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Physiological evaluation of ventilation perfusion mismatch and respiratory mechanics at different positive end expiratory pressure in patients undergoing protective one-lung ventilation.

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Physiological evaluation of ventilation perfusion mismatch and respiratory mechanics at different positive end expiratory pressure in patients undergoing protective one-lung ventilation.

Dear Professor Kavanagh,

Please find enclosed our answer to the reviewer’s comments.

We would like to thank you and the reviewers for your constructive criticism. We have considered each of your points in turn, below.

Yours sincerely

Savino Spadaro and all co-authors.

- Responses to reviewers –

Reviewer #1: The authors have examined the effect of 3 different PEEP levels on ventilation perfusion (V/Q) ratios and driving pressure in adult patients undergoing one lung ventilation (OLV) during VATS lung resection surgery. A low tidal volume strategy (4-5 ml/kg PBW) was used during OLV. They employed a novel technique for the assessment of V/Q ratios that is much less cumbersome than the classic Multiple Inert Gas Elimination Technique (MIGET). They found that the application of 10 cm H2O of PEEP during OLV reduced shunt fraction and driving pressure and improved lung compliance when compared with PEEP=0 and PEEP=5 cm H2O.

The findings are novel and potentially important for the provision of support during OLV. The finding of reduced driving pressure is interesting and of potential importance given the link between driving pressure and outcomes in patients with ARDS. To assess the importance of driving pressure in patients undergoing OLV will require direct examination of outcomes in a larger patient population, however.

We thank the reviewer for his or her comment and fully agree that further studies are needed to assess the potential impact of driving pressure on outcome in patients undergoing OLV for thoracic surgery. In order to assess this comment, we changed our discussion as follows:

“*We speculate that the combination of low VT and relatively high PEEP levels during OLV could be beneficial in reducing PPC. However, our physiological study was not designed to investigate the impact of the ventilator strategy on clinical outcomes, and we* *point out that further studies are needed to confirm this hypothesis.”* – Discussion, page 14

Q1.) The described technique for assessing V/Q ratios employs a 3-compartment lung model as opposed to a 50 compartment model in the classic MIGET technique. This probably warrants highlighting in either the methods or the discussion.

A1.) According to the Reviewer’s comment, we modified the limitations section of the discussion to quote previous publications where the technique used in the present study was compared to “gold” standard MIGET:

“The technique for assessing ventilation perfusion matching used in the present study employs a 3-compartment lung model. This model has been shown to be a substantial improvement in describing data when compared to oxygenation indices such as the PaO_2/F_IO_2*,*[46] *but does not include the complexity of the 50 compartments model used in the Multiple Inert Gas Elimination Technique (MIGET), the reference method for assessing gas exchange.*[47] *Though this technique is simpler than the reference one,*[47] *it has been shown to provide a good fit to MIGET data,* [48,49] *and to simulate arterial oxygenation with accuracy comparable to the MIGET model.* [49] *Accordingly, considering that the MIGET technique is costly for routine clinical use,*[50] *the presented technique could be regarded as suitable for bedside estimation of the* $\dot{V}/\dot{Q}$ *ratio*”

46. Karbing DS, Kjaergaard S, Smith BW, Espersen K, Allerød C, Andreassen S, Rees SE: Variation in the PaO2/FiO2 ratio with FiO2: mathematical and experimental description, and clinical relevance. Crit Care 2007;11:R118.

47. Wagner PD, Saltzman HA, West JB: Measurement of continuous distributions of ventilation/perfusion ratios: theory. J Appl Physiol 1974, 36:588-599

48. Rees SE, Kjaergaard S, Andreassen S, Hedenstierna G: Reproduction of MIGET retention and excretion data using a simple mathematical model of gas exchange in lung damage caused by oleic acid infusion. J Appl Physiol 2006; 101:826-32.

49. Rees SE, Kjaergaard S, Andreassen S, Hedenstierna G: Reproduction of inert gas and oxygenation data: a comparison of the MIGET and a simple model of pulmonary gas exchange. Intensive Care Med 2010; 36:2117-24.

50. Wagner PD: Assessment of gas exchange in lung disease: balancing accuracy against feasibility. Crit Care 2007;11:182.

Q2.) The authors comment that 10 cm H_2O PEEP did not result in hyperinflation (line 8 page 13). However, hyperinflation as commonly defined in the ARDS literature is usually assessed using CT Hounsfield units and the relationship between hyperinflation and physiologic dead space ventilation (West zone 1) and other high V/Q lung units is likely variable and it is unclear what the relationship between the authors' technique for assessment of high V/Q ratios and CT measurement of hyperinflation is. This is likely worth underscoring in their discussion.

A2.) We fully agree with the Reviewer and we thank for this comment. Based on this reasoning, we eliminated the statement:

"Of note, this PEEP level did not induce hyperinflation, as indicated by the stability of the high $\dot{V}/\dot{Q}$ fraction throughout the protocol" (discussion, page 13, top) and changed our statement that high V/Q is "a marker of over-distention" and as follows:

"One may argue that, despite the low V_T, the application of PEEP can over-distend the lung parenchyma during OLV. In our study we measured the high $\dot{V}/\dot{Q}$, as a marker of hyperinflation,[40] and found that it did not change neither at PEEP 5 or 10 cm H_2O (Table 3). This indicates that PEEP 10 cm H_2O, when associated with low V_T does not result in an increase in dead space ventilation. Based on these data we speculate that PEEP did not cause alveolar hyperinflation in our patients. However, hyperinflation, as commonly defined in the ARDS literature, is usually assessed using CT Hounsfield units and the relationship between hyperinflation and physiologic dead space ventilation (West zone 1).[41]"

Further, according to the Reviewer's suggestion, we added the following sentence in the limitations paragraph of the discussion.

"It should also be noted that the high V/Q values reported in table 2 represent a functional description of the gas exchange at the lung level, rather than an anatomical description, which is usually derived from CT measurements [41]"

40. Murias G, Blanch L, Lucangelo U: The physiology of ventilation. Respir Care 2014;59:1795-807

41. Gattinoni L, Caironi P, Pelosi P, et al: What has computed tomography taught us about the acute respiratory distress syndrome? Am J Respir Crit Care Med. 2001;164:1701-11.

Q3.) A figure plotting individual vales of high V/Q versus PEEP level and grouped by change in driving pressure (no change and decreased) might assist the reader in interpreting these data.

A3) According to this suggestion, we have added a supplementary figure illustrating the modification of high V/Q grouped by variation in driving pressure. A small non-significant tendency for an increase in high V/Q is seen in patients who do not decrease driving pressure on increasing PEEP.

We have modified the manuscript as detailed below to highlight these results:

1) Results, page 11

"High $\dot{V}/\dot{Q}$ ratio was not significantly different between TLV and OLV, regardless of the PEEP level (Table 2). We found a tendency for high $\dot{V}/\dot{Q}$ to increase at PEEP 10 cm H_2O in those patients where ΔP increased with PEEP (Supplemental figure 1)."

2) Discussion, page 15

"Interestingly, we recorded a non-significant trend for PEEP-induced increase in high $\dot{V}/\dot{Q}$ ratio only in the few patients (6/41; 15%) in which the driving pressure did not decrease by increasing PEEP (Supplemental figure 1)."

Q4.) The manuscript would benefit from review for spelling and language use.

A4) The language and spelling has been checked by a native speaker.

Reviewer #2: **This physiological study Dr. Spadaro and his coworkers provides PEEP-dependent estimations of ventilation-perfusion mismatch during OLV in context of anesthesia for thoracic surgery. I have some comments mainly on the description of methods and its limitations.**

MAJOR COMMENTS

Q1.) Abstract: Some relevant information in the Abstract is missing. The method (principle, not the monitor) to determine ventilation-perfusion matching should be mentioned here. Furthermore, it should be mentioned that patients were studied under general anesthesia for thoracic surgery. The number of patients should be mentioned. In order to keep the word count, the Background section may be shortened.

A1.) Thank you for this comment. The abstract has now been modified to include these points and the background information reduced so as to remain within the word count.

Q2.) The description of the method of shunt calculation by the commercially available device is mainly focused on the use of the device and less on details of underlying principles. Instead of solely referring to computing journals, where the method was published, some brief information would be helpful for the reader. Was Riley's approximation of physiological shunt used? Did the method consider non-linearity? A recent paper suggested to use log-transformed PaO_2/FIO_2 data which improved correlations of PaO_2/FIO_2 with physiological shunt considering varying levels of hemoglobin, cardiac output, $\Delta Ca\text{-}vO_2$, and airway pressures (Reske AW et al., Bedside Estimation of Nonaerated Lung Tissue Using Blood Gas Analysis, Crit Care Med 2013).

A1) Thank you for letting us clarify this relevant aspect.

Below is the text to which your comments refer:

"*At each FiO_2 level, the ALPE system identifies steady state, and measures ventilation, SpO_2, oxygen consumption, CO_2 production, and inspiratory and expiratory fractions of O_2 and CO_2. These measurements are taken automatically by inserting a sampling tube in the respiratory circuit for measurement of flow, O_2 and CO_2 and by placing the pulse oximeter on a finger. In principle, the system uses oxygen as tracer to separate the effects of shunt and low V/Q. In the case of true pulmonary shunt, SpO_2 will change little when changing FiO_2. In contrast, in the case of areas with low V/Q, SpO_2 will change greatly with FiO_2. In* *addition, an arterial blood gas sample was drawn and analyzed to obtain arterial acid–base and oxygenation status including partial pressure of* CO_2 *(*$PaCO_2$*). By combining measurements of oxygenation response to varying* FiO_2 *with measured end tidal* CO_2 *and* $PaCO_2$*, we retrospectively calculated the combined gas exchange status as due to shunt, low V/Q and high V/Q, using a previously outlined method.*[23] *The principle for determination of high V/Q was that the exchange of oxygen is primarily dependent on shunt and lung areas with low V/Q with the exchange of* CO_2 *predominantly being affected by areas with high V/Q. Low V/Q mismatch is represented as an index constituting the difference in* O_2 *partial pressure between end-tidal gas and blood leaving lung capillaries. A low V/Q index of 10 kPa can be interpreted as a need for an increase in* FiO_2 *of approximately 10% to counter the effect of low V/Q on oxygenation of non-shunted blood. High V/Q mismatch is represented as an index constituting the difference in* CO_2 *partial pressure between end-tidal gas and blood leaving lung capillaries. A high V/Q index >0 kPa can be interpreted as insufficient removal of* CO_2 *due to high V/Q and a potential need for increasing minute ventilation.”*

This text has now been modified to be more explicit concerning the principles of the method. The new text is based on the need of:

1) Highlight the model structure and the inclusion of extra-pulmonary effects including the non-linearities of blood buffering and oxygen binding. The technique presented here includes a complex and extensively validated model of acid-base buffering. We are happy to include citations to that here.
2) Highlight the link to the standard technique for obtaining shunt at $FiO_2 = 1$, and why that might not be desirable.
3) Highlight how there exists sufficient information in oxygen variation, arterial blood gas and capnography so as to separate the effects of shunt, low V/Q and high V/Q. In doing so we have also tried to address one of the comments of reviewer 3, highlighting that our high V/Q region has taken into account the effects on end-tidal arterial CO_2 gradient of shunt and low V/Q regions.
4) Highlight the indices we are using to describe low and high V/Q.

