" instead of clinic.

Reply 4: Thank you very much for your meticulous work, and we are very sorry for our incautiousness. We double-checked our manuscript again, and grammatical errors were all corrected in the revised manuscript.

Changes in the text:

a. Line 269 was modified as "Although measuring Pes might be of great interest to identify the optimal PEEP required to prevent alveolar collapse, with the aim for an end-expiratory PL of 0, it could always be obtained with an incremental PEEP titration, but patients were put at high risk of hemodynamic instability and lung overdistension, especially in the nondependent regions"

b. Line 275 was revised as "This could also be the reasons why EPVent 2 Trial failed to demonstrate the superiority of esophageal pressure guided PEEP strategy."

C. Line280 was changed into "We tried to sum up some potential rules to better serve the clinicians"

Reviewer B

The authors present a clinical study of 33 patients with moderate to severe ARDS. They sought to determine the association between changes in PEEP to changes in esophageal pressure measured by manometry. They hypothesized that patients would differentiate into two groups based on the esophageal pressure response to PEEP changes – those without any change in esophageal pressure and those with a change to esophageal pressure.

The authors are really commended on performing a clinical study in an ARDS population. These are quite difficult to perform. I think the relationship between how PEEP/airway pressure is dispersed to the pleural is an important question but I think the authors missed opportunities to include other relevant physiologic data to better define this complex relationship. Decisions to dichotomize responses and the choice of PEEP changes seem arbitrary and the relevance of the results to clinical practice is overstated.

My major concerns are below:

Comment 1: The rationale of choosing to increase/decrease PEEP at a level of 3 is not well ◦ specified. Can the authors better explain their choice? Would it not have been better to look at the relationship of esophageal pressure to PEEP across a broader range of PEEP – either during incremental and/or decremental PEEP trials and reporting lung/chest wall compliance and driving pressure at each PEEP level. This would provide some insight into how airway closure and lung recruitment effects the dissipation of applied pressure to the pleural space.

Reply 1: Thank you very much for your valuable comments. We fully agree with the Reviewer that altering PEEP to a broader range may explore the airway closure and lung recruitment effects. On the other hand, the aim of our study was to evaluate the transmission of airway pressure. Therefore, we would like to have less influence on the ARDS status of the lung, such as compliance, resistance, consolidation, heterogeneity, et al. Recruitment Maneuvers or extremely low PEEP that might cause the reinflation or collapse of lung were not considered in the current study. Regarding the level of 3 cmH_2O, it was reported that 24 to 37 percent of airway pressure was transmitted to pleural space (**Chest 1985,**

85(5):653-658; PMID: 3902386)). A 30% transmission of 3 cmH_2O could cause approximately 1cmH_2O change of Pes. Therefore, the PEEP levels were designed to increase or decrease for average of 3 cmH_2O. During the study performance, hemodynamics was severely affected in certain patients, so the PEEP level was changed 2 to 5cmH_2O, and the median change of PEEP was 3cmH_2O. The methodology of the manuscript has been detailed described in the revised manuscript and the changes of PEEP for each patient has been added to the Additional File 5 (eTable 1).

Changes in the text:

1) Abstract-Methods: "PEEP was increased and decreased for 3cmH2O" was changed into "PEEP level was decreased and increased subsequently (with an average change of 3 cmH2O)"
2) Methods: 3) Respiratory Mechanics measurements "PEEP was increased (PEEP+3cmH2O) and decreased (PEEP-3cmH2O) for 3cmH2O from the baseline PEEP (PEEPbaseline)" was changed to "In accordance with the study aim, the Pes response to PEEP changes was evaluated, and it was previously reported that 24 to 37 percent of airway pressure was transmitted to pleural space. Besides, hemodynamics could be severely affected when high airway pressure was applied, and the ARDS Status of the lung, such as compliance, resistance, consolidation, heterogeneity, et al, was not intended to disrupt, so recruitment maneuvers (RM) and extremely low PEEP that might cause the reinflation or collapse of lung was not chosen. As a 30% transmission of 3 cmH2O PEEP could cause 1cmH2O change of Pes, the PEEP level was designed to change with an average of 3cmH2O."
3) in the Result Section we added the PEEP changes in the second paragraph in the revised. manuscript, which reads

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Comment 2: Similar to the above comment, what is the rationale to choose a Pes of > 30% as. the cutoff for the outcome? Why even dichotomize the outcome at all instead of evaluating Pes response as a continuous outcome and evaluating factors associated with higher Pes response using regression? The dichotomization results in a loss of data – there could be other patterns of variability in the outcome that are missed.

Reply 2: Many thanks for highlighting this import issue. Airway pressure could transmit to pleura, but the transmission was rarely studied, Jardin et al. found that 24 to 37 percent of airway pressure was transmitted to pleural space and the transmission was influenced by the lung stiffness because of the damping effect (**Chest 1985, 85(5):653-658;** PMID: 3902386)). Since then, no more relevant studies were identified. so we chose the 30% of transmission empirically. Actually, airway pressure could transmit to the pleural space heterogeneously, **i.e. transmit to the dependent regions where esophageal manometry correlates well to**. We fully agree with the Reviewer that Pes response to PEEP change was a continuous variable, and dichotomization could result in a loss of data. But due to the small sample of our study, regression or other analysis of Pes as continuous variables could be severely biased. We really appreciate your comments, and in our future study, the Pes response to PEEP change in more ARDS patients shall be assessed, where more delicate statistics can be performed. The manuscript has been revised as below.

