Original Article

The effect of low versus high tidal volume ventilation on inflammatory markers in animal model undergoing lung ventilation: A prospective study

ABSTRACT

Background and Aims: Mechanical ventilation (MV) with high tidal volume (Vt.) may induce or aggravate lung injury in critically ill patients. It might also cause an overwhelming systemic inflammation leading to acute lung injury (ALI), diffuse alveolar damage (DAD) and multiple organ failure (MOF) with subsequent high mortality. The objective of this study was to compare the effects of different Vt. on the inflammatory markers of the broncho-alveolar lavage (BAL) fluid and lung biopsy in a group of animal model (Beagle dogs).

Methods: A two-phased prospective study involving 30 Beagle dogs (15 dogs/phase), each phase divided into three groups (each 5 dogs/group). In the first phase each group received MV with Vt. of 8 (low), 10 (normal, control group), and 12 (high) ml/kg body weight (b.w.) respectively. BAL fluid was obtained at the time of induction of anesthesia immediately following tracheal intubation and one hour later following MV to count the macrophages, neutrophils and lymphocytes. In the second phase of the experiment, in addition to obtaining (BAL) fluid similar to the phase one, mini thoracotomy and lung biopsy obtained from the upper lobe of the right lung at same timings for histopathological examination study. Mann-Whitney-Wilcoxon test was used for statistical analysis of the data obtained.

Results: BAL fluid analysis showed increase in the counts of macrophages and lymphocytes with Vt. of 12 ml/kg b.w. compared to the control group (10 ml/kg b.w.) ($P < 0.05$). in the second phase, similar findings obtained. The histopathological study of the lung tissue obtained in the second phase of the study from the group that received a high Vt. of 12 ml/kg b.w. showed significant inflammatory changes with presence of neutrophil infiltration and edema in the bronchial wall compared to the control group (10 ml/kg b.w.) ($P < 0.05$).

Conclusions: The use of high Vt. in ventilated animal lung model may increase the risk of inflammation and subsequent damage in healthy lungs, these findings may help physicians to avoid using high Vt. in short-term mechanically ventilated patients in the operating room setting.

Key words: Inflammation; lung injury; lymphocytes; macrophages; mechanical ventilation; neutrophils; polymorphs nuclear leukocyte

Access this article online	
Website: www.saudija.org	**Quick Response Code**
DOI: 10.4103/sja.SJA_650_20	

How to cite this article: Hajjar WM, Eldawlatly A, Alnassar SA, Ahmed I, Alghamedi A, Shakoor Z, *et al.* The effect of low versus high tidal volume ventilation on inflammatory markers in animal model undergoing lung ventilation: A prospective study. Saudi J Anaesth 2021;15:1-6.

Waseem M. Hajjar, Abdelazeem Eldawlatly[1], Sami A. Alnassar, Iftikhar Ahmed, Alaa Alghamedi, Zahid Shakoor[2], Ammar C. Alrikabi[2], Adnan W. Hajjar, Abdulaziz Ejaz Ahmad[1]

Departments of Surgery, [1]Anesthesia and [2]Pathology, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia

Address for correspondence: Dr. Waseem M. Hajjar, Department of Surgery, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia, P.O.BOX 7805, Riyadh 11472. E-mail: washajjar@yahoo.com

Submitted: 20-Jun-2020, **Revised:** 30-Jun-2020, **Accepted:** 05-Jul-2020, **Published:** 05-Jan-2021

