Lung protective mechanical ventilation
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Chapter 9

Mechanical Ventilation with Lower Tidal Volumes and PEEP Prevents Pulmonary Inflammation in Patients without Preexisting Lung Injury

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Abstract

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

**Methods:** Patients scheduled for an elective surgical procedure (lasting ≥ 5 hours) were randomized to mechanical ventilation with either higher tidal volumes of 12 ml/kg predicted body weight (PBW) and no PEEP or lower tidal volumes of 6 ml/kg PBW and 10 cmH₂O PEEP. After induction of anesthesia and 5 hours 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-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, interleukin–1α, interleukin–1β, interleukin–6, macrophage inflammatory protein–1α and macrophage inflammatory protein–1β in the bronchoalveolar lavage fluid were not affected by mechanical ventilation. Plasma levels of interleukin–6 and interleukin–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.
Introduction

Mechanical ventilation (MV) may aggravate pulmonary inflammation which may be a factor in the additional morbidity/mortality associated with non–protective forms of MV [1,2]. Indeed, MV with lower tidal volumes (VT) 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. demonstrated a reduction in the number of polymorphonuclear cells and pro–inflammatory mediators in the bronchoalveolar lavage fluid (BALF) with a lung–protective MV strategy as compared to conventional MV in patients with ARDS [4]. In addition, lung–protective MV attenuated systemic levels of inflammatory mediators [3,4], which may be of importance for clinical outcome since higher systemic levels of these mediators were associated with higher multi–organ 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 earlier studies in animals demonstrated that MV with higher VT causes pulmonary inflammation and functional injury [8–10], the clinical implications of these studies are unclear because VTs in these studies were unphysiologically large. Using a more physiological VT (10 ml/kg) and no PEEP (zero end expiratory pressure, ZEEP) demonstrated that MV for 6 h can induce a pro-inflammatory reaction in non-injured lungs [11]. Even MV for 1 h with lower tidal volumes (6 ml/kg) and ZEEP resulted in a pro-inflammatory and pro-fibrogenic response in normal rats [12]. Deleterious effects of higher VT in patients without preexisting lung injury, however, have been suggested by retrospective studies [13–15]. Fernandez et al. demonstrated that higher intra–operative VTs are more associated with respiratory failure after pneumonectomy [15]. Protective MV with lower VTs and PEEP during esophagectomy resulted in a decrease in systemic pro-inflammatory response, improved lung function and earlier extubation [16]. Higher VT in a surgical intensive care unit was associated with more pulmonary infection, longer duration of intubation and longer length of stay on the intensive care unit as compared to lower VT [17].

The purpose of this study was to investigate the effects of short–term (i.e., for 5 hours) MV on pulmonary inflammation and apoptosis. A randomized controlled trial was performed comparing two different mechanical ventilation strategies in patients without preexisting lung injury, who were scheduled for a major surgical procedure.
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 for a surgical procedure of ≥ 5 hours, and all involved physicians (surgeon, anesthesiologist, pulmonologist) consented with the study procedures. Exclusion criteria consisted of any severe lung disease (chronic obstructive pulmonary disease requiring medication, lung fibrosis, pneumonia, acute lung injury (ALI)/ARDS, pulmonary thromboembolism, previous pneumonectomy or lobectomy) and/or 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/kg/h), 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 mechanical ventilation, 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 pre-sealed envelope; patients were randomized to mechanical ventilation with either tidal volumes of 12 ml/kg predicted bodyweight ([PBW] high Vτ, HVτ) and ZEEP or 6 ml/kg PBW (low Vτ, LVτ) and 10 cmH₂O PEEP. The PBW of male patients was calculated as equal to 50 + 0.91 (centimeters of height – 152.4); that of female patients 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 on patient’s safety. If the surgical procedure exceeded 5 hours, 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 mechanical ventilation in the right middle lobe or lingula, the second performed in the contralateral lung 5 hours thereafter, either peri-operatively 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 pre-warmed saline were instilled and aspirated immediately with low suction (recovery 71 ± 18.4 ml). Arterial blood samples were drawn prior to 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 cytopsin preparation for differential counting.