According to the above 4 points, we modified our manuscript as follows:

"At each F_IO_2 level, the ALPE system identifies steady state, and measures ventilation, SpO_2, oxygen consumption, CO_2 production, and inspiratory and expiratory fractions of O_2 and CO_2. These measurements are taken automatically by inserting a sampling tube in the respiratory circuit for measurement of flow, O_2 and CO_2 and by placing the pulse oximeter on a finger. In addition, the system estimates the acid–base and oxygenation status including arterial partial pressure of CO_2 ($PaCO_2$) taking into account the results of an arterial blood gas sample. These parameters are then used to identify the fractions of ventilation and perfusion in a three compartment model of the lung, including two ventilated and perfused compartments and a further perfused only compartment, describing pulmonary shunt. The model takes into account also some extra-pulmonary factors including acid-base status, hemoglobin concentration, the non-linearity of hemoglobin oxygen binding, cardiac output and the measured oxygen consumption. The system assumes a cardiac index of 3.7 l/min/m^2, as previously reported in intensive care patients.[22] Body surface area was calculated from height and weight as previously performed by Gehan and George. [23] The estimation of ventilation and perfusion parameters is performed as follows. It is well known that variation in F_IO_2 can be used to identify shunt, with oxygenation problems at $F_IO_2 = 1$ being due to shunt alone. As F_IO_2 values of 1 may increase the risk of absorption atelectasis[24] and may therefore be undesirable, the ALPE algorithm applies the principle that in the case of true pulmonary shunt, SpO_2 will change little when changing F_IO_2. This is in contrast to areas with low $\dot{V}/\dot{Q}$, where SpO_2 will change greatly with F_IO_2. Accordingly, through variation of F_IO_2 in 3-4 steps the system mathematically estimates shunt and low $\dot{V}/\dot{Q}$ ratios. Further, the ALPE algorithm takes into account the end-tidal to arterial CO_2 gradient to account for the part of this gradient due to shunt and low $\dot{V}/\dot{Q}$ and the one due to high $\dot{V}/\dot{Q}$ ratio. For ease of understanding, the estimates of ventilation and perfusion obtained from ALPE analysis are converted into indices describing low and high $\dot{V}/\dot{Q}$ regions. Low $\dot{V}/\dot{Q}$ mismatch is represented as the difference in O_2 partial pressure between

end-tidal gas and blood leaving lung capillaries in the low $\dot{V}/\dot{Q}$ areas. As an example, a low $\dot{V}/\dot{Q}$ index of 10 kPa indicates the need for an increase in F_IO_2 of approximately 10% to counter the effect of low $\dot{V}/\dot{Q}$ on oxygenation of non-shunted blood. High $\dot{V}/\dot{Q}$ mismatch is represented as an index constituting the difference in CO_2 partial pressure between end-tidal gas and blood leaving lung capillaries. A high $\dot{V}/\dot{Q}$ index >0 kPa can be interpreted as insufficient removal of CO_2 due to high $\dot{V}/\dot{Q}$. The ALPE technique has been validated and applied in varied patient populations. [25-28]"

22. Gattinoni L, Brazzi L, Pelosi P, Latini R, Tognoni G, Pesenti A, Fumugalli R: A trial of goal-oriented hemodynamic therapy in critically ill patients. N Engl J Med 1995, 333:1025–1032.

23. Gehan EA, George SL: Estimation of human body surface area from height and weight. Cancer Chemother Rep 1970, 54:225–235.

24. Edmark L, Auner U, Enlund M, Ostberg E, Hedenstierna G: Oxygen concentration and characteristics of progressive atelectasis formation during anaesthesia. Acta Anaesthesiol Scand 2011;55:75-81.

25. Karbing DS, Kjærgaard S, Andreassen HS, Espersen K, Rees, SE: Minimal model quantification of pulmonary gas exchange in intensive care Patients. Medical Engineering & Physics 2011;33: 240–248

26. Spadaro S, Karbing DS, Mauri T, Marangoni E, Mojoli F, Valpiani G, Carrieri C, Ragazzi R, Verri M, Rees SE, Volta CA: Effect of positive end-expiratory pressure on pulmonary shunt and dynamic compliance during abdominal surgery. Br J Anaesth 2016;116:855-61

27. Kjaergaard S, Rees SE, Grønlund J, Nielsen EM, Lambert P, Thorgaard P, Toft E, Andreassen S: Hypoxaemia after cardiac surgery: clinical application of a model of pulmonary gas exchange. Eur J Anaesthesiol 2004;21:296-301

28. Kjaergaard S, Rees S, Malczynski J, Nielsen JA, Thorgaard P, Toft E, Andreassen S: Non-invasive estimation of shunt and ventilation-perfusion mismatch. Intensive Care Med 2003; 29:727-34.

Q3.) Introduction: The authors state that "…the aim of our study was to investigate whether patients could benefit from a higher PEEP during low VT OLV." This study is clearly a physiological study and not a study addressing clinically more meaningful outcomes such as postoperative complications, length of stay… I would thus suggest to focus the research question on the studied primary outcome and secondary physiological variables.

A3.) We fully agree and, accordingly, modified the introduction to focalize the physiological aim of our study on the physiological outcomes analyzed. The phrase has been modified and now the text is:

"Since atelectasis more likely occurs with low VT,[17] the aim of our study was to investigate whether higher PEEP during low VT OLV can improve both oxygenation through reduction in shunt, and lung mechanics through reduced driving pressure"

Q4.) **The Discussion does not address limitations of the study. These include the technique of pulmonary shunt estimation. Relevant parameters to calculate pulmonary shunt were not measured (e.g. venous admixture, cardiac output) and a simplified method was used. In addition to providing more detailed information on the method, the authors should systematically discuss their limitations. A major limitation is the absence of direct cardiac output measurements, since CO is expected to relevantly change due to PEEP-induced changes in cardiac preload. Other limitations include non-linearity of correlations of PaO_2/FIO_2 and physiological shunt, changes in hemoglobin concentration due to blood loss and so on.**

A4.) Thanks to the Reviewer comment, in the revised manuscript we modified the methods section to include more information on the ALPE technique as well as the discussion section to assess the limitations of the model and (as requested by reviewer 1) the differences between the ALPE and the "gold standard" MIGET technique.

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"Second, the technique for assessing ventilation perfusion matching used in the present study employs a 3-compartment lung model. This model has been shown to be a substantial improvement in describing data when compared to oxygenation indices such as the PaO_2/F_IO_2,[46] but does not include the complexity of the 50 compartments model used in the Multiple Inert Gas Elimination Technique (MIGET), the reference method for assessing gas exchange.[47] Though this technique is simpler that the reference one,[47] it has been shown to provide a good fit to MIGET data, [48,49] and to simulate arterial oxygenation with accuracy comparable to the MIGET model. [49] Accordingly, considering that the MIGET technique is

costly for routine clinical use,[50] *the technique presented could be regarded as bedside technique to estimate the $\dot{V}/\dot{Q}$ ratio. While the model used here accounts for several extra-pulmonary parameters, cardiac output (CO) was not measured and the system assumes a fixed cardiac index. This may be a potential source of errors in the calculation of pulmonary shunt in our patients, since PEEP may impact on CO with several mechanisms, for example by decreasing the cardiac preload or by increasing right ventricular afterload. However, previous studies have showed no significant changes in CO after the application of PEEP in the dependent lung during OLV,*[8,51-52] *and even aggressive recruitment maneuvers have been shown to have slight and transient effects on CO in this context.* [7,53] *Furthermore, a previous validation study of this model showed that its estimate of shunt varies by an average of 2% per liter of CO change.*[54] *It should also be noted that the high $\dot{V}/\dot{Q}$ values reported in table 2 represent a functional description of the gas exchange at the lung level, rather than an anatomical description, which is usually derived from CT measurements.*[41]”

46. Karbing DS, Kjaergaard S, Smith BW, et al: Variation in the PaO_2/FiO2 ratio with FiO_2: mathematical and experimental description, and clinical relevance. Crit Care. 2007;11(6):R118.

47. Wagner PD, Saltzman HA, West JB. Measurement of continuous distributions of ventilation/perfusion ratios: theory. J Appl Physiol 1974, 36:588-599

48. Rees SE, Kjaergaard S, Andreassen S, et al: Reproduction of MIGET retention and excretion data using a simple mathematical model of gas exchange in lung damage caused by oleic acid infusion. J Appl Physiol. 2006; 101:826-32.

49. Rees SE, Kjaergaard S, Andreassen S, et al: Reproduction of inert gas and oxygenation data: a comparison of the MIGET and a simple model of pulmonary gas exchange. Intensive Care Med. 2010; 36:2117-24.

50. Wagner PD. Assessment of gas exchange in lung disease: balancing accuracy against feasibility. Crit Care. 2007;11(6):182.

51. Fujiwara M, Abe K, Mashimo T: The effect of positive end-expiratory pressure and continuous positive airway pressure on the oxygenation and shunt fraction during one-lung ventilation with propofol anesthesia. J Clin Anesth 2001;13:473-7.

52. Ferrando C, Mugarra A, Gutierrez A, Carbonell JA, García M, Soro M, Tusman G, Belda FJ: Setting individualized positive end-expiratory pressure level with a positive end-

expiratory pressure decrement trial after a recruitment maneuver improves oxygenation and lung mechanics during one-lung ventilation. Anesth Analg 2014;118:657-65

53. Garutti I, Martinez G, Cruz P, Piñeiro P, Olmedilla L, de la Gala F: The impact of lung recruitment on hemodynamics during one-lung ventilation. J Cardiothorac Vasc Anesth 2009; 23:506-8

54. Karbing DS, Allerød C, Thomsen LP, Espersen K, Thorgaard P, Andreassen S, Kjærgaard S, Rees SE: Retrospective evaluation of a decision support system for controlled mechanical ventilation. Med Biol Eng Comput 2012; 50:43-51

41. Gattinoni L, Caironi P, Pelosi P, Goodman LR: What has computed tomography taught us about the acute respiratory distress syndrome? Am J Respir Crit Care Med 2001;164:1701-11.

Q5.) How can the authors exclude that even higher PEEP than 10cmH$_2$0 would have been better (in terms of their physiological outcomes)?

A5.) We agree and have modified the limitations section to acknowledge that in some patients an even higher PEEP might be beneficial, the text now reading:

‘‘…. or whether an even higher PEEP level might be beneficial in some patients.”

