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Mechanical Ventilation with Lower Tidal Volumes and Positive End-expiratory Pressure Prevents Pulmonary Inflammation in Patients without Preexisting Lung Injury

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Background: **Mechanical ventilation with high tidal volumes aggravates lung injury in patients with acute lung injury or acute respiratory distress syndrome. The authors sought to determine the effects of short-term mechanical ventilation on local inflammatory responses in patients without preexisting lung injury.**

Methods: **Patients scheduled to undergo an elective surgical procedure (lasting ≥5 h) were randomly assigned to mechanical ventilation with either higher tidal volumes of 12 ml/kg ideal body weight and no positive end-expiratory pressure (PEEP) or lower tidal volumes of 6 ml/kg and 10 cm H_2O PEEP. After induction of anesthesia and 5 h thereafter, bronchoalveolar lavage fluid and/or blood was investigated for polymorphonuclear cell influx, changes in levels of inflammatory markers, and nucleosomes.**

Results: **Mechanical ventilation with lower tidal volumes and PEEP (n = 21) attenuated the increase of pulmonary levels of interleukin (IL)-8, myeloperoxidase, and elastase as seen with higher tidal volumes and no PEEP (n = 19). Only for myeloperoxidase, a difference was found between the two ventilation strategies after 5 h of mechanical ventilation ($P < 0.01$). Levels of tumor necrosis factor α, IL-1α, IL-1β, IL-6, macrophage inflammatory protein 1α, and macrophage inflammatory protein 1β in the bronchoalveolar lavage fluid were not affected by mechanical ventilation. Plasma levels of IL-6 and IL-8 increased with mechanical ventilation, but there were no differences between the two ventilation groups.**

Conclusion: **The use of lower tidal volumes and PEEP may limit pulmonary inflammation in mechanically ventilated patients without preexisting lung injury. The specific contribution of both lower tidal volumes and PEEP on the protective effects of the lung should be further investigated.**

MECHANICAL ventilation (MV) may aggravate pulmonary inflammation, which may be a factor in the additional morbidity/mortality associated with nonprotective forms of MV.[1,2] Indeed, MV with lower tidal volumes (V_Ts) has been found to improve survival of patients with acute lung injury or acute respiratory distress syndrome (ARDS).[3] This so-called "ventilator-associated lung injury" can be characterized by local attraction of inflammatory cells, which produce inflammatory mediators. These locally produced mediators can subsequently disseminate into the systemic compartment. Ranieri *et al.*[4] demonstrated a reduction in bronchoalveolar lavage fluid (BALF) number of polymorphonuclear cells and proinflammatory mediators with a lung-protective MV strategy as compared with conventional MV in patients with ARDS. In addition, lung-protective MV attenuated systemic levels of inflammatory mediators,[3,4] which may be of importance for clinical outcome because higher systemic levels of these mediators were associated with higher multiorgan failure scores.[5] Furthermore, it has been shown in experimental studies that lung-protective MV limits end-organ epithelial cell apoptosis, protecting organ function during MV.[6,7]

Whether MV *per se* initiates pulmonary inflammation is an ongoing debate. Although previous studies in animals demonstrated that MV with higher V_T causes pulmonary inflammation and functional injury,[8–10] the clinical implications of these studies are unclear because V_Ts in these studies were unphysiologically large. Using a more physiologic V_T (10 ml/kg) and no PEEP (zero end-expiratory pressure [ZEEP]) demonstrated that MV for 6 h can induce a proinflammatory reaction in noninjured lungs.[11] Even MV for 1 h with lower V_Ts (6 ml/kg) and ZEEP resulted in a proinflammatory and profibrogenic response in normal rats.[12] Deleterious effects of higher V_T in patients without preexisting lung injury, however, have been suggested by retrospective studies.[13–15] Fernandez *et al.* demonstrated that higher intraoperative V_Ts are more associated with respiratory failure after pneumonectomy.[15] Protective MV with lower V_Ts and PEEP during esophagectomy resulted in a decrease in systemic proinflammatory response, im-

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proved lung function, and earlier extubation.[16] Higher V_T in a surgical intensive care unit was associated with more pulmonary infection, longer duration of intubation, and longer duration of stay in the intensive care unit as compared with lower V_T.[17]

The purpose of this study was to investigate the effects of short-term (*i.e.*, for 5 h) MV on pulmonary inflammation and apoptosis. A randomized controlled trial was performed comparing two different MV strategies in patients without preexisting lung injury who were scheduled to undergo a major surgical procedure.

Materials and Methods

This study represents a part of a large study. Another part has already been published.[18]

Patients

The study protocol was approved by the Medical Ethics Committee of the University of Amsterdam, and informed consent was obtained from all patients. Adult patients were eligible if scheduled to undergo a surgical procedure of 5 h or longer and all involved physicians (surgeon, anesthesiologist, pulmonologist) consented with the study procedures. Exclusion criteria included a history of any lung disease, use of immunosuppressive medication, recent infections, previous thromboembolic disease, recent ventilatory support, and participation in another clinical trial.

Study Protocol

All patients received routine anesthesia according to the local protocol, including intravenous propofol (2–3 mg/kg, thereafter 6–12 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}), fentanyl (2–3 μg/kg, thereafter as required), and rocuronium (as required), and epidural bupivacaine (0.125%)–fentanyl (2.5 μg/ml). The ventilatory protocol consisted of volume-controlled MV, at an inspired oxygen fraction of 0.40, inspiratory-to-expiratory ratio of 1:2, and a respiratory rate adjusted to achieve normocapnia. Randomization was performed by drawing a presealed envelope; patients were randomly assigned to MV with either V_Ts of 12 ml/kg ideal body weight (high V_T [HV_T]) and ZEEP or 6 ml/kg (low V_T [LV_T]) and 10 cm H_2O PEEP. The ideal body weight of male patients was calculated as equal to 50 + 0.91 (centimeters of height − 152.4); that of female patients was calculated as 45.5 + 0.91 (centimeters of height − 152.4).[3] Anesthesiologists were allowed to change the ventilation protocol at any time point upon surgeon's request or if there was any concern for the patient's safety. If the surgical procedure exceeded 5 h, anesthesiologists were allowed to change the ventilation strategy after the second sampling (blood and bronchoalveolar lavage).