Introduction

Surgical interventions continue to grow with its known complications. According to the Center for Disease Control and Prevention (CDC), 51.4 million surgical procedures were performed in the United States alone, with a crude mortality rate as high as 0.5-5%.[1] Manecke G. *et al*. assessed the costs of post-surgical complications in patients who underwent 10 major abdominal, urologic, and orthopedic procedures and found that there was a 72% increase in hospital costs.[1] One of the postoperative complications that researchers opted to investigate, was the ventilation associated pulmonary complications.[2,3] However, the prevention of such complication via manipulating the ventilation settings is a controversial matter.[2,3] MV may injure healthy lungs, a phenomenon known as ventilator-induced lung injury (VILI), or it may worsen pre-existing lung disease.[2] Pneumonia, for example, is a frequent complication of long term MV that is better known as ventilator-associated pneumonia (VAP). It is one of the known risk factors of acute lung injury (ALI) and adult respiratory distress syndrome (ARDS).[4] ALI and ARDS might lead to a serious complication known as multiple organ dysfunction syndrome (MODS) with subsequent high mortality rate.[5,6]

There was one animal study reported conflicting findings of VILI with high Vt. which did not support stretch-induced injury in the lungs of healthy mice.[7] MV can influence the inflammatory response in patients with pre-existent lung injury.[6,8,9] In those individuals, being ventilated with high Vt. of >10 ml/kg b.w. and low to moderate positive end expiratory pressure (PEEP) led to increased levels of intra-alveolar and systemic inflammatory mediators release.[9] However, it is not clear if MV is solely responsible for triggering lung inflammation in patients without any previous lung injury.[10]

The aim of this study was to compare the injurious effects of ventilation on healthy animal model lungs using conventional, low and high Vt. We hypothesized that low Vt. does not cause significant inflammatory changes. On the other hand, high Vt. can cause and enhance inflammatory reactions in healthy lungs. In this study, the counts of macrophages, neutrophils and lymphocytes were examined in the BAL fluid along with the examination of lung tissue samples taken during this experiment. This is one of the few studies conducted using animal models of a large size with the ability of performing proper ventilation at different Vt. settings.

Although there are, few similar reports on humans studied the effect of low versus high tidal volume ventilation on inflammatory markers in healthy individuals undergoing surgeries and ventilation.[11,12]

Methods

An IRB approval from the Research Committee Chair of the Department of Surgery, College of Medicine, King Saud University, was obtained. This study was conducted in the surgical experimental laboratory in our institute. International Guidelines of using Animals in Scientific Procedures was observed fully according to the ARRIVE Guidelines for Reporting Animal Research.[13]

A total of 30 healthy Beagle dogs weighing 16-18 kg were recruited in this two phased study (15 dogs/each phase), all the dogs, included in this study, were all a similar age and weight.

Preoperative1g I.M. cefuroxime was given to all dogs. Anesthesia was induced with I.M. ketamine and xylazine (2 mg/kg b.w.) followed by tracheal intubation and MV with Fi O2 of 100% to maintain tissue oxygen saturation 92-100% monitored using pulse oximetry fixed at the tongue to prevent hypoxia, as the relationship between the hypoxia and the inflammation is very well documented in the literature.[14] However, pulse and the oxygen saturation were monitored throughout the experiment.

After tracheal intubation, the Beagle dogs were randomly connected to MV with different Vt. In both phases, the control group ($n = 5$) received Vt. of 10 ml/kg b.w. The two other groups received a low ($n = 5$) and high ($n = 5$) Vt. of 8 and 12 ml/kg b.w., respectively. The respiratory rate was adjusted to 25-30 breath/min monitored with capnography and the duration of volume-controlled ventilation was 60 min with FiO_2 of 0.5.

In both phases, 30 cc sterile normal saline (NS) was instilled through the tracheal tube at the time of induction and one hour after MV. The recovered (BAL) fluid was sent to the immunology and cytology laboratory to measure the macrophages, neutrophils and lymphocytes counts.

Biopsy from the upper lobe of the right lung were obtained during the second phase of the experiment, at the time of induction and one hour after MV via right-sided mini-thoracotomy. The tissues were handled very carefully to the histopathology laboratory and examined immediately by staff histopathologist, who was blinded of the samples, to identify any signs of inflammation and for polymorphs and neutrophils infiltrations counts. All the dogs were

post-operatively looked after and had not been sacrificed, and all survived [Figure 1].