Assays

Myeloperoxidase was determined by enzyme-linked immuno sorbent assay [22]. BALF–levels of human neutrophil elastase were assessed with a sandwich-type enzyme-linked immuno sorbent assay (Hycult biotechnology, Uden, the Netherlands). The detection limit of the assay was 4.0 ng/ml. Tumor necrosis factor (TNF)–α, interleukin–1α, interleukin–6, interleukin–8, macrophage inflammatory protein–1α, and macrophage inflammatory protein–1β were measured by enzyme-linked immuno sorbent assay (TNF-α, interleukin–6, interleukin–8, Sanquin, Amsterdam, the Netherlands; interleukin–1α, macrophage inflammatory protein–1α, macrophage inflammatory protein–1β, R&D Systems, Minneapolis, MN). Nucleosomes were measured by enzyme-linked immuno sorbent assay as described previously with slight modifications [23]. One unit (U) 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 Student’s t test, Mann-Whitney U test, or χ² 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 6 time points per patient. The fixed effects were hour of MV (0 – 5) and MV-group (LV/PEEP or HV/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 samples comparing t = 5 versus t = 0 hours. 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 (figure 1).

![Diagram](image.png)

**Figure 1** CONSORT diagram. BAL = bronchoalveolar lavage; HV,/ZEEP = tidal volumes of 12 ml/kg predicted bodyweight and no positive end-expiratory pressure; LV,/PEEP = tidal volumes of 6 ml/kg predicted body weight and 10 cmH2O positive end-expiratory pressure; MV = mechanical ventilation

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).
Table 1: Baseline characteristics of patients

<table>
<thead>
<tr>
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<th>LV-/PEEP (n = 21)</th>
<th>HV-/ZEEP (n = 19)</th>
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<tbody>
<tr>
<td>Age, mean ± SD, yr</td>
<td>62 ± 9.8</td>
<td>61 ± 9.3</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>14 (67)</td>
<td>14 (74)</td>
</tr>
<tr>
<td>ASA, median (range)</td>
<td>2 (1-4)</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>Height, mean ± SD, cm</td>
<td>176 ± 8.7</td>
<td>174 ± 10.0</td>
</tr>
<tr>
<td>Weight, mean ± SD, kg</td>
<td>79 ± 14.4</td>
<td>76 ± 13.7</td>
</tr>
<tr>
<td>Tobacco use, n (%)</td>
<td>9 (43)</td>
<td>6 (32)</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whipple’s Procedure*</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Laparoscopic Radical Prostatectomy</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Hemihepatectomy</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Retropitoneal Tumor Resection</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total Pancreatectomy</td>
<td>1</td>
<td></td>
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</tbody>
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*Whipple’s procedure is a pancreatico-duodenectomy. †The open prostatectomy was performed after an initial laparoscopic approach.

Beside from the mechanical ventilator settings (Vt, 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-/PEEP group as compared to 4.86 [95% CI: 4.61 – 5.12] in the HV-/ZEEP group (p < 0.001). Accordingly, pH was significantly lower in the LV-/PEEP group (7.36 [95% CI: 7.34 – 7.38] as compared to the HV-/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 mechanical ventilation (figure 2). Perioperative hemodynamic parameters, including number of patients being transfused and the number of transfusions (red blood cells and plasma) were not different between the two ventilation groups (table 2).

Table 2: Peri-operative parameters

<table>
<thead>
<tr>
<th></th>
<th>LV-/PEEP (n = 21)</th>
<th>HV-/ZEEP (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV duration, mean ± SD, min</td>
<td>304 ± 35</td>
<td>308 ± 52</td>
</tr>
<tr>
<td>Blood loss, median [IQR], ml</td>
<td>1550 [800-2325]</td>
<td>1000 [463-1675]</td>
</tr>
<tr>
<td>Number of patients receiving RBC (%)</td>
<td>7 (33.3)</td>
<td>5 (26.3)</td>
</tr>
<tr>
<td>RBC, median [IQR], units</td>
<td>0 [0-1.5]</td>
<td>0 [0-1]</td>
</tr>
<tr>
<td>Number of patients receiving plasma (%)</td>
<td>3 (14.3)</td>
<td>0 [0-0]</td>
</tr>
<tr>
<td>Transfused plasma, median [IQR], units</td>
<td>0 [0-0]</td>
<td>0 [0-0]</td>
</tr>
<tr>
<td>Colloids, median [IQR], l</td>
<td>0.5 [0.5-1.5]</td>
<td>0.5 [0.5-1.5]</td>
</tr>
<tr>
<td>Chrystalloids, median [IQR], l</td>
<td>4.5 [2.75-5.75]</td>
<td>4.0 [2.5-5.5]</td>
</tr>
<tr>
<td>Lowest Hb, mean ± SD, mmol/l</td>
<td>6.0 ± 1.2</td>
<td>6.2 ± 1.0</td>
</tr>
<tr>
<td>Lowest SBP, mean ± SD, mmHg</td>
<td>82 ± 9.6</td>
<td>87 ± 14.9</td>
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MV = mechanical ventilation; IQR = interquartile range; Hb = hemoglobin; HV-/ZEEP = higher tidal volumes/zero positive end-expiratory pressure; LV-/PEEP = lower tidal volumes/positive end-expiratory pressure; RBC = (transfused) red blood cells; SBP = systolic blood pressure. †Hemoglobin, 1 mmol/l = 1.61 g/dL.
Figure 2 Peri-operative parameters. Tidal volumes (V_t), respiratory rate (Respir. Rate), maximal airway pressures (P_max), PaO_2, PaCO_2 and pH in patients ventilated with lower tidal volumes and positive end-expiratory pressure (open symbols, n = 21) and patients ventilated with higher tidal volumes and no positive end-expiratory pressure (closed symbols, n = 19). MV = mechanical ventilation. Data are mean ± SD.