Bronchoscopy and bronchoalveolar lavage were performed twice on all patients: the first directly after induction of anesthesia and start of MV in the right middle lobe or lingula, and the second performed in the contralateral lung 5 h thereafter, either perioperatively or directly postoperatively. BALF was obtained and processed as previously described.[19–21] In short, bronchoalveolar lavage was performed by an experienced pulmonologist in a standardized fashion according to the guidelines of the American Thoracic Society, using a flexible fiberoptic video-bronchoscope. Seven successive 20-ml aliquots of prewarmed saline were instilled and aspirated immediately with low suction (recovery, 71 ± 18.4 ml). Arterial blood samples were drawn before both lavages, and hourly blood gas analyses were performed. Cell-free supernatants from BALF and blood were stored at −80°C until analysis. BALF cells were resuspended in ice-cold phosphate-buffered saline. The resuspended cells were partially used for absolute cell counts (using a Bürker-Turk hemocytometer; Emergo, Landsmeer, The Netherlands) and Giemsa-stained cytospin preparation for differential counting.

Assays

Myeloperoxidase was determined by enzyme-linked immunosorbent assay.[22] BALF levels of human neutrophil elastase were assessed with a sandwich-type enzyme-linked immunosorbent assay (Hycult Biotechnology, Uden, The Netherlands). The detection limit of the assay was 4.0 ng/ml. Tumor necrosis factor (TNF)-α, interleukin (IL)-1α, IL-6, IL-8, macrophage inflammatory protein 1α, and macrophage inflammatory protein 1β were measured by enzyme-linked immunosorbent assay (TNF-α, IL-6, IL-8, Sanquin, Amsterdam, The Netherlands; IL-1α, macrophage inflammatory protein 1α, macrophage inflammatory protein 1β, R&D Systems, Minneapolis, MN). Nucleosomes were measured by enzyme-linked immunosorbent assay as described previously with slight modifications.[23] One unit was arbitrarily set at the amount of nucleosomes released by 100 Jurkat cells. Detection limit of the assay is 0.1 U/ml. Nucleosomes are generated by internucleosomal cleavage of chromatin, during apoptotic cell death. We used the release of nucleosomes as measurement for apoptotic cell death.

Statistical Analysis

Baseline characteristics of the randomized patient groups were compared with the Student *t* test, Mann–Whitney U test, or chi-square test as appropriate. Linear mixed model analysis was used to detect differences between respiratory variables. This type of analysis takes the association between values for individual patients measured at each time point into account. This implies a maximum of six time points per patient. The fixed effects were hour of MV (0–5) and MV group (LV_T/PEEP or HV_T/ZEEP). Data obtained with linear mixed model analysis are presented as mean and 95% confidence interval (CI). All measured inflammatory mediators were not normally distributed. Differences within groups were analyzed with a Wilcoxon signed-rank test for paired sam-

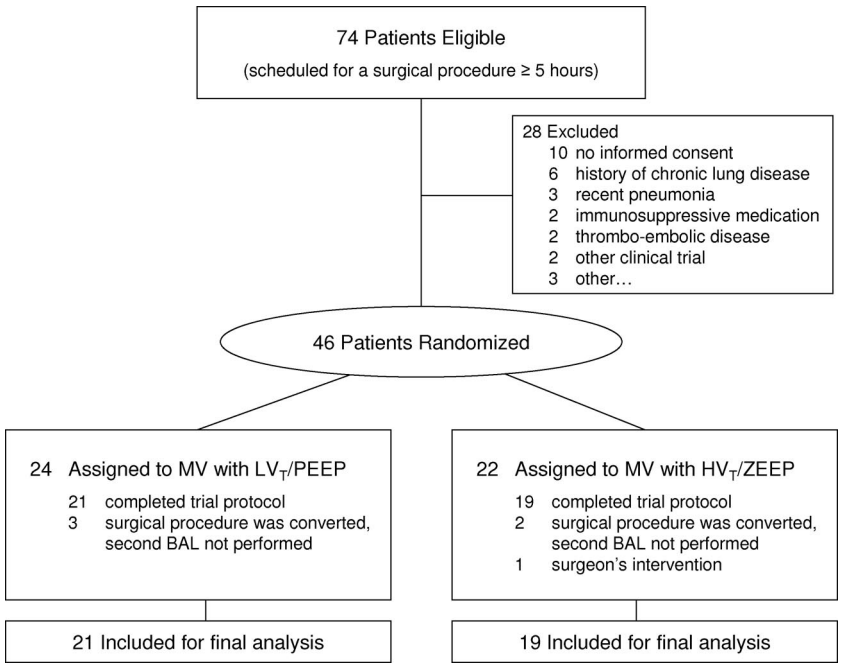

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) diagram. BAL = bronchoalveolar lavage; HV_T/ZEEP = tidal volumes of 12 ml/kg ideal body weight and no positive end-expiratory pressure; LV_T/PEEP = tidal volumes of 6 ml/kg ideal body weight and 10 cm H_2O positive end-expiratory pressure; MV = mechanical ventilation.

ples comparing t = 5 *versus* t = 0 h. The Mann–Whitney U test was used to compare the changes over time between the two randomization groups. We corrected for multiple testing using the Benjamini–Hochberg false discovery rate adjustment.[24] A *P* value of less than 0.05 was considered statistically significant. All statistical analyses were performed with Statistical Package for the Social Sciences 12.0.2 (SPSS, Chicago, IL).

Results

Patients

Seventy-four consecutive patients who were scheduled to undergo an elective surgical procedure of 5 h or more were screened (fig. 1). Twenty-eight patients were excluded, leaving 46 patients for randomization. Five patients were randomized but excluded from final analysis, because the initial surgical procedure was converted by the surgeon into another shorter operation (<3 h), and only one bronchoalveolar lavage was performed. One patient was randomized, but no lavages were performed upon the surgeon's request after induction of anesthesia. In total, 40 patients completed the study protocol. There were no major differences between the two randomization groups with regard to baseline characteristics (table 1). Besides the mechanical ventilator settings (V_T, PEEP, and respiratory rate), there were significant differences in partial pressure of carbon dioxide and pH between the two MV strategies. Partial pressure of carbon dioxide was 5.60 (95% CI, 5.35–5.84) in the LV_T/PEEP group as compared with 4.86 (95% CI, 4.61–5.12) in the HV_T/ZEEP group ($P < 0.001$). Accordingly, pH was significantly lower in the LV_T/PEEP group (7.36; 95% CI, 7.34–7.38) as compared with the HV_T/ZEEP group (7.40; 95% CI, 7.39–7.42; $P < 0.001$). Maximum airway pressures were not different between the study groups during 5 h of MV (fig. 2). Perioperative hemodynamic parameters, including number of patients being transfused and the number of transfusions (erythrocytes and plasma) (table 2) were not different between the two ventilation groups.