Statistical analysis

Our statistical analysis aim was to detect any statistical difference in the (BAL) fluid samples' cell counts in the pre and post ventilation status during the first and the second phase of the study. All statistical analyses were performed using a statistical software package (SAS 9.3 for Windows 8; SAS Institute, Cary NC). Mann-Whitney-Wilcoxon test was done using the PROC NPAR1WAY procedure. $P < 0.05$ was considered statistically significant.

Results

During the first phase, the counts of macrophages, neutrophils and lymphocytes obtained from the BAL fluid were comparable between normal versus low Vt. groups ($P > 0.05$) [Table 1]. Comparing between the high versus normal Vt. showed significant increase of the macrophages and lymphocytic counts in the high Vt. group ($P < 0.05$) at both induction and one-hour later in all the three groups [Table 2].

During the second phase of the experiment, there was no statistical significance found between the control groups with Vt. 10 ml/kg b.w. compared with the group that received a low Vt. of 8 ml/kg b.w. However, the BAL fluid one hour after induction showed a statistically significant increase in the counts of macrophages and lymphocytes with Vt. of 12 ml/kg b.w. ($P < 0.05$) [Tables 3 and 4].

During the second phase of the study, lung biopsies obtained showed that the samples acquired from the groups that received Vt. of 8 and 10 ml/kg b.w. didn't show any significant signs of inflammation [Figure 2a and b]. However, those obtained from the group that received high Vt. of 12 ml/kg b.w. showed an inflammatory picture with neutrophils invading the lamina propria of the bronchioles [Figure 3a and b].

Discussion

During the first phase of the study, the BAL fluid content showed no differences between using a low versus normal Vt. This indicates that using low Vt. is as safe as using normal one. However, using higher Vt. of 12 ml/kg b.w. caused an

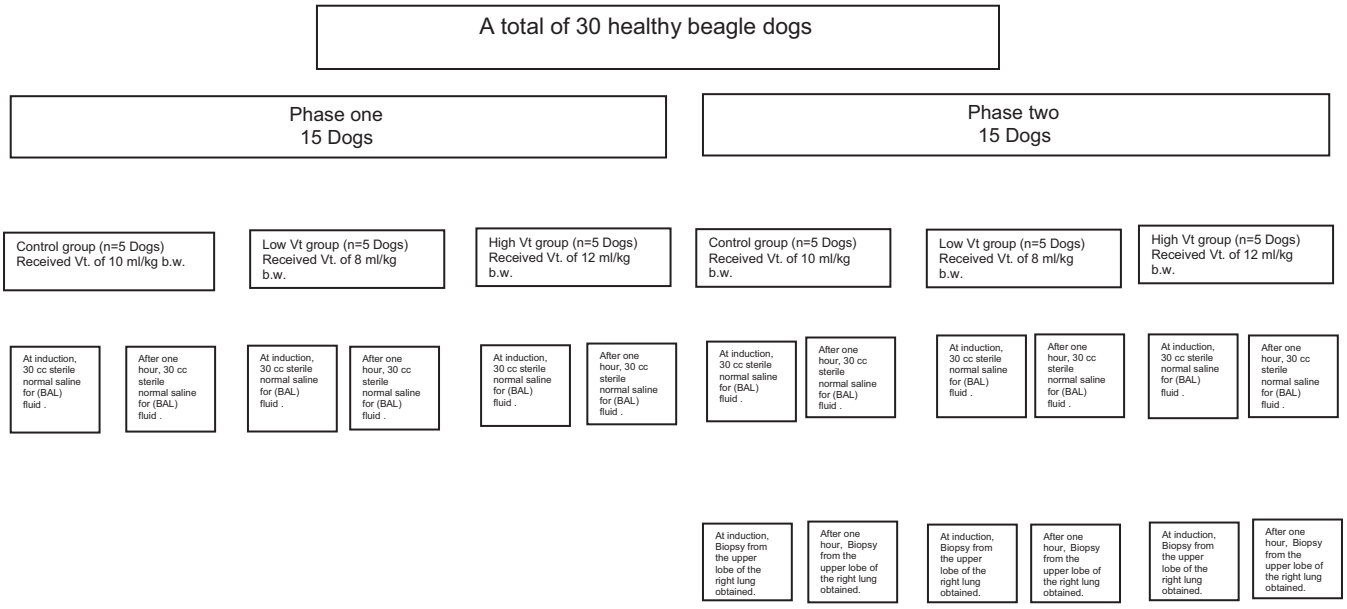

Figure 1: The Flow chart of the Beagle dogs which were recruited in this two phased study