**Cellular composition of BALF, myeloperoxidase and elastase in BALF**

99% 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 hours of MV with higher tidal volumes and ZEEP as compared to baseline levels. Median myeloperoxidase levels increased from 2.80 [IQR: 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 HVT/ZEEP group (p = 0.013). No increase in myeloperoxidase and elastase levels were observed with the use of lower tidal volumes and PEEP (figure 3). Only for myeloperoxidase there was a statistically significant difference between the two ventilation strategies (p = 0.004).
Figure 3 Myeloperoxidase (MPO, A) and elastase (B) in bronchoalveolar lavage fluid recovered at baseline (t = 0) and after 5 hours (t = 5) from patients mechanically ventilated with 6 ml/kg and 10 cmH₂O positive end-expiratory pressure (LV₁/PEEP, open symbols) or with 12 ml/kg and zero positive end-expiratory pressure (HV₁/ZEEP, closed symbols). Horizontal lines represent median values. Wilcoxon signed-rank test: #p < 0.01 vs. t = 0. †p < 0.05 vs. t = 0. Mann-Whitney U test: ‡p < 0.01 between groups.

Protein levels of inflammatory mediators in the BALF and plasma

MV minimally influenced cytokine and chemokine levels in BALF (figure 4). BALF levels of TNF-α and interleukin-8 were influenced by the way patients were ventilated. TNF-α increased in the LV₁/PEEP group (p = 0.028), while interleukin-8 increased in the HV₁/ZEEP group (p = 0.015) after 5 hours of MV. Plasma levels of interleukin-6 and interleukin-8 did significantly increase during the surgical procedure, but this increase in cytokine generation was similar in both groups (figure 5).
Figure 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 hours (t = 5) from patients mechanically ventilated with 6 ml/kg and 10 cmH2O positive end–expiratory pressure (LV/PEEP, open symbols) or with 12 ml/kg and zero positive end–expiratory pressure (HV/ZEER, closed symbols). For all data points below the detection limit, the data point was given the an arbitrary value of 7.8 pg/mL. Horizontal lines represent median values. Wilcoxon signed–rank test: #p < 0.05 vs. t = 0.
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Figure 5 Plasma interleukin–6 (A) and interleukin–8 (B) recovered at baseline (t = 0) and after 5 hours (t = 5) from patients mechanically ventilated with 6 ml/kg and 10 cmH₂O positive end-expiratory pressure (LV₆/PEEP, open symbols) or with 12 ml/kg and zero positive end-expiratory pressure (HV₁₂/ZEEP, closed symbols). Horizontal lines represent median values. Wilcoxon signed-rank test: †p = 0.001, ‡p < 0.001 vs. t = 0.

Nucleosome levels in BALF and plasma

MV with higher tidal volumes and ZEEP caused an increase in BALF nucleosomes as compared to lower tidal volumes and 10 cmH₂O PEEP (p = 0.028; figure 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.