Table 1. Baseline Characteristics of Patients

	LV_T/PEEP (n = 21)	HV_T/ZEEP (n = 19)
Age, mean ± SD, yr	62 ± 9.8	61 ± 9.5
Male, n (%)	14 (67)	14 (74)
ASA, median (range)	II (I–V)	II (I–III)
Height, mean ± SD, cm	176 ± 8.7	174 ± 10.0
Weight, mean ± SD, kg	79 ± 14.4	76 ± 13.7
Tobacco use, n (%)	9 (43)	6 (32)
Surgical procedure	5 Whipple procedure* 5 Laparoscopic radical prostatectomy 6 Hemihepatectomy 2 Retroperitoneal tumor resection 2 Total pancreatectomy 1 Open prostatectomy†	8 Whipple procedure* 7 Laparoscopic radical prostatectomy 3 Hemihepatectomy 1 Colon conduit

* The Whipple procedure is a pancreaticoduodenectomy. † The open prostatectomy was performed after an initial laparoscopic approach.

ASA = American Society of Anesthesiologists (physical status); HV_T/ZEEP = higher tidal volumes/zero end-expiratory pressure; LV_T/PEEP = lower tidal volumes/positive end-expiratory pressure.

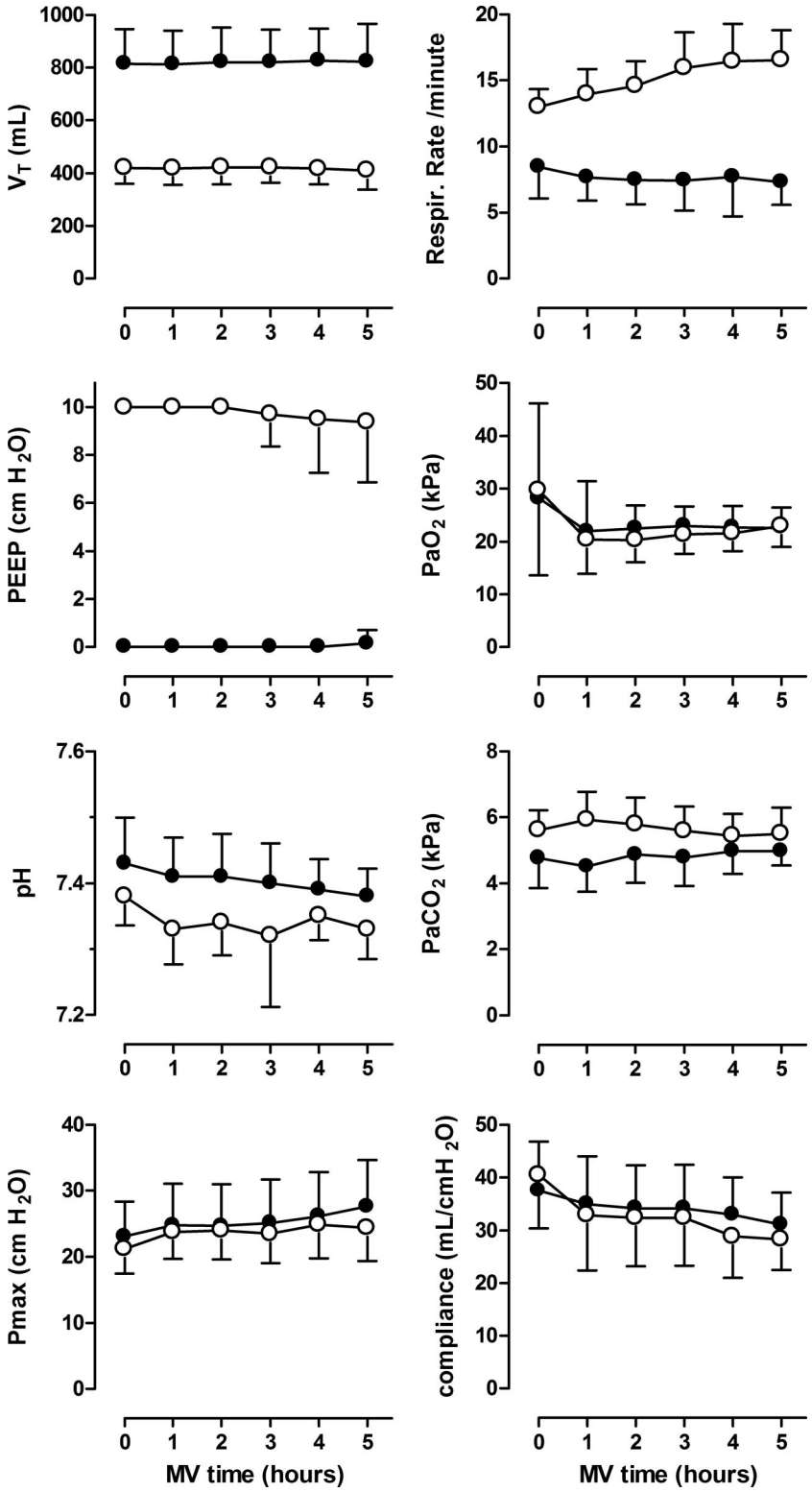

Fig. 2. Respiratory variables. Tidal volume (V_T), respiratory rate (respir. rate), positive end-expiratory pressure (PEEP), maximal pressure (Pmax), compliance, and arterial blood gas analyses in patients ventilated with lower tidal volumes and 10 cm H_2O PEEP (*open symbols*, n = 21) and patients ventilated with higher tidal volumes and no PEEP (*closed symbols*, n = 19). Data are mean ± SD. 1 kPa = 7.5 mmHg. MV = mechanical ventilation; $Paco_2$ = partial pressure of arterial carbon dioxide; Pao_2 = partial pressure of arterial oxygen.

Cellular Composition of BALF, Myeloperoxidase, and Elastase in BALF

Ninety-nine percent of the cells from the BALF were macrophages. MV did not alter cell content, and no differences in neutrophil influx were found between groups. Myeloperoxidase and elastase levels in BALF, however, were significantly higher after 5 h of MV with higher V_Ts and ZEEP as compared with baseline levels. Median myeloperoxidase levels increased from 2.80 [interquartile range, 0.0–7.80] to 8.80 [2.35–25.0] ng/ml (P = 0.009) and elastase levels increased from 7.10 [1.60–14.5] to 17.4 [5.70–21.2] ng/ml in the HV_T/ZEEP

Table 2. Perioperative Parameters

	LV_T/PEEP (n = 21)	HV_T/ZEEP (n = 19)
MV duration, mean ± SD, min	304 ± 35	308 ± 52
Blood loss, median [IQR], ml	1,550 [800–2,325]	1,000 [463–1,675]
Number of patients receiving erythrocytes (%)	7 (33.3)	5 (26.3)
Transfused erythrocytes, median [IQR], units	0 [0–1.5]	0 [0–1]
Number of patients receiving plasma (%)	3 (14.3)	0 (0)
Transfused plasma, median [IQR], units	0 [0–0]	0 [0–0]
Colloids, median [IQR], l	0.5 [0.5–1.5]	0.5 [0.5–1.5]
Crystalloids, median [IQR], l	4.5 [2.75–5.75]	4.0 [2.5–5.5]
Lowest hemoglobin, mean ± SD, mm*	6.0 ± 1.2	6.2 ± 1.0
Lowest SBP, mean ± SD, mmHg	82 ± 9.6	87 ± 14.9

* Hemoglobin, 1 mm = 1.61 g/dl.