MINOR COMMENTS

Q6.) Abstract: Was the ventilator mode volume or pressure controlled? After reading the methods section of the main manuscript, the reader understands that it was VCV. Thus, mentioning both changes in Compliance and driving pressure seems duplicate information, since dP=VT/C and VT was likely being kept constant during VCV.

A6.) We agree with the reviewer. Since Driving Pressure and Compliance return the same information, we decided to delete Compliance in the abstract.

Q7.) Results, study population: please quote fig.1 here which mentions the reasons why 9 patients did not complete the study.

A7.) The reviewer correctly pointed out that Figure 1, previously cited only in the methods, contains information useful to understand why some patients were excluded from the dataset. We therefore added the quote in the Results section.

Reviewer #3: **This is a study of V/Q mismatch in the setting of low tidal volume ventilation during one lung ventilation for lung resection. The authors explore the level of PEEP necessary to maintain best gas exchange and respiratory system compliance during OLV while using protective low tidal volumes. To measure gas exchange efficiency they use a proprietary methodology of V/Q analysis and find that zero and five cmH_2O PEEP are inferior to 10 cmH_2O PEEP in reducing shunt and improving compliance and as a result lowering driving pressure. In addition to better gas exchange, the authors cite other work to suggest less lung injury and complications with a PEEP level of 10 cmH_2O. While the results as reported are plausible for the gas exchange and V/Q effects, the quantitation is not standard. The blood gases alone tell the story as to what is the most effective PEEP.**

Major concerns:

Q1.) The ALPE system to the best of my knowledge has never been validated with the multiple inert gas elimination technique to assess its accuracy in measuring shunt and the contributions of other elements of V/Q mismatching. While the shunt estimates seem reasonable the values of low V/Q and high V/Q do not seem convincing. I am not sure what the units of mmHg mean for the low and high V/Q values. Any estimates of differences in CO_2 as they may be based on the calculation of the Bohr-Enghoff dead space and their interpretation need to be considered in the light of the fact that shunt and low V/Q add to dead space as well as do high V/Q and true dead space.

A1.) We understand the concerns of the reviewer on the importance of validation of the method employed in the present study. Regarding the accuracy and reproducibility of the method, it has previously been compared to the reference technique, i.e. the MIGET. This was performed in two studies that demonstrated that the two techniques were in substantial agreement. It was shown that the limits of agreement for the shunt values calculated using the ALPE method compared to MIGET Shunt was 5% (Rees SE, S Kiaergaard, S Andreassen and G Hedenstierna, J Appl Physiol 101: 826-832, 2006; Rees SE, Kjaergaard S, Andreassen S, et al: Reproduction of inert gas and oxygenation data: a comparison of the MIGET and a simple model of pulmonary gas exchange. Intensive Care Med. 2010; 36:2117-24). The following text has been added to the discussion.

"Second, the technique for assessing ventilation perfusion matching used in the present study employs a 3-compartment lung model. This model has been shown to be a substantial improvement in describing data when compared to oxygenation indices such as the PaO_2/F_IO_2,[46] *but does not include the complexity of the 50 compartments model used in the Multiple Inert Gas Elimination Technique (MIGET), the reference method for assessing gas exchange.*[47] *Though this technique is simpler that the reference one,*[47] *it has been shown to provide a good fit to MIGET data,* [48,49] *and to simulate arterial oxygenation with accuracy comparable to the MIGET model.* [49] *Accordingly, considering that the MIGET technique is*

costly for routine clinical use,[50] *the technique presented could be regarded as bedside technique to estimate the $\dot{V}/\dot{Q}$ ratio."*

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47. Wagner PD, Saltzman HA, West JB. Measurement of continuous distributions of ventilation/perfusion ratios: theory. J Appl Physiol 1974, 36:588-599

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49. Rees SE, Kjaergaard S, Andreassen S, et al: Reproduction of inert gas and oxygenation data: a comparison of the MIGET and a simple model of pulmonary gas exchange. Intensive Care Med. 2010; 36:2117-24.

50. Wagner PD. Assessment of gas exchange in lung disease: balancing accuracy against feasibility. Crit Care. 2007;11(6):182.

In addition, we have modified the methods section to provide a more explicit description of the ALPE technique for measuring gas exchange and describing the values of low and high V/Q. In particular we have explained how the end-tidal to arterial gradient is used to calculate high V/Q accounting for shunt and low V/Q.

"At each F_IO_2 level, the ALPE system identifies steady state, and measures ventilation, SpO_2, oxygen consumption, CO_2 production, and inspiratory and expiratory fractions of O_2 and CO_2. These measurements are taken automatically by inserting a sampling tube in the respiratory circuit for measurement of flow, O_2 and CO_2 and by placing the pulse oximeter on a finger. In addition, the system estimates the acid–base and oxygenation status including arterial partial pressure of CO_2 ($PaCO_2$) taking into account the results of an arterial blood gas sample. These parameters are then used to identify the fractions of ventilation and perfusion in a three compartment model of the lung, including two ventilated and perfused compartments and a further perfused only compartment, describing pulmonary shunt. The model takes into account also some extra-pulmonary factors including acid-base status, hemoglobin concentration, the non-linearity of hemoglobin oxygen binding, cardiac output and the measured oxygen consumption. The system assumes a cardiac index of 3.7

l/min/m^2, as previously reported in intensive care patients.[22] Body surface area was calculated from height and weight as previously performed by Gehan and George. [23] The estimation of ventilation and perfusion parameters is performed as follows. It is well known that variation in F_IO_2 can be used to identify shunt, with oxygenation problems at $F_IO_2 = 1$ being due to shunt alone. As F_IO_2 values of 1 may increase the risk of absorption atelectasis[24] and may therefore be undesirable, the ALPE algorithm applies the principle that in the case of true pulmonary shunt, SpO_2 will change little when changing F_IO_2. This is in contrast to areas with low $\dot{V}/\dot{Q}$, where SpO_2 will change greatly with F_IO_2. Accordingly, through variation of F_IO_2 in 3-4 steps the system mathematically estimates shunt and low $\dot{V}/\dot{Q}$ ratios. Further, the ALPE algorithm takes into account the end-tidal to arterial CO_2 gradient to account for the part of this gradient due to shunt and low $\dot{V}/\dot{Q}$ and the one due to high $\dot{V}/\dot{Q}$ ratio. For ease of understanding, the estimates of ventilation and perfusion obtained from ALPE analysis are converted into indices describing low and high $\dot{V}/\dot{Q}$ regions. Low $\dot{V}/\dot{Q}$ mismatch is represented as the difference in O_2 partial pressure between end-tidal gas and blood leaving lung capillaries in the low $\dot{V}/\dot{Q}$ areas. As an example, a low $\dot{V}/\dot{Q}$ index of 10 kPa indicates the need for an increase in F_IO_2 of approximately 10% to counter the effect of low $\dot{V}/\dot{Q}$ on oxygenation of non-shunted blood. High $\dot{V}/\dot{Q}$ mismatch is represented as an index constituting the difference in CO_2 partial pressure between end-tidal gas and blood leaving lung capillaries. A high $\dot{V}/\dot{Q}$ index >0 kPa can be interpreted as insufficient removal of CO_2 due to high $\dot{V}/\dot{Q}$. The ALPE technique has been validated and applied in varied patient populations. [25-28]”

22. Gattinoni L, Brazzi L, Pelosi P, Latini R, Tognoni G, Pesenti A, Fumugalli R: A trial of goal-oriented hemodynamic therapy in critically ill patients. N Engl J Med 1995, 333:1025–1032.

23. Gehan EA, George SL: Estimation of human body surface area from height and weight. Cancer Chemother Rep 1970, 54:225–235.

24. Edmark L, Auner U, Enlund M, Ostberg E, Hedenstierna G: Oxygen concentration and characteristics of progressive atelectasis formation during anaesthesia. Acta Anaesthesiol Scand 2011;55:75-81.

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Q2.) It is not clear whether the approach in estimating shunt includes placing the patient on 100% O2, which would be the 'gold' standard for shunt estimation.

A2.) The text included in response to your previous question also includes the answer to this point, i.e.

*"*It is well known that variation in F_IO_2 can be used to identify shunt, with oxygenation problems at F_IO_2 = 1 being due to shunt alone. As F_IO_2 values of 1 may increase the risk of absorption atelectasis[24] and may therefore be undesirable, the ALPE algorithm applies the principle that in the case of true pulmonary shunt, SpO_2 will change little when changing F_IO_2.*"*

[24] Edmark L, Auner U, Enlund M, Ostberg E, Hedenstierna G: Oxygen concentration and characteristics of progressive atelectasis formation during anaesthesia. Acta Anaesthesiol Scand 2011;55:75-81..

Minor comments

Q3.) The authors need to denote fractional concentration of oxygen as FIO$_2$ with both the capital I and 2 subscripted. Also end-tidal CO2 should have et subscripted.

A3.) We modified the abbreviation according to the suggestion

Q4.) Units are inconsistently used. For example ml/Kg, mL per kg-hr. Please make all unit measurements consistent in their abbreviation. I would suggest there is no need for capitals involving mass and volume.

A4.) We thank the reviewer for this suggestion. All the unit measurements are now consistent and without capitals

Statistical Reviewer/Editor #4:

Abstract

Q1.) **Please report some aspect of sample size (e.g., n = 45) in the abstract.**

A1.) Done

Introduction

Q2.) Providing a focused experimental question will allow your readers to properly evaluate the methods used in the study. Please report the actual hypothesis of the trial (e.g., "We hypothesized that...").

A2.) Done

Methods

Q3.) To allow your readers to interpret the context of this analysis in light of previous examinations of these data, it is important to report the nature of the current analysis (e.g., "This is the primary analysis of these data..."), or to explicitly report if this is a secondary or subgroup analysis of these data (e.g., "This analysis is a subgroup analysis of previously collected data...").

A3.) “This is the primary analysis of these data” has been added to the methods.

Q4.)The pre-planned (i.e., a priori) versus post hoc (i.e., derived after initial examination of these data) nature of this analysis should also be reported. Finally, to allow evaluation of the previous reports of these data, please cite any published manuscripts that report any aspects of the data used in this study.

A4.) We reported that all the primary and secondary outcomes presented in the study were pre-planned. The only analysis performed after initial examination of the data was the description of physiological variables in patients where PEEP did not decrease the Driving Pressure. Thus, this text was added to methods: *All the analysis performed for the primary and secondary outcomes were pre-planned; furthermore, a post-hoc, sub-group, analysis was performed to describe the behavior of physiological variables, following identification of a subgroup of patients where ΔP did not decrease with increased PEEP.*

Statistical Analyses

Q5.) Please report the nature of the hypothesis testing (e.g., two-tailed testing is used by convention).

A5.) We have now added in the statistics section that we performed two-tailed hypothesis testing.