Changes in the text:

1) Methods: 3) Respiratory Mechanics measurements "PEEP was increased (PEEP+3cmH2O) and decreased (PEEP-3cmH2O) for 3cmH2O from the baseline PEEP (PEEPbaseline)" was changed to "In accordance with the study aim, the Pes response to PEEP changes was evaluated, and it was previously reported that 24 to 37 percent of airway pressure was transmitted to pleural space [16]. Besides, hemodynamics could be severely affected when high airway pressure was applied, and the ARDS Status of the lung, such as compliance, resistance, consolidation, heterogeneity, et al, was not intended to disrupt, so recruitment maneuvers (RM) and extremely low PEEP that might cause the reinflation or collapse of lung was not chosen. As a 30% transmission of 3 cmH2O PEEP could cause 1cmH2O change of Pes, the PEEP level was designed to change with an average of 3cmH2O."

Comment 3: The authors make a lot of declarative statements in the discussion regarding. physiology that are actually speculative and not based on the physiologic data presented. For example, the discussion of airway closure is relevant. However, airway closure was not measured which is a missed opportunity and a major limitation. Moreover, there are several statements on the clinical utility of the results as a way to improve the impact of esophageal manometry which I fail to see based on the data provided. Clinically, I would assess airway closure and lung recruitment as methods to determine how PEEP effects Pes, not the arbitrary cutoff established in this trial. The authors should focus more on explaining this relationship rather than eluding to any clinical reference.

Reply 3: Thank you very much for your valuable comments. We measured esophageal pressure in clinical routine during PEEP titration and evaluated the inspiratory effort of ARDS patients. We found the different Pes change with PEEP adjustment. We were so excited about this phenomenon, so we performed PEEP adjustment just as before to test the findings, although we did not know what physiology behind this phenomenon. Regrettably, just as you commented, when sorting through the data, there was no physiologic data to explain the different response of Pes to PEEP changes. We only speculated that the findings were caused by the airway closure or less transmission to the pleura where esophageal manometry was measured. But as a major limitation of our study, both the speculations were not supported by the current data. As you addressed, all the statement was revised as speculation, and in our future studies, we will try our best to explore the underlying mechanisms of this finding.

Changes in the text:

The third paragraph of the Discussion section was revised as below:

"Another theory to explain this phenomenon was the relation between Pes and Ppl and the pleural pressure gradient due to heterogeneity of ARDS lung. Pes was found to correlate well with pleural pressure in dependent lung regions (PMID: 29323931). It was showed in our study that PEEP-dependent patients had lower E_L and E_{RS}, as well the E_L/E_{RS} ratio, while there was no significant difference in E_{CW} between the two groups (Table 1). The PEEP-dependent patients showed better lung compliance, which could exhibit better conductive transmission. Endotracheal pressure could transmit to pleura in dependent regions which reflected as Pes changed with PEEP adjustment. Decades ago, Jardin et al. (PMID: 3902386) evaluated the airway pressure transmission using the relationship between tracheal pressure and Pes, and they found that the lung stiffness influenced the transmission because of the damping effect, with higher transmission in patients with higher Crs. On the other hand, PEEP might transmit much less to pleura in dependent regions in PEEP-independent type patients which showed little or no changes of Pes with PEEP titration, as the pressure was more distributed to the pleura in non-dependent regions. Our study further confirmed this transmission differences, with more accurate Pes measurements and focusing on end-expiratory phase. Even more, taking into account the change of PL simultaneously, the results were more conducive to the clinical application of PEEP titration guided by Pes."

Comment 4: This statement on line 279-281 is concerning. "We just tried to sum up some potential rules to better serve the clinic, that was, simple phenotypes of esophageal pressure without complicated analysis" As stated above, these relationships are very complex and deserve attention to collecting all relevant physiologic data. The conclusion here made by the authors is not at all appropriate justification to not provide this data. The focus on "simple phenotypes" here is a significant limitation.

Reply 4: Thank you very much for your meticulous work, and we are really sorry for our incautiousness. As you commented, the relationship between Pes and PEEP was very complicated, but we were not able to illustrate the underlying mechanism with the current data. The study was designed to distinguish the two types of Pes responses to PEEP changes, and for the PEEP-independent patients, no matter what reasons, relatively high PEEP should be cautiously used to achieve reopening of collapsed lung tissues. As esophageal manometry became widely used, we wanted to deliver the message through our study that Pes responded differently with PEEP changes, and Pes-guided PEEP titration should be cautious, especially for the PEEP-independent patients. The relationship between Pes and PEEP deserved further

attention with additional relevant physiologic data, and our study was just a beginning to explore the sophisticated physiology.

Changes in the text:

The last paragraph of Discussion Section was rephrased:

As one of the few PEEP titration methods available in clinical practice, esophageal manometry has its unique value, but the results should be cautiously interpreted. The barotrauma, hemodynamic impact of higher PEEP is always the issue. As illustrated in our study, two types of Pes responded to PEEP changes were found, and especially for the PEEP-independent patients, relatively high PEEP should be cautiously used to achieve reopen of collapsed lung, as Pes value could not reflect the pressure transmission accurately. The relationship between Pes and PEEP is very complicated and deserves attention with additional relevant physiologic data to explore. Airway closure and less transmission to the pleura where esophageal manometry was located are two different reasons and correspond to two different titration strategies. Our study might provide a potential method to solve the problems of applying esophageal manometry to guide PEEP setting. Through the Pes responses to PEEP increase or decrease of 3-5cmH_2O, two types were identified without sophisticated calculation and analysis. Regrettably, we were not able to illustrate the underlying mechanisms, and it was just the first attempt to explore the sophisticated physiology.