HV_T/ZEEP = higher tidal volumes/zero end-expiratory pressure; IQR = interquartile range; LV_T/PEEP = lower tidal volumes/positive end-expiratory pressure; MV = mechanical ventilation; SBP = systolic blood pressure.

group ($P = 0.013$). No increase in myeloperoxidase and elastase levels was observed with the use of lower V_Ts and PEEP (fig. 3). Only for myeloperoxidase was there a statistically significant difference between the two ventilation strategies ($P = 0.004$).

Protein Levels of Inflammatory Mediators in BALF and Plasma

Mechanical ventilation minimally influenced cytokine and chemokine levels in BALF (fig. 4). BALF levels of TNF-α and IL-8 were influenced by the way patients were ventilated. TNF-α increased in the LV_T/PEEP group ($P = 0.028$), whereas IL-8 increased in the HV_T/ZEEP group ($P = 0.015$) after 5 h of MV. Plasma levels of IL-6 and IL-8 did significantly increase during the surgical procedure, but this increase in cytokine generation was similar in both groups (fig. 5).

Nucleosome Levels in BALF and Plasma

Mechanical ventilation with higher V_Ts and ZEEP caused an increase in BALF nucleosomes as compared with lower V_Ts and 10 cm H_2O PEEP ($P = 0.028$; fig. 6). There was also a statistically significant difference between the two ventilation strategies ($P = 0.043$). In plasma, nucleosome levels were equally increased in both groups.

Postoperative Complications and Clinical Outcome

In the postoperative recovery, 28 patients had follow-up chest radiographs. There were no differences in postoperative arterial blood gas analysis (HV_T/ZEEP *vs.* LV_T/PEEP): partial pressure of oxygen, 117 ± 42 *versus* 123 ± 53 mmHg; partial pressure of carbon dioxide, 43 ± 5 *versus* 42 ± 5 mmHg; and pH, 7.36 ± 0.053 *versus* 7.34 ± 0.051. There were no differences in the incidence of pulmonary complications (*e.g.*, acute lung injury, pneumonia) between the two study groups; in each study group, there was one patient requiring prolonged MV for respiratory failure after surgery. One patient ventilated with LV_T/PEEP died postoperatively of multiple organ failure after complicated hemihepatectomy. All other patients were discharged home.

Multiple Testing

Every measured mediator was tested three times (differences within groups comparing t = 5 *vs.* t = 0 and changes between randomization groups). Because this approach serves to inflate type I error, we corrected for multiple testing. As a consequence, three *P* values were no longer significant ($P > 0.05$). There was only a trend for higher levels of BALF nucleosomes in the HV_T/ZEEP group after 5 h of MV ($P = 0.084$). There was no statistical significant difference between the two MV

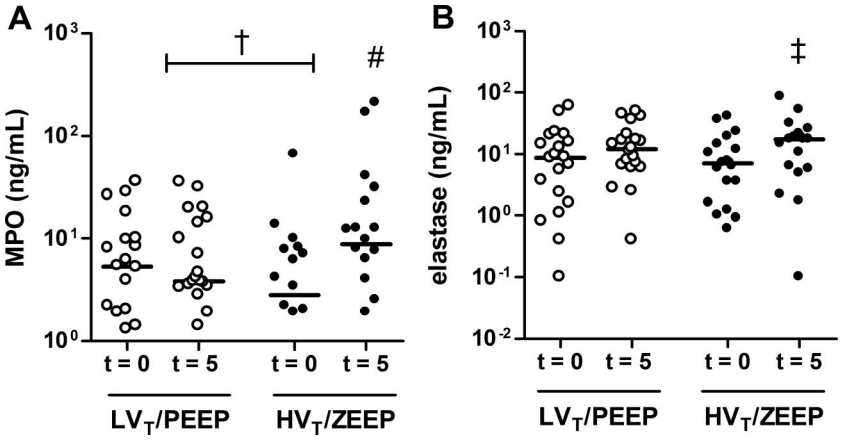

Fig. 3. Myeloperoxidase (MPO; *A*) and elastase (*B*) in bronchoalveolar lavage fluid recovered at baseline (t = 0) and after 5 h (t = 5) from patients mechanically ventilated with 6 ml/kg and 10 cm H_2O positive end-expiratory pressure (LV_T/PEEP; *open symbols*) or with 12 ml/kg and zero end-expiratory pressure (HV_T/ZEEP; *closed symbols*). *Horizontal lines* represent median values. Wilcoxon signed-rank test: # $P < 0.01$ *versus* t = 0. ‡ $P < 0.05$ *versus* t = 0. Mann–Whitney *U* test: † $P < 0.01$ between groups.