Q6.) Please ensure that the Bonferroni correction was applied to post hoc testing for the ANOVA as well as the Friedman tests. The table caption seems to refute this idea.

A6.) The post hoc analysis was indeed performed with Bonferroni correction. We have reformulated the table caption to make this clearer.

Results

Q7.) To properly interpret the study, a reader must be able to evaluate potential bias due to lost, missing, or excluded data. In that regard, please:- Report why data were missing or lost for any reason. If there were no missing data of any kind, simply state this fact somewhere in the results section.

A7) There were no missing data in the dataset and this fact was reported at the beginning of the results section

Q8.) Please double check the numerical reporting for accuracy in the results section. There is at least one typo (e.g., "…and 11% [516]").

A8.) We apologize for this typo which is now corrected. All the data were double checked and no other error were retrieved.

Q9.) Please reconsider the number of decimal places used to report the measurements in the tables and text. Most statistical software packages output descriptive statistics using many decimal places (e.g., 4.5476), but reporting more decimal places than actually observed gives a false sense of precision to your readers. For example, age is often measured to one year of accuracy (e.g., 45 years), so mean age should not be reported using decimal places, as this level of precision was not available in the original measurement.

A9.) Thank you for this advice. We changed all the measurement, both in the tables and in the manuscript, in which the decimal places reported initially did not reflect the original level of precision.

Physiological evaluation of ventilation perfusion mismatch and respiratory mechanics at different positive end expiratory pressure in patients undergoing protective one-lung ventilation.

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Clinical.gov trial number: NCT02968550

Words count: Abstract 239 Introduction 271 Discussion 1443

Running head: Shunt evaluation during protective one lung ventilation

Competing Interests: The authors declare no competing interests

Abstract.

Background: Arterial oxygenation is often impaired during one-lung ventilation, both due to pulmonary shunt and atelectasis. The use of low V_T (5 ml/kg PBW) in the context of a lung-protective approach exacerbates atelectasis. We sought to describe the combined physiological effects of PEEP and low V_T during one lung ventilation.

Methods: Data from forty-one patients studied during general anesthesia for thoracic surgery were collected and analyzed. Shunt fraction, high $\dot{V}/\dot{Q}$ and respiratory mechanics were measured at PEEP 0 cm H_2O during bilateral lung ventilation and one-lung ventilation and, subsequently, during one-lung ventilation at 5 or 10 cm H_2O of PEEP. Shunt fraction and high $\dot{V}/\dot{Q}$ were measured using variation of inspired oxygen fraction, and measurement of respiratory gas concentration and arterial blood gas. The level of PEEP was applied in random order and maintained for 15 min prior to measurements.

Results: During one-lung ventilation, increasing PEEP from 0 cm H_2O, to 5 cm H_2O and 10 cm H_2O resulted in a shunt fraction decrease of 5% [0 – 11] and 11% [5 - 16], respectively ($p<0.001$). The PaO_2/F_IO_2 ratio increased significantly only at PEEP 10 cm H_2O ($p<0.001$). Driving pressure decreased from 16 ± 3 cm H_2O at PEEP 0 cm H_2O to 12 ± 3 cm H_2O at PEEP 10 cm H_2O; $p<0.001$. High $\dot{V}/\dot{Q}$ ratio did not change.

Conclusion: During low V_T one-lung ventilation, high PEEP levels improve pulmonary function without increasing high $\dot{V}/\dot{Q}$, and reduce driving pressure.

Introduction

Arterial oxygenation is impaired during one-lung ventilation (OLV) in lateral decubitus due to the obligatory shunt through the non-dependent lung.[1,2] The generation of atelectasis in the dependent, ventilated lung, further decreases oxygenation by reducing the aerated lung volume and inducing ventilation–perfusion mismatch.[3,4] Applying a positive end-expiratory pressure (PEEP) to the dependent lung could ameliorate intrapulmonary shunt.[2,4-6] However, studies have shown conflicting results, with some showing sustained improvement,[5-8] others no effects[9] or even worsening of oxygenation.[10,11] These conflicting results might be at least partially explained by the different interplay between PEEP and tidal volume (V_T) in the different studies. Indeed, the previous quoted studies report the use of different V_T during OLV, ranging between 6 and 10 ml/kg. High V_T have *per se* the potential of decreasing shunt by recruiting the atelectatic lung areas, but this strategy may be deleterious, both causing lung injury,[12,13] and augmenting cytokine production.[12,13] Thus, considerable attention has been paid to identify the correct V_T to be used during OLV[13-16] and recent evidence suggests that a lung protective tidal volume of 4-5 ml/kg predicted body weight (PBW) should be applied during OLV.[16]

Since atelectasis more likely occurs with low V_T, [17] the aim of our study was to investigate whether higher PEEP during low V_T OLV can improve both oxygenation through reduction in shunt, and lung mechanics through reduced driving pressure. Thus, we applied different PEEP levels (0, 5 and 10 cm H_2O) and measured ventilation/perfusion matching, and respiratory mechanics in patients undergoing thoracoscopic surgery ventilated with a V_T of 4-5 ml/kg PBW during OLV. We therefore sought to describe the physiological effects of increased PEEP.

Materials and Methods

This study was performed in the Department of Anesthesia and Intensive Care at the University Hospital of Ferrara (Italy) from January to November 2016. Our trial was approved by the Ethics Committee of our institution (Protocol n. 140495) and registered in Clinicaltrial.gov (NCT02968550). Written informed consent was obtained from each patient before surgery.

Patients scheduled for elective lobectomy or lung resection through Video Assisted Thoracoscopic Surgery (VATS) requiring OLV and lateral position were enrolled if >18 year of age and with an American Society of Anesthesiologists (ASA) physical status I to III. Patients were excluded in case of hemodynamic instability (defined as a decrease in systolic arterial pressure of >20% from baseline), severe chronic respiratory failure (COPD patients with GOLD stage 3 or 4,[18] preoperative hemoglobin <10 g ml^{-1}), procedures requiring unplanned conversion to thoracotomy surgery or planned to be shorter than 30 min.

As ~~a~~ routine practice in our institution, patients underwent ~~a~~ pre-operative spirometry performed in sitting position according to the American Thoracic Society’s standards, using SpiroPro spirometer (SpiroPro, Jaeger, Würzburg, Germany). Spirometry measurement of vital capacity (VC), forced expiratory volume in the 1st second (FEV_1), forced vital capacity (FVC), expiratory reserve volume (ERV) and transfer coefficient (KCO) were performed.

Before anesthesia induction, a thoracic epidural catheter (Tuohy; Braun Laboratories, Melsungen AG, Germany) was placed between T3 and T6 and 3 mL bupivacaine 0.25% was administered.

All patients breathed an inspiratory oxygen fraction (F_IO_2) of 0.8 during the induction of general anesthesia, to maintain adequate oxygenation while reducing the risk of absorption atelectasis.[19] Anesthesia was induced with propofol (1.5–2 mg/kg), fentanyl (3μg/kg) and rocuronium (0.6

mg/kg) to facilitate tracheal intubation. The trachea was intubated with an appropriately sized and side double lumen tube (Broncho-part; Rush, Kermen, Germany). Tube position was confirmed by bronchoscopy in the supine and lateral positions. Anesthesia was maintained with a continuous infusion of propofol (150–200 μg kg $^{-1}$ min^{-1}), remifentanil (0.1–0.2 μg kg $^{-1}$ min^{-1}) and cis-atracurium (2 μg kg^{-1} min^{-1}). Balanced crystalloid solutions[20] were infused at a rate of 3 ml kg^{-1} h^{-1}.

Patients were ventilated with a square flow waveform using Dräger Primus ventilator (Drägerwerk AG & Co. KGaA, Lübeck, Germany). During 2-lung (bilateral) ventilation V_T was set to 6–8 ml/kg PBW and zero PEEP. During OLV, V_T was reduced to 4-5 ml/kg PBW and PEEP varied from 0 to 10 cm H_2O, according to the experimental protocol (see below). F_IO_2 was set to maintain peripheral oxygen saturation (SpO_2) equal to or greater than 92%. Inspiratory-to-expiratory ratio was set to 1:2 and frequency adjusted to maintain an arterial CO_2 partial pressure ($PaCO_2$) between 40 and 60 mmHg.

Respiratory mechanics were assessed by the constant V'/rapid occlusion method previously described in detail.[21] End-inspiratory occlusion was obtained by increasing end-inspiratory pause to 40%. Driving pressure (ΔP), was calculated as plateau pressure minus PEEP, while respiratory system compliance (C_{RS}) was calculated as V_T / (end inspiratory plateau pressure – PEEP).

Patients were monitored using a Dräger Infinity C700™ monitor (Dräger Medical GmbH, Lübeck, Germany) with an electrocardiogram, pulse oximetry, $_{ET}CO_2$, and continuous arterial pressure monitoring via a catheter inserted into the radial artery. The latter was placed under local anesthesia before induction of general anesthesia, in line with the standard practice of our institution, for invasive blood pressure and to obtain samples for blood gas monitoring. Analysis of arterial blood gases were performed within 3 min from sampling (Cobas 123 POC; Roche diagnostics Rotkreuz, Switzerland). Depth of anesthesia was monitored using bispectral index (Aspect A-2000; Aspect Medical System, Newton, MA, USA).

Shunt and $\dot{V}/\dot{Q}$ matching were assessed by the ALPE system (ALPE Integrated, Mermaid Care A/S, Nr. Sundby, Denmark). To assess $\dot{V}/\dot{Q}$ matching, the ALPE system instructs the user to modify F_IO_2 in 3-4 steps. At each F_IO_2 level, the ALPE system identifies steady state, and measures ventilation, SpO_2, oxygen consumption, CO_2 production, and inspiratory and expiratory fractions of O_2 and CO_2. These measurements are taken automatically by inserting a sampling tube in the respiratory circuit for measurement of flow, O_2 and CO_2 and by placing the pulse oximeter on a finger. In addition, the system estimates the acid–base and oxygenation status including arterial partial pressure of CO_2 ($PaCO_2$) taking into account the results of an arterial blood gas sample. These parameters are then used to identify the fractions of ventilation and perfusion in a three compartment model of the lung, including two ventilated and perfused compartments and a further perfused only compartment, describing pulmonary shunt. The model takes into account also some extra-pulmonary factors including acid-base status, hemoglobin concentration, the non-linearity of hemoglobin oxygen binding, cardiac output and the measured oxygen consumption. The system assumes a cardiac index of 3.7 $l/min/m^2$, as previously reported in intensive care patients.[22] Body surface area was calculated from height and weight as previously performed by Gehan and George. [23] The estimation of ventilation and perfusion parameters is performed as follows. It is well known that variation in F_IO_2 can be used to identify shunt, with oxygenation problems at $F_IO_2 = 1$ being due to shunt alone. As F_IO_2 values of 1 may increase the risk of absorption atelectasis[24] and may therefore be undesirable, the ALPE algorithm applies the principle that in the case of true pulmonary shunt, SpO_2 will change little when changing F_IO_2. This is in contrast to areas with low $\dot{V}/\dot{Q}$, where SpO_2 will change greatly with F_IO_2. Accordingly, through variation of F_IO_2 in 3-4 steps the system mathematically estimates shunt and low $\dot{V}/\dot{Q}$ ratios. Further, the ALPE algorithm takes into account the end-tidal to arterial CO_2 gradient to account for the part of this gradient due to shunt and low $\dot{V}/\dot{Q}$ and the one due to high $\dot{V}/\dot{Q}$ ratio. For ease of understanding, the estimates of ventilation and perfusion obtained from ALPE analysis are converted into indices describing low and high $\dot{V}/\dot{Q}$ regions. Low $\dot{V}/\dot{Q}$ mismatch is represented as the difference in O_2 partial pressure between end-tidal gas and blood leaving lung capillaries in the low $\dot{V}/\dot{Q}$ areas. As an example, a low $\dot{V}/\dot{Q}$ index of 10 kPa indicates the need for an increase in F_IO_2 of approximately 10% to counter the effect of low $\dot{V}/\dot{Q}$ on oxygenation of non-shunted blood. High $\dot{V}/\dot{Q}$ mismatch is represented as an index constituting the difference in CO_2 partial pressure between end-tidal gas and blood leaving lung capillaries. A high $\dot{V}/\dot{Q}$ index >0 kPa can be interpreted as insufficient removal of CO_2 due to high $\dot{V}/\dot{Q}$. The ALPE technique has been validated and applied in varied patient populations. [25-28]