Table 1: Comparison of Macrophages, Neutrophils and Lymphocytes counts of Control group and Low Vt. group of First Phase

Inflammatory Cell Types	Group	*n*	Minimum	Maximum	Mean rank	Sum of ranks	*P*
Macrophages	Control	5	10	38	5.9	29.5	0.655
	Low TV	5	15	30	5.1	25.5	
Neutrophils	Control	5	50	80	4.9	24.5	0.514
	Low TV	5	60	80	6.1	30.5	
Lymphocytes	Control	5	0	30	5.5	27.5	1.000
	Low TV	5	5	20	5.5	27.5	

Table 2: Comparison of Macrophages, Neutrophils, and Lymphocytes counts of the Control group and High Vt. group of the First Phase

Inflammatory Cell Types	Group	*n*	Minimum	Maximum	Mean rank	Sum of ranks	*P*
Macrophages	Control	5	10	38	4	20	**0.015***
	High TV	5	5	80	7	35	
Neutrophils	Control	5	50	80	4	20	0.114
	High TV	5	10	95	7	35	
Lymphocytes	Control	5	0	30	4.5	22.5	**0.046***
	High TV	5	0	10	6.5	32.5	

*Significant *P*. $P < 0.05$ was considered statistically significant, as all these bold values are less than 0.05, ($P < 0.05$)

Table 3: Comparison of Macrophages, Neutrophils and Lymphocytes counts in the Control group and Low Vt. group of Second Phase

Inflammatory Cell Types	Group	*n*	Minimum	Maximum	Mean rank	Sum of ranks	*P*
Macrophages	Control	5	35	50	5.9	29.5	0.674
	Low TV	5	10	63	5.1	25.5	
Neutrophils	Control	5	40	55	5.7	28.5	0.832
	Low TV	5	18	80	5.3	26.5	
Lymphocytes	Control	5	3	13	4.5	22.5	0.281
	Low TV	5	8	20	6.5	32.5	

Table 4: Comparison of Macrophages, Neutrophils, and Lymphocytes counts in the Control group and High Vt. group of Second Phase

Inflammatory Cell Types	Group	*n*	Minimum	Maximum	Mean rank	Sum of ranks	*P*
Macrophages	Control	5	35	50	3	15	**0.009***
	High TV	5	10	34	8	40	
Neutrophils	Control	5	40	55	4	20	0.104
	High TV	5	40	80	7	35	
Lymphocytes	Control	5	3	13	3.5	17.5	**0.031***
	High TV	5	10	50	7.5	37.5	

*Significant *P*. $P < 0.05$ was considered statistically significant, as all these bold values are less than 0.05, ($P < 0.05$)

exaggerated inflammatory response, with a statistically significant increase in the levels of macrophages and lymphocytes. During the second phase, no difference in the levels of macrophages and lymphocytes between low versus normal Vt. When comparing high Vt. with the normal one, higher levels of macrophages and lymphocytes were obtained with a statistically significance changes, which meant that high Vt. can caused an inflammatory response in healthy lungs.