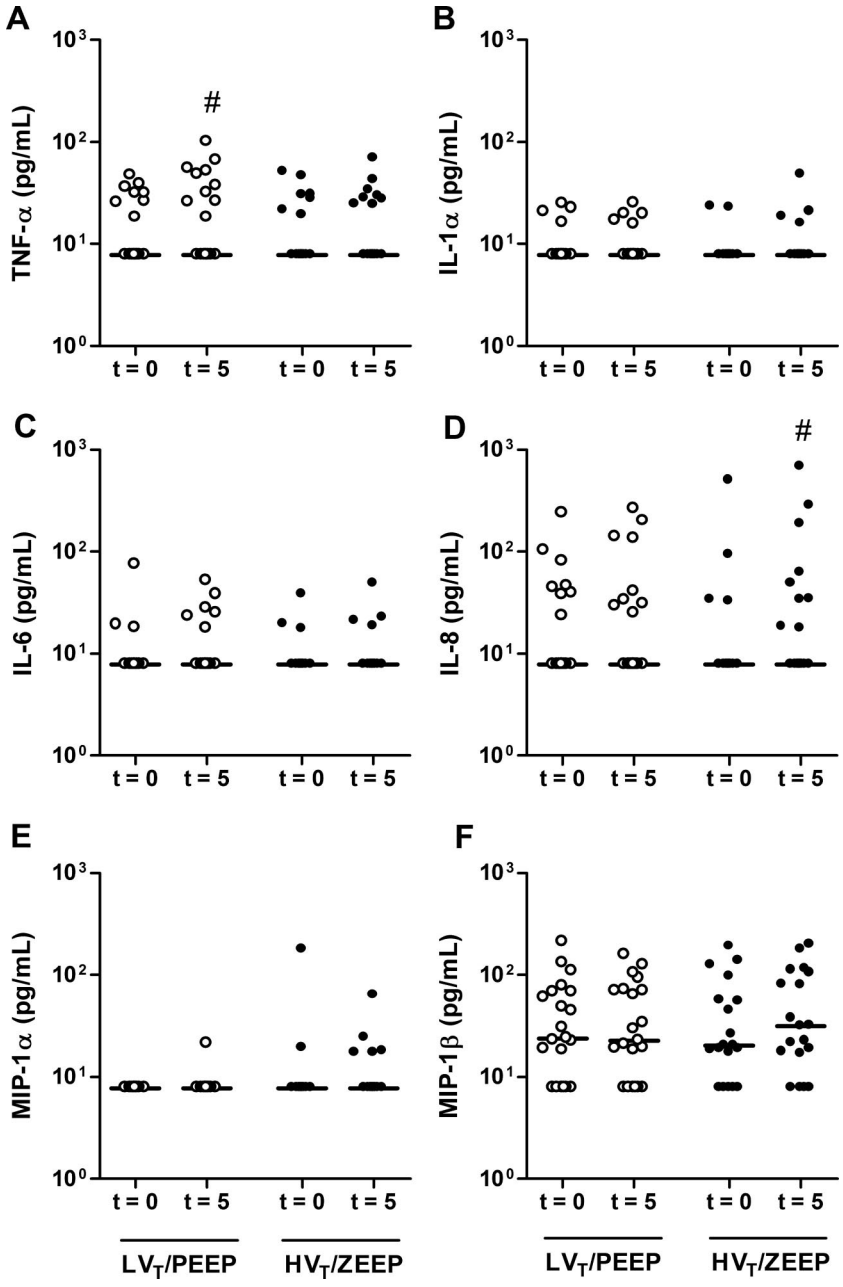

Fig. 4. Tumor necrosis factor (TNF)-α (*A*), interleukin (IL)-1α (*B*), IL-6 (*C*), IL-8 (*D*), macrophage inflammatory protein (MIP)-1α (*E*), and MIP-1β (*F*) in bronchoalveolar lavage fluid recovered at baseline (t = 0) and after 5 h (t = 5) from patients mechanically ventilated with 6 ml/kg and 10 cm H_2O positive end-expiratory pressure (LV_T/PEEP; *open symbols*) or with 12 ml/kg and zero end-expiratory pressure (HV_T/ZEEP; *closed symbols*). For all data points below the detection limit, the data point was given an arbitrary value of 7.8 pg/ml. *Horizontal lines* represent median values. Wilcoxon signed-rank test: $^{\#}P < 0.05$ *versus* t = 0.

strategies, regarding nucleosome levels in the BALF ($P = 0.12$). Also, the level of TNF-α in the LV_T/PEEP group was not significantly increased after 5 h of MV ($P = 0.084$).

Discussion

In the current study, we demonstrate that short-term MV is associated with significant inflammatory changes in the pulmonary compartment and that a lung-protective strategy attenuates these changes. Based on our findings, it seems that MV is a proinflammatory stimulus in noninjured lungs.

Myeloperoxidase (and also elastase) in the BALF is higher after 5 h of MV with higher V_Ts and ZEEP as compared with baseline levels. No increase in myeloperoxidase and elastase was seen after 5 h of MV with lower V_Ts and PEEP. This implies activation of polymorphonuclear cells, which were recruited to the pulmonary compartment or already present there. Higher concentrations of IL-8 in the BALF of patients ventilated with higher V_Ts and ZEEP support the first idea. However, in the differential cell count, we do not see an increase in neutrophils, which can be explained by the fact that the concentration of IL-8 in the plasma is very high, and thus there is a chemotactic gradient not favoring migration of neutrophils into the lung. Another possibility is that the

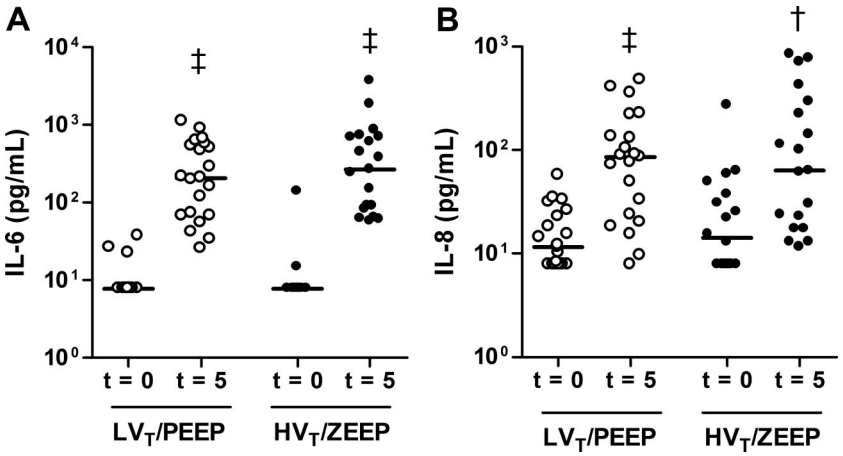

Fig. 5. Plasma interleukin (IL)-6 (*A*) and IL-8 (*B*) recovered at baseline (t = 0) and after 5 h (t = 5) from patients mechanically ventilated with 6 ml/kg and 10 cm H_2O positive end-expiratory pressure (LV_T/PEEP; *open symbols*) or with 12 ml/kg and zero end-expiratory pressure (HV_T/ZEEP; *closed symbols*). *Horizontal lines* represent median values. Wilcoxon signed-rank test: † P = 0.001, ‡ $P < 0.001$ *versus* t = 0.

neutrophils remained in the subepithelium and did not migrate further into the alveoli. Neutrophil count in the BALF is a well-established method to observe neutrophil influx into the lung. However, neutrophils can accumulate in alveolar septa after MV.[25] A practical limitation was that we did not have reliable methods to obtain and isolate viable lung epithelial cells from our patients, and we could not investigate them in more detail. From a scientific point of view, it would also have been interesting to have obtained lung tissue for specific staining and identification of apoptotic cells. However, we have not performed these assays, because we thought that many patients would not consent to more invasive procedures perioperatively or postoperatively.