Study protocol

Measurements were made 1) before surgery, when patients were ventilated at zero PEEP during bilateral lung ventilation in the supine position, (TLV baseline), and 2) during OLV in the lateral decubitus, after collapse of the nondependent lung. OLV, (Figure 1) immediately opening the lumen of the endotracheal tube of the non-ventilated lung to room air. After the assessment of shunt, respiratory mechanics and gas exchange at ZEEP, we applied in random order 5 or 10 cm H_2O of PEEP. Randomization was obtained by using a computer-generated number. Each level of PEEP was maintained for 15 min, allowing the effects of PEEP to reach an equilibrium.[29] Parameters describing respiratory mechanics, hemodynamic and gas exchange were measured at each PEEP step. The design of the study is summarized in Figure 1.

Statistical analysis

Normal distribution of data was tested by the Shapiro–Wilk Normality Test. Data are reported as mean ± standard deviation or median [interquartile range] as appropriate. Differences between measurements at different PEEP levels were analyzed using repeated measures ANOVA or Friedman’s rank analysis for normally or not normally distributed variables, respectively. When multiple comparisons were made, p-values were adjusted by the Bonferroni post hoc procedure. Treatment effect is expressed as mean difference and 95% Confidence interval (CI) or median difference (interquartile range). Pearson correlation with R square was used to analyze the correlation. Correlation strength were considered based on the absolute value of the r (0.20-0.39 “weak”, 0.40-0.59 “moderate”, 0.60-0.79 “strong”).[30] All the analysis performed for the primary and secondary outcomes were pre-planned; furthermore, a post-hoc, sub-group, analysis was performed to describe the behavior of physiological variables, following identification of a subgroup of patients where ΔP did not decrease with increased PEEP. Two-tailed statistical hypothesis testing was performed with p-values $\leq$0.05 considered statistically significant. Statistical analysis was performed with using SPSS Statistics for Windows, Version 20.0 (IBM, Armonk, NY, USA). This is the primary analysis of these data.

Sample size calculation

An a priori sample size was calculated according to the primary end-point: the improvement in shunt fraction by increasing PEEP levels in patients undergoing OLV in lateral decubitus. Based on at least 90% power, 40 patients were required to detect a mean difference in shunt fraction from 38%±5 to 34%±7 after the application of 5 cm H_2O of PEEP using paired t tests with an α=0.05. This is consistent with the observed difference in shunt fraction seen previously when investigating the effects of PEEP during OLV at a V_T of 10 ml/kg.[8] Finally, 50 patients were required to account for an anticipated dropout of 20% due to declining participation, interruption of intervention and unplanned thoracotomic conversion. Sample size analysis was performed using MedCalc software (MedCalc software 9.3.6.0, Mariakerke, Belgium).

Results

Study population

Among the 50 patients assessed for eligibility, 41 completed the study (Figure 1); their clinical and demographic characteristics are described in Table 1. There were no missing data in the dataset. The median shunt during TLV at zero PEEP was 19% [9-23], with a C_{RS} of 36.2 ± 10 ml/cm H_2O and a ΔP of 13 ± 4 cm H_2O. The average shunt raised to 33% [27–45] during OLV at ZEEP, while C_{RS} to 22 ± 5 ml/cm H_2O and ΔP increased to 16 ± 3 cm H_2O. Hemodynamic parameters did not change throughout the protocol, irrespective of the applied PEEP level (Table 2).

Effects of PEEP on ventilation/perfusion and respiratory mechanics (Table 2)

The median decrease in shunt fraction was 5% [0–11] at PEEP 5 cm H_2O and 11% [5-16] at PEEP 10 cm H_20 ($p<0.001$); while the C_{RS} increased by 3 ml/cm H_2O [CI 1.4 – 4.6] at PEEP 5 cm H_2O and 6.7 ml/cm H_2O [CI 4.7 – 8.5] at PEEP 10 cm H_2O ($p<0.001$). Similarly, ΔP decreased from 16 ± 3 cm H_2O to 14 ± 3 cm H_2O at PEEP 5 and to 12 ± 3 cm H_2O at PEEP 10; $p<0.001$ (Figure 2). High $\dot{V}/\dot{Q}$ ratio was not significantly different between TLV and OLV, regardless of the PEEP level (Table 2). We found a tendency for high $\dot{V}/\dot{Q}$ to increase at PEEP 10 cm H_2O in those patients where ΔP increased with PEEP (Supplemental figure 1). The PaO_2/F_IO_2 ratio increased significantly only at PEEP 10 cm H_2O compared to zero PEEP (281 [129-243] mmHg vs 142 [96-168] mmHg; $p<0.001$).

Predictors of shunt severity during OLV

There was a strong inverse correlation between ERV and the amount of shunt developed during OLV at ZEEP ($r = -0.79$; $r^2 = 0.62$; $p<0.001$) (Figure 3). A similar but weaker correlation was found at PEEP 5 ($r=-0.72$; $r^2=0.52$; $p<0.001$) (Supplemental Figure 2) and PEEP 10 ($r = -0.58$; $r^2=0.40$)

(Supplemental Figure 3). Furthermore, there was a moderate correlation between KCO and shunt ($r=-0.47$; $r^2=0.23$; $p=0.04$) and a weak correlation between body mass index (BMI) ($r=0.33$; $r^2=0.12$; $p=0.03$) and shunt.

Discussion

The main finding of this study is that a PEEP of 10 cm H_2O is needed to decrease the shunt fraction and the driving pressure while increasing oxygenation in patients ventilated with "protective" low V_T during OLV.

In patients undergoing general anesthesia and muscle paralysis, the decrease of FRC associated with the development of atelectasis impairs the matching of ventilation and perfusion. During OLV, the absence of ventilation in the non-dependent lung and the atelectasis induced by anesthesia in the dependent lung, results in further ventilation/perfusion mismatch and hypoxia. However, no conclusive data are available on the correct amount of PEEP that should be applied during OLV to ameliorate oxygenation. This probably reflects the fact that shunt is highly influenced by the ventilatory pattern and in particular by the interplay between V_T and PEEP.[15] The recent extension of the "lung protective ventilation" concept from the ARDS to the anesthesia field, underlines the need for minimizing both atelectasis and over-distension,[31] suggesting the use of low V_T and a "adequate" PEEP levels. However, OLV might deserve even lower V_T as compared to those recommended for protective ventilation during TLV.[13,16] In thoracic surgery, a V_T of 5 ml/kg was shown to decrease postoperative levels of tumor necrosis factor-alpha, interleukin (IL) 8 and IL-10 as compared to 10 ml/kg.[13] Of note, in an animal study, a V_T of 10 ml/kg compared to one of 5 ml/kg resulted in inhomogeneous distribution of aeration predisposing to postoperative lung injury.[32] The role played by low V_T during OLV is further supported by a study from Qutub et al.[33] demonstrating higher extravascular lung water with a V_T of 8 or even 6 mL/kg as compared to a V_T of 4 ml/kg. Hence, as suggested by Losher et al, the adequate V_T during OLV should be around 4 or 5 ml/kg PBW.[16] However, the use of low V_T may exacerbate the atelectasis in the dependent, ventilated lung. In patients with acute lung injury, Cereda and colleagues demonstrated that low V_T could induce a progressive decrease in compliance, which could be prevented by setting an adequate PEEP level.[34] Indeed, the use of low V_T without setting an appropriate PEEP level could likely exacerbate atelectasis and favor postoperative pulmonary complications (PPC).[3,35-37]

Our results suggest that 10 cm H_2O of PEEP are needed when a V_T of 4-5 ml/kg is used. Indeed, 5 cm H_2O of PEEP were not able to improve oxygenation or to reduce both shunt and driving pressure (Table 2). Recently, Neto et al. [38] demonstrated that the higher the intraoperative driving pressure, the greater the incidence of PPC and this is likely true also in patients undergoing thoracic surgery.[37] Of note, a relatively high percentage of our patients (65%) had a value of ΔP higher than 14 cm H_2O during OLV at zero PEEP, and recent studies described a significant association between this ΔP cut-off and mortality in patients with ARDS. [39] Since in our patients 10 cm H_2O of PEEP applied during OLV decreased ΔP from 16 ± 3 cm H_2O to 12 ± 3 cm H_2O; $p<0.001$ and decreased the percentage of patients with ΔP >14 cm H_2O (29%), we speculate that the combination of low V_T and relatively high PEEP levels during OLV could be beneficial in reducing PPC. However, our physiological study was not designed to investigate the impact of the ventilator strategy on clinical outcomes, and further studies are needed to confirm this hypothesis.

One may argue that, despite the low V_T, the application of PEEP can over-distend the lung parenchyma during OLV. In our study we measured the high $\dot{V}/\dot{Q}$, as a marker of hyperinflation,[40] and found that it did not change neither at PEEP 5 or 10 cm H_2O (Table 2). This indicates that PEEP 10 cm H_2O, when associated with low V_T does not result in an increase in dead space ventilation. Based on these data we speculate that PEEP did not cause alveolar hyperinflation in our patients. However, hyperinflation, as commonly defined in the ARDS literature, is usually assessed using CT Hounsfield units and the relationship between hyperinflation and physiologic dead space ventilation (West zone 1).[41] Interestingly, we recorded a non-significant trend for PEEP-induced increase in high $\dot{V}/\dot{Q}$ ratio only in the few patients (6/41; 15%) in which the driving pressure did not decrease by increasing PEEP (Supplemental figure 1). The lack of positive physiological response in patients where driving pressure did not decrease by increasing PEEP was also seen in shunt, where the median value changed little on increasing PEEP (PEEP 0: 32% [29 - 45]; PEEP 5: 33% [22 – 40]; PEEP 10: 28% [22 – 34]).