Lung tissues obtained during the second phase of this study at the time of induction and one-hour after ventilation, showed that using a high Vt. of 12 ml/kg b.w. caused a polymorphs nuclear leukocytes and neutrophils invasion. This can be explained by the release of mediators that send a signal to the immune system, which plays an important role in starting the inflammatory process. Those cells (neutrophils) interact with other inflammatory cells (such as macrophages and lymphocytes) as well as alveolar lining cells by releasing more chemical mediators, which causes an up regulation of the inflammatory response.[15,16] This interaction plays a crucial role in the pathogenesis of VILI.[17]

Few studies have addressed the effects of MV using a high Vt. strategy on pulmonary inflammatory response in patients without lung disease, mostly during major surgery.[11,12,18-20]

Evidence suggests that low Vt. used for ventilating patients with ALI and ARDS (6-8 ml/kg b.w.) decreases mortality in comparison to using high Vt. of 12 ml/kg b.w.[21] In those patients Vt. reduction is the standard of care.[22] However, there is no consensus of opinion on the effects on patients with healthy lungs. Some researchers push for the use of protective lung ventilation strategy.[21,23]

The association between the use of high Vt. and the initiation of inflammation in patients with normal lungs has been demonstrated in many studies.[18-21] On the other hand, such association was contradicted by other studies that did not make similar observations.[17,24]

Lung injury that occurs during MV is attributed to cyclic opening and closing of the airways. It coincides with the generation of uncoordinated shear forces that are responsible for the histological damage of the bronchioles. Those shear

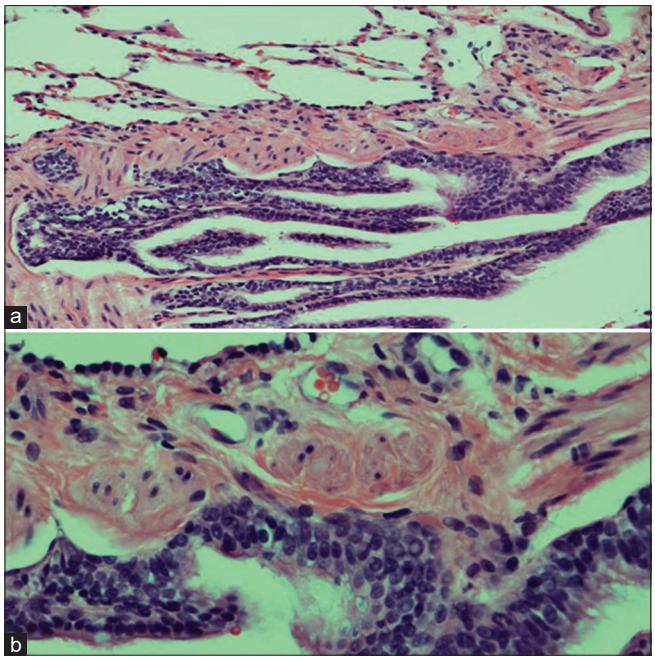

Figure 2: (a and b) H/E stain ×200 and 400 respectively. Section of Lung obtained from the animal group, which received low tidal volume ventilation. Note the absence of significant inflammation in alveolar and bronchial walls and lining

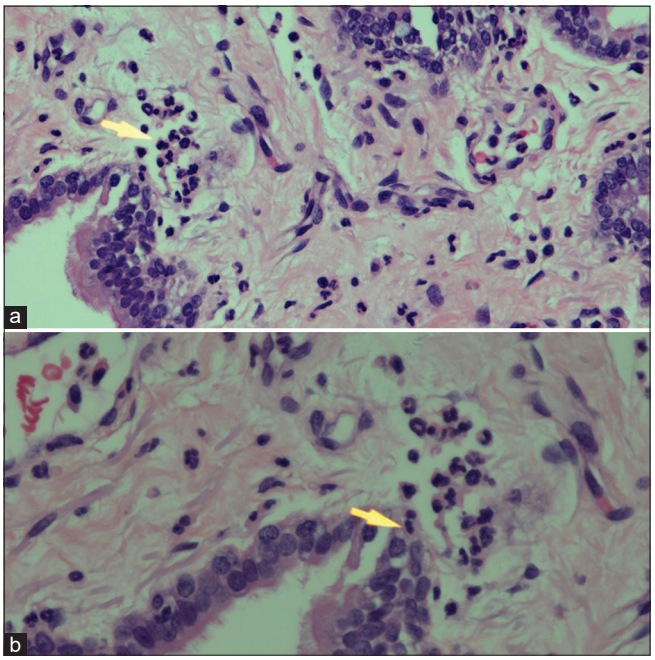

Figure 3: (a and b) H/E stain X200 and 400 respectively. High power view of a section of lung obtained from animals receiving high tidal volume ventilation. Note the presence of neutrophils infiltration (arrowhead) and edema in bronchial wall

forces happen in accordance with an increase in the airway resistance.[17]