For all other measured inflammatory protein levels in BALF, there were no differences between the groups. It should be noted that a period of 5 h is probably too short to detect differences in certain protein levels due to modified transcriptional and translational processes. We hypothesize that most inflammatory mediators measured in BALF were made in alveolar macrophages and lung epithelial cells and released upon stimulation.[26,27]

Furthermore, we have shown that there is a trend for higher BALF levels of nucleosomes after 5 h of MV with higher V_T ventilation and ZEEP as compared with baseline levels. During apoptotic cell death, nucleosomes are generated by internucleosomal cleavage of chromatin. The nucleosomes are then packed in apoptotic blebs along with other nuclear components. We used the release of nucleosomes as a measurement for apoptotic cell death. The rapid increase in BALF nucleosomes (*i.e.*, within hours after initiation of MV) most likely reflects apoptosis of pneumocytes. As far as we know, this is the first study showing an association between MV and alveolar apoptosis in humans. *In vitro* experiments have shown that mechanical strain induces proapoptotic changes in human lung epithelial cells.[27,28] Furthermore, *in vivo* animal experiments have shown that impairment of apoptosis pathways limited pulmonary inflammation and lung injury, and also protected against multiple organ failure and death.[6,7] Therefore, it has been proposed that intraalveolar apoptosis is a potentially harmful process that could be targeted in the treatment of (ventilator-associated) lung injury.[29] On the other hand, apoptosis may be a pivotal process involved in alveolar repair mechanisms. More research is needed before clinical application of antiapoptotic strategies.

Both surgical stimuli and general anesthesia are associated with increased plasma levels of proinflammatory markers.[30,31] In the current study, we extended these

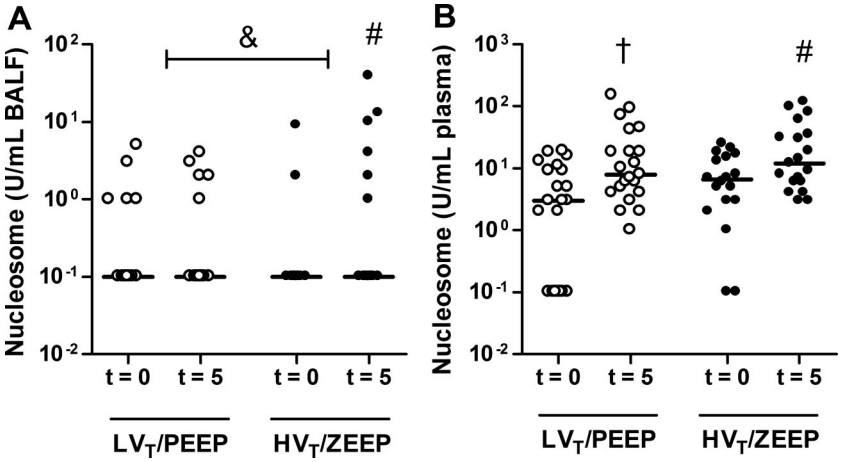

Fig. 6. Nucleosome levels in bronchoalveolar lavage fluid (BALF; *A*) and plasma (*B*) recovered at baseline (t = 0) and after 5 h (t = 5) from patients mechanically ventilated with 6 ml/kg and 10 cm H_2O positive end-expiratory pressure (LV_T/PEEP; *open symbols*) or with 12 ml/kg and zero end-expiratory pressure (HV_T/ZEEP; *closed symbols*). *Horizontal lines* represent median values. Wilcoxon signed-rank test: # $P < 0.05$, † $P < 0.01$ *versus* t = 0. Mann–Whitney U test: & $P < 0.05$ between groups.

findings by showing higher concentrations of IL-6 and IL-8 after 5 h of MV in both ventilation strategies. In patients with acute lung injury, systemic cytokine concentrations increase after initiating MV with low PEEP and higher V_T.[32] We hypothesize, however, that in patients with noninjured lungs, there is no translocation of inflammatory mediators because much higher levels of inflammatory mediators in the systemic compartment were found as compared with the pulmonary compartment.

One limitation of our study is that our study protocol does not allow us to differentiate the effects of lower V_Ts from those by higher PEEP levels. We chose to combine lower V_Ts with PEEP and higher V_Ts with no PEEP, because these settings result in similar maximum airway pressures. Recent studies in open chest rabbits demonstrated that MV with V_Ts of 8–12 ml/kg and ZEEP may cause permanent mechanical alterations and histologic damage to peripheral airways and inflammation in noninjured lungs.[25,33] Surfactant inactivation or depletion seems to play a major role during ventilation with V_Ts of 10 ml/kg and ZEEP.[34] Another animal study demonstrated that atelectasis caused increased alveolar–capillary protein leakage and disruption of the vascular endothelium, possibly *via* shear stress.[35] During general anesthesia, atelectasis is potentiated by anesthesia and muscle relaxants altering diaphragmatic position. Also, tidal airway closure can occur and cause peripheral airway injury. This may be a common but unrecognized complication in patients undergoing general anesthesia.[36] Cyclic opening and closing from ZEEP leads to greater increases in bronchoalveolar lavage cytokines than atelectasis.[37] Therefore, patients ventilated with ZEEP in our study could have gross atelectasis and peripheral airway injury, caused by tidal airway closure. Of note, no recruitment maneuver was performed in either MV strategies.

Our data are different from those from previous studies in which MV strategies were investigated in patients with noninjured lungs undergoing surgery. Indeed, Wrigge *et al.*[38] demonstrated that MV with V_T of 15 ml/kg ideal body weight and ZEEP for 1 h caused no consistent changes in plasma levels of measured cytokines. In a study of patients undergoing thoracic or abdominal surgery, no differences in inflammatory responses were found between two ventilation strategies similar to the ones used in our study after MV for 3 h.[39] These studies, however, looked at inflammatory mediators only after 1 and 3 h of MV, respectively. In other studies in which MV during or after cardiopulmonary bypass surgery was investigated, increased levels of proinflammatory mediators were reported, but not consistently.[40–43] Wrigge *et al.*[40] showed that ventilation for 6 h with lower V_T (6 ml/kg ideal body weight) had no or only minor effect on systemic and pulmonary inflammatory responses in patients after cardiopulmonary bypass surgery as compared with higher V_T (12 ml/kg). Only TNF-α levels in the BALF were significantly higher in the high V_T group than in the low V_T group. Koner *et al.*[43] investigated different ventilation strategies during cardiopulmonary bypass and did not find any changes in systemic cytokine levels, postoperative pulmonary function, or duration of hospitalization with either MV strategy. Unfortunately, no pulmonary cytokine levels were measured in that study. In contrast, two other studies did find a difference between different ventilation strategies in patients undergoing cardiopulmonary bypass.[41,42]

Considering the minor differences in pulmonary inflammatory mediators caused by the two different ventilation strategies in patients during general anesthesia, it seems that the inflammatory response plays a minor role. From experimental studies, it is known that the inflammatory response occurs after 4–6 h or the damage being mainly mechanical without any relevant inflammatory response.[8,11,44] MV with moderate V_T and ZEEP can cause mechanical injury with alveolar–bronchiolar uncoupling.[25] Therefore, in our patient group, there may be lung injury in the absence of a relevant inflammatory response.