Since patients undergoing thoracic surgery can have very different levels of shunt, usually ranging between 20 and 30%,[42-43] we investigated the possible pre-operative determinants of shunt in order to predict a higher risk of intraoperative hypoxia. Interestingly, we found a strong negative correlation ($r = -0.79$; $r^2 = 0.62$) between the pre-operative ERV and the shunt fraction (fig. 3). This was not true for other spirometry parameters, such as FEV1, FVC and Tiffenau Index, while other clinical or spirometry variables showed only weak (BMI, FVC/VC) to moderate (KCO) predicting values for intraoperative shunt. The relationship between pre-operative ERV and per-operative shunt can be explained by two factors. First, it is known that FRC and hence ERV is reduced during induction of anesthesia.[44] Secondly, a pre-existing low ERV would therefore be reduced further during anesthesia and may result in an FRC below closing volume. Rothen et al, previously demonstrated that pulmonary shunt is increased when the closing volume is greater than FRC.[45] PEEP should increase per-operative ERV above closing volume reducing shunt and as a consequence weaken the relationship between pre-operative ERV and peri-operative shunt as observed in this study (Supplemental Figure 2 and 3 shows the relationship between ERV and shunt at PEEP 5 and 10 cm H_2O, respectively).

Our study has some limitations. *First,* although the overall shunt levels were similar to those previously reported in the literature,[43] this was a single center study and thus our results may be dependent on local surgical and anesthesiological practice. *Second,* the technique for assessing ventilation perfusion matching used in the present study employs a 3-compartment lung model. This model has been shown to be a substantial improvement in describing data when compared to oxygenation indices such as the PaO_2/F_IO_2,[46] but does not include the complexity of the 50 compartments model used in the Multiple Inert Gas Elimination Technique (MIGET), the reference method for assessing gas exchange.[47] Though this technique is simpler than the reference one,[47] it has been shown to provide a good fit to MIGET data, [48,49] and to simulate arterial oxygenation with accuracy comparable to the MIGET model. [49] Accordingly, considering that the MIGET technique is costly for routine clinical use,[50] the presented technique could be regarded as suitable for bedside estimation of the $\dot{V}/\dot{Q}$ ratio. While the model used here accounts for several extra-pulmonary parameters, cardiac output (CO) was not measured and the system assumes a fixed cardiac index. This may be a potential source of errors in the calculation of pulmonary shunt in our patients, since PEEP may impact on CO with several mechanisms, for example by decreasing the cardiac preload or by increasing right ventricular afterload. However, previous studies have showed no significant changes in CO after the application of PEEP in the dependent lung during OLV,[8,51-52] and even aggressive recruitment maneuvers have been shown to have slight and transient effects on CO in this context. [7,53] Furthermore, a previous validation study of this model showed that its estimate of shunt varies by an average of 2% per liter of CO change.[54] It should also be noted that the high $\dot{V}/\dot{Q}$ values reported in table 2 represent a functional description of the gas exchange at the lung level, rather than an anatomical description, which is usually derived from CT measurements.[41] *Finally*, while our results showed positive short term physiological effects of increasing PEEP, further studies are required to see if the application of protective OLV combined with a PEEP of 10 cm H_2O would translate to improved postoperative outcome or whether an even higher PEEP level might be beneficial in some patients.

In conclusion, this study has shown that when using low V_T during one lung ventilation, it is important to apply a proper amount of PEEP to prevent intra-operative increases in driving pressure and intrapulmonary shunt. It is likely that a PEEP of 10 cm H_2O is required. Our results indicate that this level of PEEP could be applied without compromising high $\dot{V}/\dot{Q}$. These results are of potential clinical interest for designing lung-protective ventilatory protocols to be applied during OLV.

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Table 1

Table 1: Characteristic's patients.

Variable	Patients n=41
Age (years)	68 [60 – 74]
BMI (kg m^{-2})	26 ± 4
ASA score, n	
II	10
III	31
MRC dyspnea scale	2 [1.5 - 3]
Sex (M/F), n	30/11
Surgery side (L/R)	23/18
Type of surgery, n	
• Lobectomy	24
• Wedge resection	17
Duration of MV (min)	236 ± 36
Duration of OLV (min)	216 ± 33
Comorbidities	
Diabetes, n (%)	9 (22)
Cardiac dysfunction, n (%)	21 (51)
COPD, n (%)	7 (17)
Smoking History	38 (93)
• Pack years	18 [14 – 23.5]
• Current smokers, n (%)	21 (49)
Preoperative spirometry	
VC (% predicted)	97 [84 – 113]

KCO (%predicted)	70 [51 – 87]
FEV_1 (%predicted)	92 [81.4 – 105.4]
FVC (%predicted)	97 [85 – 111]
FVC/VC	0.99 [0.94 - 1]
FEV_1/FVC	74 [68.3 – 79.9]
ERV (% predicted)	86 [60 – 123]

Current smoking was defined as at least 1 year from quit. ASA = American Society of Anesthesiologists; BMI = body mass index; COPD = chronic obstructive pulmonary disease; KCO = transfer coefficient; MRC=Medical Research Council Scale; MV = mechanical ventilation; OLV = one lung ventilation; VC = vital capacity; FEV_1 = Forced expiratory volume in the 1st second; FVC= Forced vital capacity; ERV=Expiratory reserve volume

Table 2. Intraoperative variables

Variable	TLV	PEEP 0	PEEP 5	PEEP 10
Shunt fraction (%)	19 [9 – 23]	33 [27-45]	31 [22-42] #	22 [14-29] # *
Low V/Q (mmHg)	31 [22 – 49]	47 [28–112]	45 [22-88]	38 [24-90]
High V/Q (mmHg)	13 ± 4	13 ± 5	13 ± 5	14 ± 6
C_{RS} (ml cm^{-1} H_2O)	36.2 ± 10	22.0 ± 5	25.5 ± 7 #	29.5 ± 8 # *
ΔP (cm H_2O)	13 ± 4	16 ± 3	14 ± 3 #	12 ± 3 # *
V_T (ml Kg^{-1})	7 ± 0.6	4.9 ±0.5	5 ± 0.4	4.8 ± 0.5
RR (breath min^{-1})	13 ± 1	14 ± 2	15 ± 2	15 ± 2
Arterial pH	7.35 ± 0.1	7.32 ± 0.01	7.31 ± 0.01	7.30 ± 0.1
$PaCO_2$ (mmHg)	46 ± 6	48 ± 7	50 ± 6	51 ± 8
PaO_2/F_IO_2 ratio (mmHg)	303 [150 – 351]	142 [96 - 168]	158 [107 - 205]	281 [129 - 243]#*
Mean arterial pressure (mmHg)	82 ± 16	76 ± 18	77± 19	77 ± 18
Heart rate (bpm)	77 ± 10	69 ± 11	70 ± 12	68 ± 10

PEEP= positive end-expiratory pressure; V/Q= Ventilation/perfusion ratio; VT = tidal volume; RR = respiratory rate; $PaCO_2$ = arterial partial pressure of carbon dioxide; PaO_2 = arterial partial pressure of oxygen; F_IO_2 = Fraction of inspired oxygen;

# p<0.05 compared to PEEP 0 * p<0.05 compared to PEEP 5 (repeated measure ANOVA or Friedman’s rank analysis, both with multiple pairwise comparisons and Bonferroni correction, comparing different PEEP levels during OLV)

FIGURE LEGENDS

Figure 1. Flowchart of the study

Figure 2. Individual Differences in driving pressure at different levels of PEEP. The horizontal dashed line shows the cut-off of 14 cm H_2O. The continuous horizontal lines show the means in each group

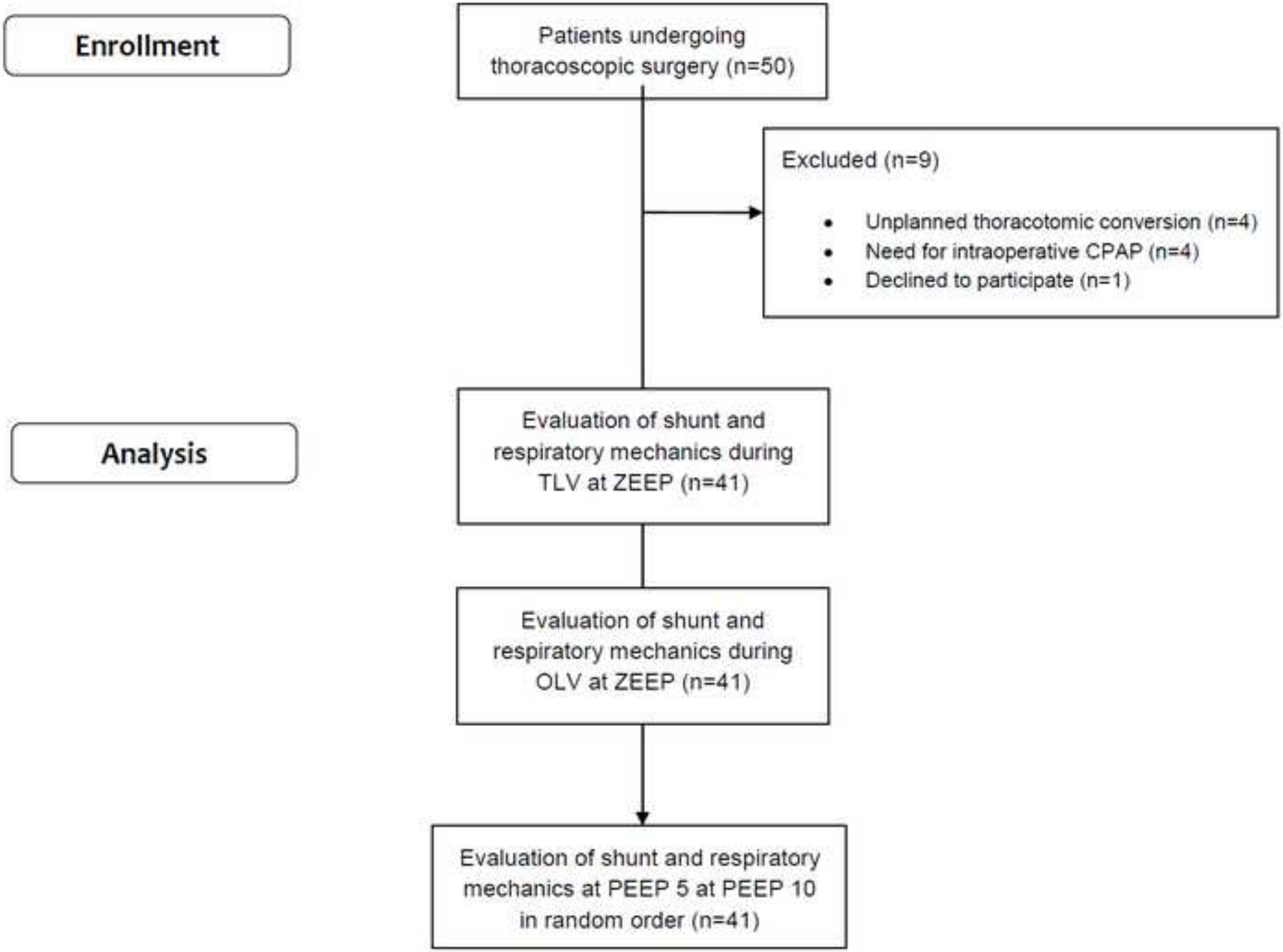
Figure 3. Correlation between Expiratory Reserve Volume (ERV) and intrapulmonary shunt measured at zero PEEP