To the best of our knowledge, this study is one of the very few studies on the adverse effects of MV conducted on larger sized animals. Using Beagle dogs as an animal model allowed us to conduct this experiment in an environment similar to the one that anesthetists and surgeons experience in the operation room (OR) while ventilating patients or in this case, Beagle dogs.

The major purpose of this study was to test the lung-protective ventilation methods. Those methods decrease the previously mentioned stretch of alveolar walls and alveolar inflammation. However, it is still unclear whether MV itself is the main trigger of inflammation or if it is merely one of the factors that aggravate the inflammatory response induced by a stimulus such as the surgery itself. However, the results of this study showed that using high Vt. can cause an inflammatory reaction in healthy lungs. Recent consensus recommendations by an international expert panel resulted in a few statements which supported our study results concerning the use of lung protective ventilation during the surgery like the use of low FiO2, low Vt ≤6-8 ml/Kg b.w. and positive end expiratory pressure (PEEP) ≃5 cm H2O.[25]

This study had some limitations, as the sample size was relatively a small number. Another limitation was that we used short-term mechanical ventilation for an hour after induction of anesthesia, which is a relatively short period.

In conclusion, conventional and low Vt. have no effect on healthy lungs in this animal model. The results of this study showed that using higher Vt. could cause an inflammatory reaction in healthy lungs. This is supported by analysis of the retrieved BAL fluid and histologically via studying the lung biopsies obtained intra-operatively. These findings may help physicians to avoid using high Vt. in short-term mechanically ventilated patients in the operation room setting.

Ethics approval and consent to participate

An IRB approval from the Research Committee Chair of the Department of Surgery, College of Medicine, King Saud University, was obtained prior to its conduction.

A written informed consent to use the animals in our study was obtained from our institution, College of Medicine, animal laboratory.

Acknowledgements

This study was supported by the College of Medicine Research Centre, Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia.

Financial support and sponsorship

This study was supported by the College of Medicine Research Centre, Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia, by providing the 30 healthy beagle dogs and the animal laboratory to conduct this study only.

Conflicts of interest

There are no conflicts of interest.