The inflammatory changes observed in healthy lungs are mere physiologic adaptations to the artificial process of MV. However, we propose that lung injury is induced by a "multiple-hit" model, whereby predisposing conditions, such as injurious MV or major surgery, may result in (weak) pulmonary inflammation. Possible second hits, such as transfusion of blood products which may cause transfusion-related acute lung injury, prolonged (injurious) MV, aspiration, shock, sepsis, and pulmonary infection, may all cause additional lung injury, finally resulting in full-blown ARDS with high morbidity and mortality. There is indeed clinical evidence supporting this multiple-hit hypothesis. High V_T ventilation was independently associated with development of ARDS in patients who did not have ARDS at the onset of MV in the intensive care unit.[13,14] During MV of pneumonectomy patients, higher intraoperative V_Ts were identified as a risk factor of postoperative respiratory failure.[15] Furthermore, postoperative patients who were ventilated with a lower V_T strategy had a lower risk of pulmonary infection, and duration of intubation and duration of stay tended to be shorter.[17] Therefore, we would like to encourage the use of lower V_Ts and PEEP according to the principle of *primum non nocere*: Ventilator-associated lung injury can be limited. However, our results do not imply that these two different ventilation strategies can lead to different postoperative complications.

Of course, the aforementioned studies, including ours, have investigated patients who underwent major surgery. Inflammatory effects of the surgical procedure itself could not be excluded but are equal in both groups. However, investigating the effects of MV in healthy humans would lack any clinical significance. Similar results are probably not reproducible if the duration of MV was less than 5 h. Also, the type of surgery could have affected the variables investigated. We do realize that further studies are needed to elucidate the effects of prolonged MV.

In conclusion, MV for 5 h with lower V_Ts and PEEP may limit pulmonary proinflammatory changes in patients with noninjured lungs during major surgery. Even during a relatively brief period of MV, patients will most likely benefit from lower V_Ts and PEEP. The specific contribution of both lower V_Ts and PEEP on the protective effects of the lung should be further investigated.