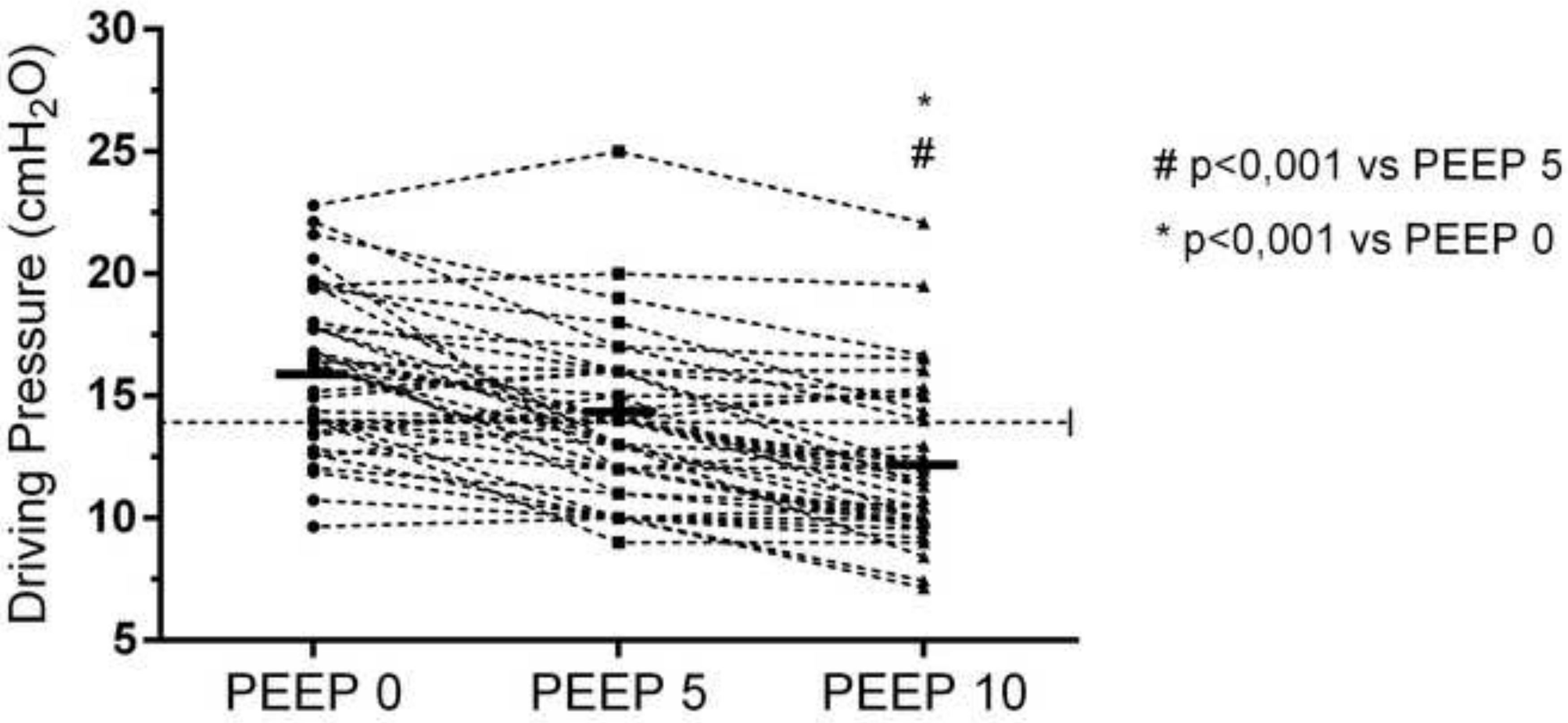
Supplemental Figure 1. Individual Differences in High $\dot{V}/\dot{Q}$ at different levels of PEEP in patients in which PEEP decreased (Δ) or not-decreased (■) the driving pressure. The continuous horizontal lines show the median in each group

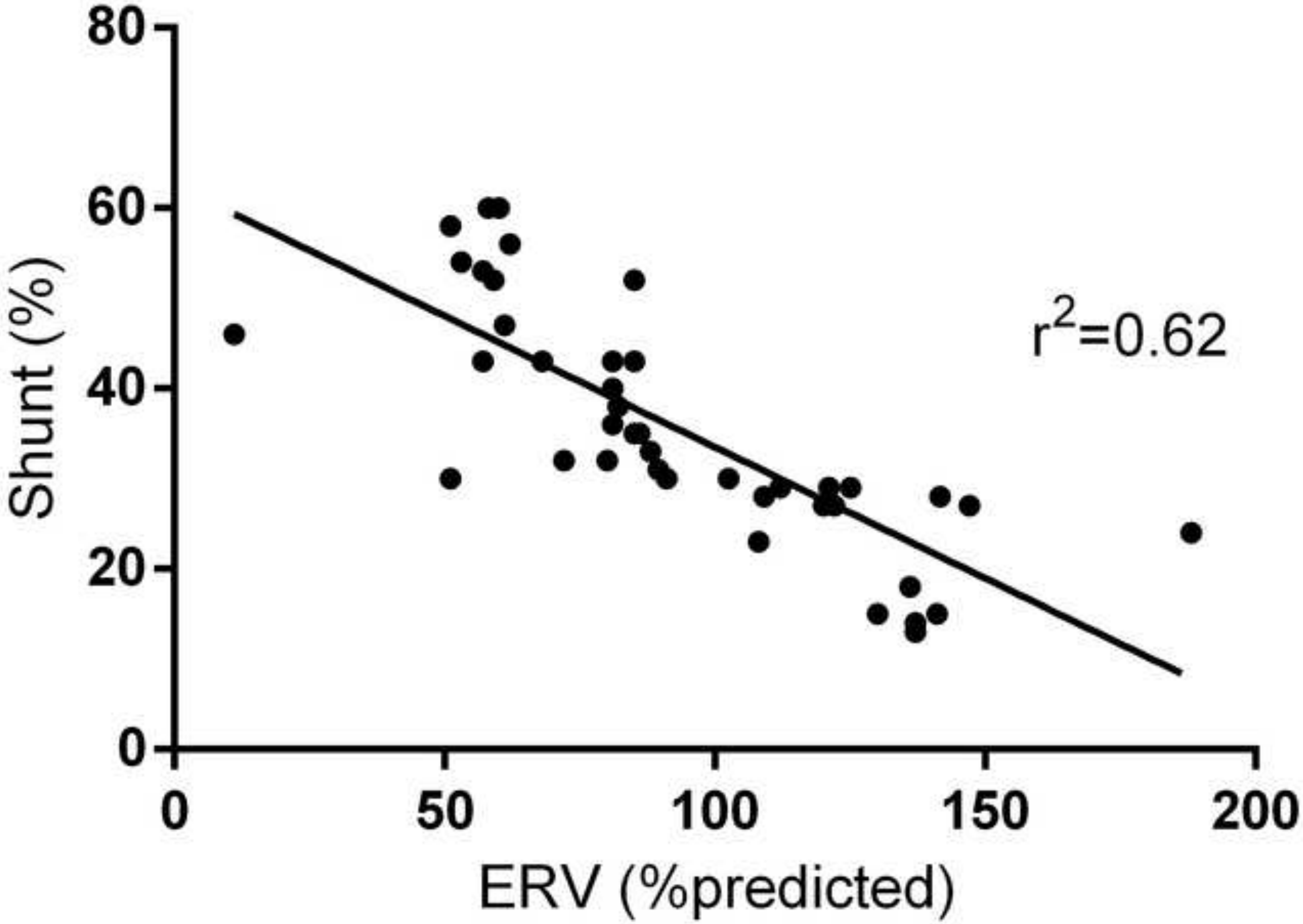
Supplemental Figure 2. Correlation between ERV and intrapulmonary shunt measured at PEEP 5 cm H_2O. ERV = Expiratory Reserve Volume