References

1. Manecke GR, Asemota A, Michard F. Tackling the economic burden of postsurgical complications: Would perioperative goal-directed fluid therapy help? Crit Care 2014;18:566.
2. Wolthuis EK, Vlaar AP, Choi G, Roelofs JJ, Juffermans NP, Schultz MJ. Mechanical ventilation using non-injurious ventilation settings causes lung injury in the absence of pre-existing lung injury in healthy mice. Crit Care 2009;13:R1.
3. Chastre J, Fagon JY. Ventilator-associated pneumonia. Am J Respir Crit Care Med 2002;165:867-903.
4. Dreyfuss D, Ricard JD. Acute lung injury and bacterial infection. Clin Chest Med 2005;26:105-12.
5. Brun-Buisson C, Minelli C, Bertolini G, Brazzi L, Pimentel J, Lewandowski K, *et al*. Epidemiology and outcome of acute lung injury in European intensive care units. Results from the ALIVE study. Intens Care Med 2004;30:51-61.
6. Walker MG, Yao LJ, Patterson EK, Joseph MG, Cepinskas G, Veldhuizen RA, *et al*. The effect of tidal volume on systemic inflammation in acid-induced lung injury. Respiration 2011;81:333-42.
7. Wilson MR, Patel BV, Takata M. Ventilation with 'clinically-relevant' high tidal volumes does not promote stretch-induced injury in the lungs of healthy mice. Crit Care Med 2012;40:2850-7.
8. Yim AP, Wan S, Lee TW, Arifi AA. VATS lobectomy reduces cytokine responses compared with conventional surgery. Ann Thorac Surg 2000;70:243-7.
9. Ranieri VM, Suter PM, Tortorella C, De Tullio R, Dayer JM, Brienza A, *et al*. Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: A randomized controlled trial. JAMA 1999;282:54-61.
10. Gajic O, Frutos-Vivar F, Esteban A, Hubmayr RD, Anzueto A. Ventilator settings as a risk factor for acute respiratory distress syndrome in mechanically ventilated patients. Intensive Care Med 2005;31:922-6.
11. Memtsoudis SG, Bombardieri AM, Ma Y, Girardi FP. The effect of low versus high tidal volume ventilation on inflammatory markers in healthy individuals undergoing posterior spine fusion in the prone position: A randomized controlled trial. J Clin Anesth 2012;24:263-9.
12. Pinheiro de Oliveira R, Hetzel MP, dos Anjos Silva M, Dallegrave D, Friedman G. Mechanical ventilation with high tidal volume induces inflammation in patients without lung disease. Crit Care 2010;14:R39.
13. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLoS Biol 2010;29;8:e1000412.
14. Eltzschig HK, Carmeliet P. Hypoxia and inflammation. N Engl J Med 2011;364:656-65.
15. Dos Santos CC. Slutsky AS. Invited review: Mechanisms of ventilator-induced lung injury: A perspective. J Appl Physiol 2000;89:1645-55.
16. Tschumperlin DJ, Oswari J, Margulies AS. Deformation-induced injury of alveolar epithelial cells. Effect of frequency, duration, and amplitude. Am J Respir Crit Care Med 2000;162:357-62.
17. D'Angelo E, Pecchiari M, Baraggia P, Saetta M, Balestro E, Milic-Emili J. Low-volume ventilation causes peripheral airway injury and increased airway resistance in normal rabbits. J Appl Physiol 2002;92:949-56.
18. Zupancich E, Paparella D, Turani F, Munch C, Rossi A, Massaccesi S, *et al*. Mechanical ventilation affects inflammatory mediators in patients undergoing cardiopulmonary bypass for cardiac surgery: A randomized clinical trial. J Thorac Cardiovasc Surg 2005;130:378-83.
19. Pinheiro de Oliveira R, Hetzel MP, dos Anjos Silva M, Dallegrave D, Friedman G. Mechanical ventilation with high tidal volume induces inflammation in patients without lung disease. Crit Care 2010;14:R39.
20. Davies JD, Senussi MH, Mireles-Cabodevila E. Should A tidal volume of 6 mL/kg be used in all patients? Respir Care 2016;61:774-90.
21. Amato MB, Barbas CS, Medeiros DM, Magaldi RB, Schettino GP, Lorenzi-Filho G, *et al*. Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. N Engl J Med 1998;338:347-54.
22. Putensen C, Theuerkauf N, Zinserling J, Wrigge H, Pelosi P. Meta-analysis: Ventilation strategies and outcomes of the acute respiratory distress syndrome and acute lung injury. Ann Intern Med 2009;151:566-76.
23. Schultz MJ, Haitsma JJ, Slutsky AS, Gajic O. What tidal volumes should be used in patients without acute lung injury? Anesthesiology 2007;106:1226-31.
24. Lellouche F, Dionne S, Simard S, Bussières J, Dagenais F. High tidal volumes in mechanically ventilated patients increase organ dysfunction after cardiac surgery. Anesthesiology 2012;116:1072-82.
25. Young CC, Harris EM, Vacchiano C, Bodnar S, Bukowy B, Elliott RR, *et al*. Lung-protective ventilation for the surgical patient: International expert panel-based consensus recommendations. Br J Anaesth 2019;123:898-913.