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References

1. Pinhu L, Whitehead T, Evans T, Griffiths M: Ventilator-associated lung injury. Lancet 2003; 361:332–40
2. Slutsky AS: Lung injury caused by mechanical ventilation. Chest 1999; 116:9S–15S
3. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. N Engl J Med 2000; 342:1301–8
4. Ranieri VM, Suter PM, Tortorella C, De Tullio R, Dayer JM, Brienza A, Bruno F, Slutsky AS: Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: A randomized controlled trial. JAMA 1999; 282:54–61
5. Ranieri VM, Giunta F, Suter PM, Slutsky AS: Mechanical ventilation as a mediator of multisystem organ failure in acute respiratory distress syndrome. JAMA 2000; 284:43–4
6. Imai Y, Parodo J, Kajikawa O, de Perrot M, Fischer S, Edwards V, Cutz E, Liu M, Keshavjee S, Martin TR, Marshall JC, Ranieri VM, Slutsky AS: Injurious mechanical ventilation and end-organ epithelial cell apoptosis and organ dysfunction in an experimental model of acute respiratory distress syndrome. JAMA 2003; 289:2104–12
7. Crimi E, Zhang H, Han RN, Sorbo LD, Ranieri VM, Slutsky AS: Ischemia and reperfusion increases susceptibility to ventilator-induced lung injury in rats. Am J Respir Crit Care Med 2006; 174:178–86
8. Belperio JA, Keane MP, Burdick MD, Londhe V, Xue YY, Li K, Phillips RJ, Strieter RM: Critical role for CXCR2 and CXCR2 ligands during the pathogenesis of ventilator-induced lung injury. J Clin Invest 2002; 110:1703–16
9. Wilson MR, Choudhury S, Goddard ME, O'Dea KP, Nicholson AG, Takata M: High tidal volume upregulates intrapulmonary cytokines in an *in vivo* mouse model of ventilator-induced lung injury. J Appl Physiol 2003; 95:1385–93
10. Webb HH, Tierney DF: Experimental pulmonary edema due to intermittent positive pressure ventilation with high inflation pressures: Protection by positive end-expiratory pressure. Am Rev Respir Dis 1974; 110:556–65
11. Bregeon F, Roch A, Delpierre S, Ghigo E, Autillo-Touati A, Kajikawa O, Martin TR, Pugin J, Portugal H, Auffray JP, Jammes Y: Conventional mechanical ventilation of healthy lungs induced pro-inflammatory cytokine gene transcription. Respir Physiol Neurobiol 2002; 132:191–203
12. Caruso P, Meireles SI, Reis LF, Mauad T, Martins MA, Deheinzelin D: Low tidal volume ventilation induces proinflammatory and profibrogenic response in lungs of rats. Intensive Care Med 2003; 29:1808–11
13. Gajic O, Dara SI, Mendez JL, Adesanya AO, Festic E, Caples SM, Rana R, St Sauver JL, Lymp JF, Afessa B, Hubmayr RD: Ventilator-associated lung injury in patients without acute lung injury at the onset of mechanical ventilation. Crit Care Med 2004; 32:1817–24
14. 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
15. Fernandez-Perez ER, Keegan MT, Brown DR, Hubmayr RD, Gajic O: Intraoperative tidal volume as a risk factor for respiratory failure after pneumonectomy. ANESTHESIOLOGY 2006; 105:14–8
16. Michelet P, D'Journo XB, Roch A, Doddoli C, Marin V, Papazian L, Decamps I, Bregeon F, Thomas P, Auffray JP: Protective ventilation influences systemic inflammation after esophagectomy: A randomized controlled study. ANESTHESIOLOGY 2006; 105:911–9
17. Lee PC, Helsmoortel CM, Cohn SM, Fink MP: Are low tidal volumes safe? Chest 1990; 97:430–4
18. Choi G, Wolthuis EK, Bresser P, Levi M, van der PT, Dzoljic M, Vroom MB, Schultz MJ: Mechanical ventilation with lower tidal volumes and positive end-expiratory pressure prevents alveolar coagulation in patients without lung injury. ANESTHESIOLOGY 2006; 105:689–95
19. Choi G, Schultz MJ, van Till JW, Bresser P, Van Der Zee JS, Boermeester MA, Levi M, van der Poll T: Disturbed alveolar fibrin turnover during pneumonia is restricted to the site of infection. Eur Respir J 2004; 24:786–9
20. Maris NA, de Vos AF, Dessing MC, Spek CA, Lutter R, Jansen HM, Van Der Zee JS, Bresser P, van der PT: Antiinflammatory effects of salmeterol after inhalation of lipopolysaccharide by healthy volunteers. Am J Respir Crit Care Med 2005; 172:878–84
21. Rijneveld AW, Florquin S, Bresser P, Levi M, De WV, Lijnen R, Van Der Zee JS, Speelman P, Carmeliet P, van der Poll T: Plasminogen activator inhibitor type-1 deficiency does not influence the outcome of murine pneumococcal pneumonia. Blood 2003; 102:934–9
22. Bresser P, Out TA, van Alphen L, Jansen HM, Lutter R: Airway inflammation in nonobstructive and obstructive chronic bronchitis with chronic haemophilus influenzae airway infection: Comparison with noninfected patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000; 162:947–52
23. van Nieuwenhuijze AE, van Lopik T, Smeenk RJ, Aarden LA: Time between onset of apoptosis and release of nucleosomes from apoptotic cells: putative implications for systemic lupus erythematosus. Ann Rheum Dis 2003; 62:10–4
24. Benjamini Y, Hochberg Y: Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Statist Soc B 2007; 57:289–300
25. D'Angelo E, Pecchiari M, Saetta M, Balestro E, Milic-Emili J: Dependence of lung injury on inflation rate during low-volume ventilation in normal open-chest rabbits. J Appl Physiol 2004; 97:260–8
26. Dunn I, Pugin J: Mechanical ventilation of various human lung cells *in vitro*: Identification of the macrophage as the main producer of inflammatory mediators. Chest 1999; 116:95S–7S
27. Dos Santos CC, Han B, Andrade CF, Bai X, Uhlig S, Hubmayr R, Tsang M, Lodyga M, Keshavjee S, Slutsky AS, Liu M: DNA microarray analysis of gene expression in alveolar epithelial cells in response to TNF-α, LPS and cyclic stretch. Physiol Genomics 2004; 19:331–42
28. Hammerschmidt S, Kuhn H, Grasenack T, Gessner C, Wirtz H: Apoptosis and necrosis induced by cyclic mechanical stretching in alveolar type II cells. Am J Respir Cell Mol Biol 2004; 30:396–402
29. Martin TR, Hagimoto N, Nakamura M, Matute-Bello G: Apoptosis and epithelial injury in the lungs. Proc Am Thorac Soc 2005; 2:214–20
30. Pirttikangas CO, Salo M, Mansikka M, Gronroos J, Pulkki K, Peltola O: The influence of anaesthetic technique upon the immune response to hysterectomy: A comparison of propofol infusion and isoflurane. Anaesthesia 1995; 50:1056–61
31. Crozier TA, Muller JE, Quittkat D, Sydow M, Wuttke W, Kettler D: Effect of anaesthesia on the cytokine responses to abdominal surgery. Br J Anaesth 1994; 72:280–5
32. Stuber F, Wrigge H, Schroeder S, Wetegrove S, Zinserling J, Hoeft A, Putensen C: Kinetic and reversibility of mechanical ventilation-associated pulmonary and systemic inflammatory response in patients with acute lung injury. Intensive Care Med 2002; 28:834–41
33. 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
34. D'Angelo E, Pecchiari M, Gentile G: Dependence of lung injury on surface tension during low-volume ventilation in normal open-chest rabbits. J Appl Physiol 2007; 102:174–82
35. Duggan M, McCaul CL, McNamara PJ, Engelberts D, Ackerley C, Kavanagh BP: Atelectasis causes vascular leak and lethal right ventricular failure in uninjured rat lungs. Am J Respir Crit Care Med 2003; 167:1633–40
36. Pelosi P, Rocco PR: Airway closure: The silent killer of peripheral airways. Crit Care 2007; 11:114–5
37. Chu EK, Whitehead T, Slutsky AS: Effects of cyclic opening and closing at low- and high-volume ventilation on bronchoalveolar lavage cytokines. Crit Care Med 2004; 32:168–74
38. Wrigge H, Zinserling J, Stuber F, von Spiegel T, Hering R, Wetegrove S, Hoeft A, Putensen C: Effects of mechanical ventilation on release of cytokines into systemic circulation in patients with normal pulmonary function. ANESTHESIOLOGY 2000; 93:1413–7
39. Wrigge H, Uhlig U, Zinserling J, Behrends-Callsen E, Ottersbach G, Fischer M, Uhlig S, Putensen C: The effects of different ventilatory settings on pulmonary and systemic inflammatory responses during major surgery. Anesth Analg 2004; 98:775–81
40. Wrigge H, Uhlig U, Baumgarten G, Menzenbach J, Zinserling J, Ernst M, Dromann D, Welz A, Uhlig S, Putensen C: Mechanical ventilation strategies and inflammatory responses to cardiac surgery: A prospective randomized clinical trial. Intensive Care Med 2005; 31:1379–87
41. Reis MD, Gommers D, Struijs A, Dekker R, Mekel J, Feelders R, Lachmann B, Bogers AJ: Ventilation according to the open lung concept attenuates pulmonary inflammatory response in cardiac surgery. Eur J Cardiothorac Surg 2005; 28:889–95
42. Zupancich E, Paparella D, Turani F, Munch C, Rossi A, Massaccesi S, Ranieri VM: 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
43. Koner O, Celebi S, Balci H, Cetin G, Karaoglu K, Cakar N: Effects of protective and conventional mechanical ventilation on pulmonary function and systemic cytokine release after cardiopulmonary bypass. Intensive Care Med 2004; 30:620–6
44. D'Angelo E, Pecchiari M, Della VP, Koutsoukou A, Milic-Emili J: Effects of mechanical ventilation at low lung volume on respiratory mechanics and nitric oxide exhalation in normal rabbits. J Appl Physiol 2005; 99:433–44