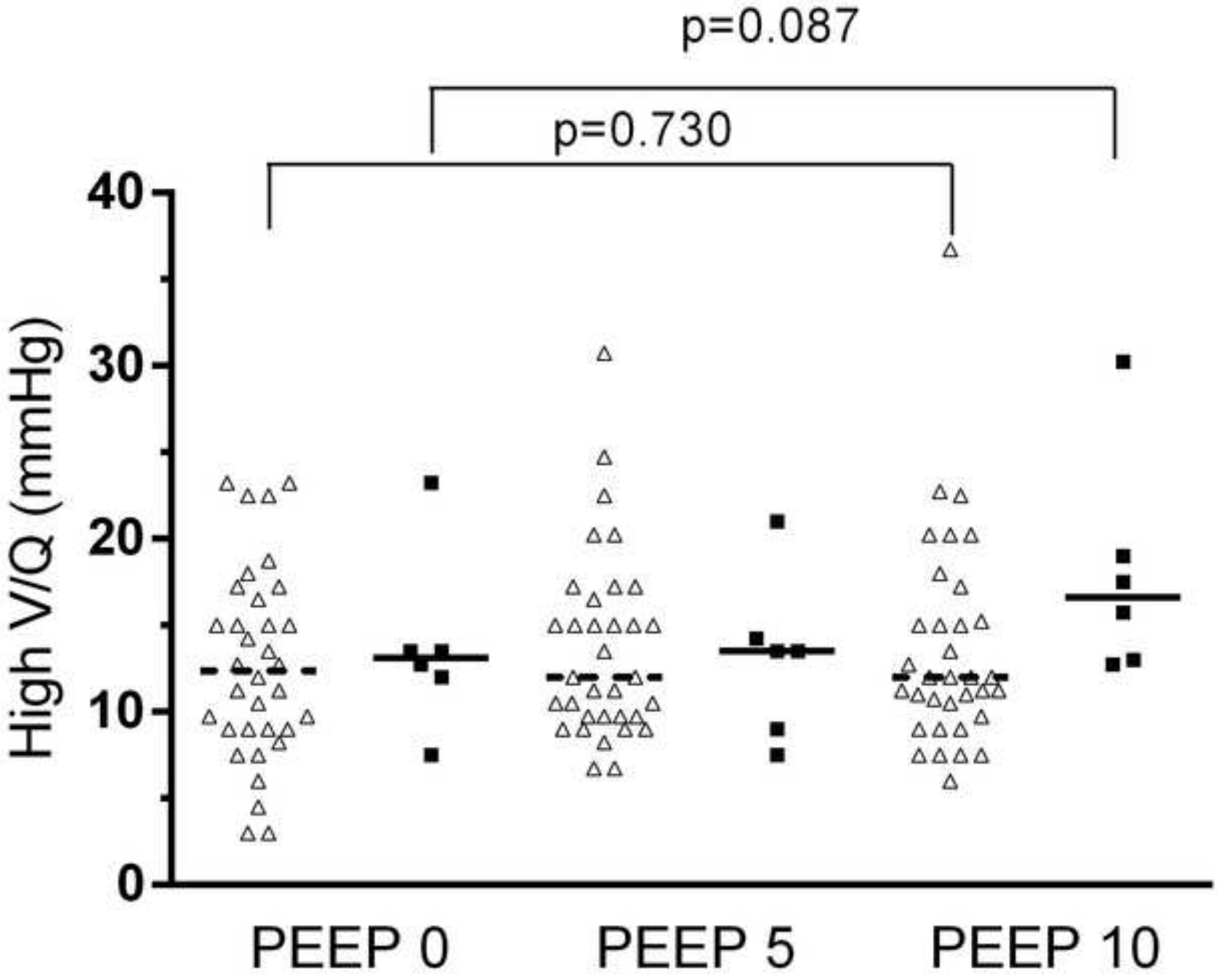
Supplemental Figure 3. Correlation between ERV and intrapulmonary shunt measured at PEEP 10 cm H_2O. ERV = Expiratory Reserve Volume

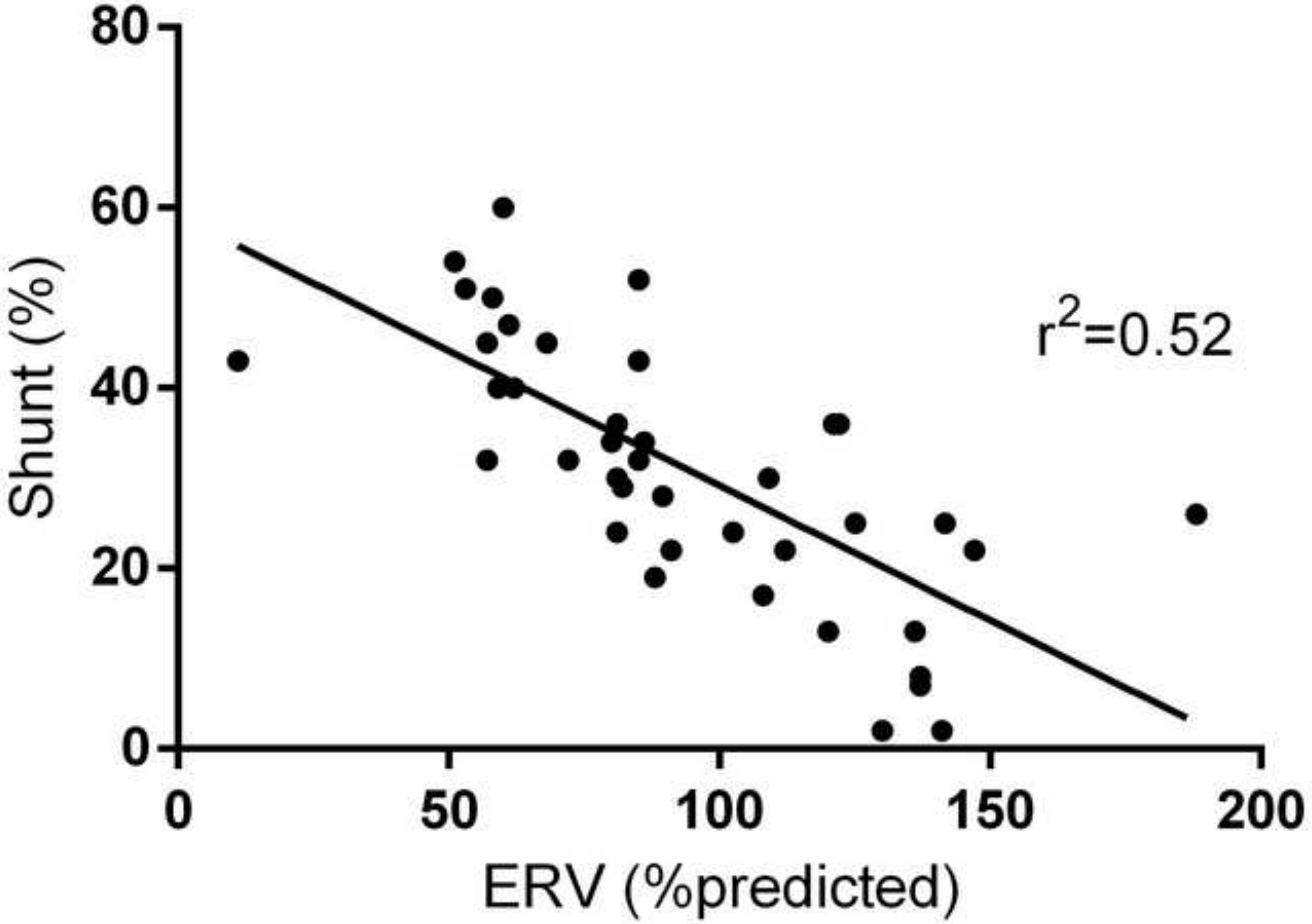
Figure 1

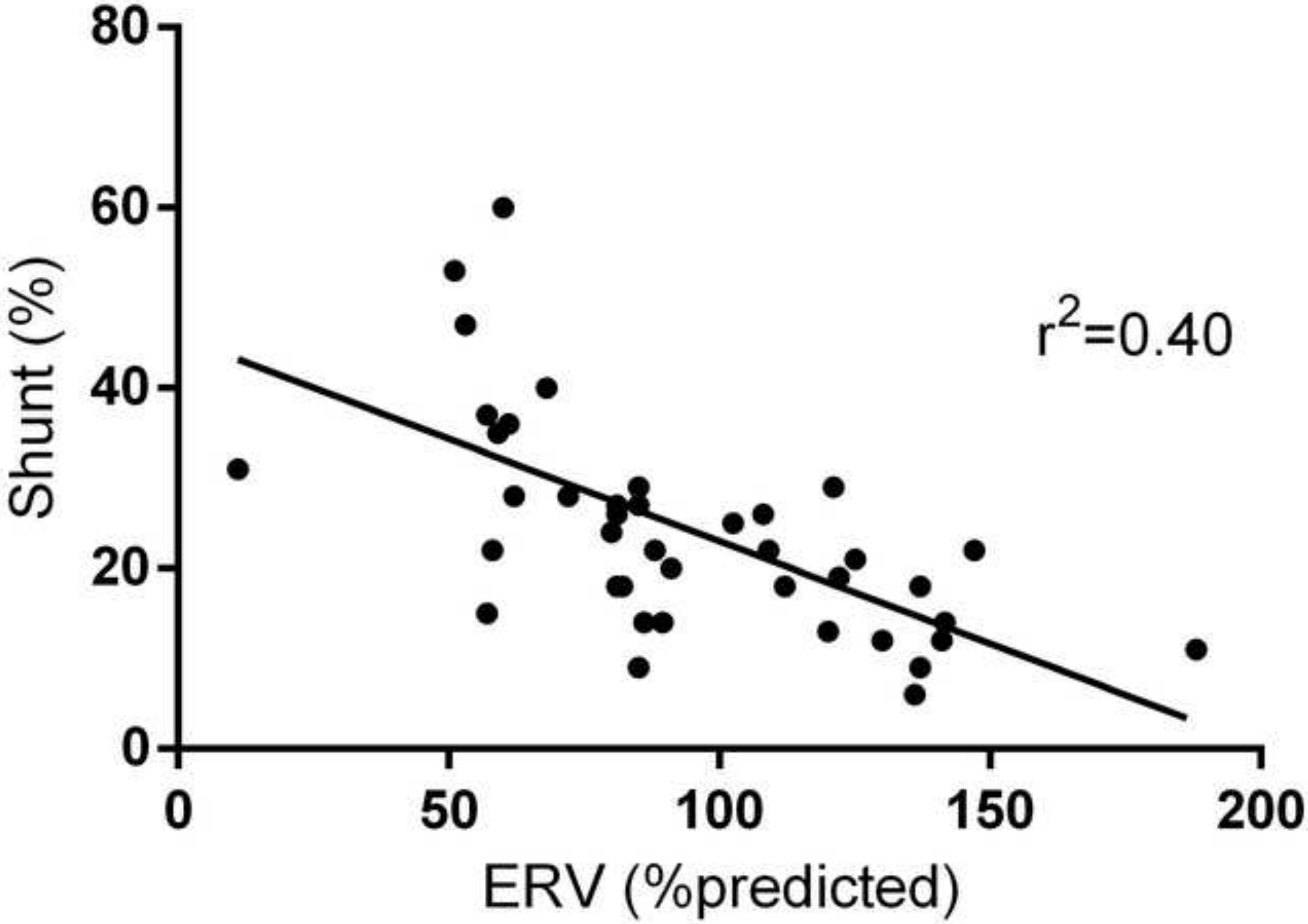

Enrollment
Patients undergoing thoracoscopic surgery (n=50)
Excluded (n=9)
• Unplanned thoracotomic conversion (n=4)
• Need for intraoperative CPAP (n=4)
• Declined to participate (n=1)
Analysis
Evaluation of shunt and respiratory mechanics during TLV at ZEEP (n=41)
Evaluation of shunt and respiratory mechanics during OLV at ZEEP (n=41)
Evaluation of shunt and respiratory mechanics at PEEP 5 at PEEP 10 in random order (n=41)


Driving Pressure (cmH2O)
30
25
20
15
10
5
PEEP 0
PEEP 5
PEEP 10
*
#
# p<0,001 vs PEEP 5
* p<0,001 vs PEEP 0


80
60
40
20
0
Shunt (%)
0
50
100
150
200
ERV (%predicted)
r²=0.62


p=0.087
p=0.730
High V/Q (mmHg)
40
30
20
10
0
PEEP 0
PEEP 5
PEEP 10


80
60
40
20
0
Shunt (%)
0
50
100
150
200
ERV (%predicted)
r²=0.52


80
60
40
20
0
Shunt (%)
0
50
100
150
200
ERV (%predicted)
r2=0.40