Figure 6 Nucleosome levels in bronchoalveolar lavage fluid (BALF, A) and plasma (B) recovered at baseline (t = 0) and after 5 hours (t = 5) from patients mechanically ventilated with 6 ml/kg and 10 cmH₂O positive end-expiratory pressure (LV₆/PEEP, open symbols) or with 12 ml/kg and zero positive end-expiratory pressure (HV₁₂/ZEEP, closed symbols). Horizontal lines represent median values. Wilcoxon signed-rank test: †p < 0.05, ‡p < 0.01 vs. t = 0. Mann-Whitney U test: &p < 0.05 between 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 (HV₁/ZEEP vs. LV₁/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 LV₁/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 versus 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 not significant any more (p > 0.05). There was only a trend for higher levels of BALF nucleosomes in the HV₁/ZEEP group after 5 h of MV (p = 0.084). There was no statistical significant difference between the two MV-strategies, regarding nucleosome levels in the BALF (p = 0.12). Also the level of TNF-α in the LV₁/PEEP group was not significantly increased after 5 h of MV (p = 0.084).

Discussion

In the present 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 appears that MV is a pro–inflammatory stimulus in non–injured lungs.

Myeloperoxidase (and also elastase) in the BALF is higher after 5 hours of MV with higher tidal volumes and ZEEP as compared to baseline levels. No increase in myeloperoxidase and elastase was seen after 5 hours of MV with lower tidal volumes and PEEP. This implies activation of polymorphonuclear cells, which were recruited to the pulmonary compartment or already present there. Higher concentrations of interleukin-8 in the BALF of patients ventilated with higher tidal volumes 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 interleukin-8 in the plasma is very high, thus there is a chemotactic gradient not favoring migration of neutrophils into the lung. Another possibility is that the neutrophils remained in the sub–epithelium, 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 felt that many patients would not consent to more invasive procedures peri- 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 hours 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 tidal volume ventilation and ZEEP as compared to 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 pro–apoptotic 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 intra–alveolar 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 anti–apoptotic strategies.

Both surgical stimuli and general anaesthesia are associated with increased plasma levels of pro–inflammatory markers [30,31]. In the present study we extended these findings by showing higher concentrations of interleukin–6 and interleukin–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 VT [32]. We hypothesize, however, that in patients with non–injured lungs there is no translocation of inflammatory mediators since much higher levels of inflammatory mediators in the systemic compartment were found as compared to the pulmonary compartment.

One limitation of our study is that our study protocol does not allow to differentiate the effects of lower tidal volumes from those by higher PEEP levels. We chose to combine
lower tidal volumes with PEEP and higher tidal volumes with no PEEP, because these settings result in similar maximum airway pressures. Recent studies in open chest rabbits demonstrated that MV with VTs of 8 – 12 ml/kg and ZEEP, may cause permanent mechanical alterations and histological damage to peripheral airways and inflammation in non-injured lungs [25,33]. Surfactant inactivation or depletion seems to play a major role during ventilation with tidal volumes 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 yet 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]. So, patients ventilated with ZEEP in our study could have gross atelectasis and peripheral airway injury, caused by tidal airway closure. Of notice, no recruitment maneuver was performed in both MV strategies.

Our data are different from those from previous studies in which MV strategies were investigated in patients with non-injured lungs undergoing surgery. Indeed, Wrigge et al. demonstrated that MV with VT of 15 ml/kg PBW and ZEEP for 1 h caused no consistent changes in plasma levels of measured cytokines [38]. 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 hours of MV, respectively. In other studies in which MV during or after cardiopulmonary bypass surgery was investigated, increased levels of pro-inflammatory mediators were reported, but not consistently [40-43]. Wrigge et al. showed that ventilation for 6 h with lower VT (6 ml/kg PBW) had no or only minor effect on systemic and pulmonary inflammatory responses in patients after cardiopulmonary bypass surgery as compared to higher VT (12 ml/kg) [40]. Only TNF-α levels in the BALF were significantly higher in the high VT group than in the low VT group. Koner et al. investigated different ventilation strategies during cardiopulmonary bypass and did not found any changes in systemic cytokine levels, postoperative pulmonary function or length of hospitalization with either MV–strategy [43]. Unfortunately, no pulmonary cytokine levels were measured in that study. Contrasting, 2 other studies did found 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 under 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 hours or the damage being mainly mechanical
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without any relevant inflammatory response [8,11,44]. MV with moderate tidal volumes and ZEEP can cause mechanical injury with alveolar-bronchiolar uncoupling [25]. So, 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 physiological 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) mechanical ventilation, 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 intra–operative V_{T}s 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 length of stay tended to be shorter [17]. Therefore, we would like to encourage the use of lower tidal volumes 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, abovementioned studies, including ours, have investigated patients who underwent major surgery. Inflammatory effects of the surgical procedure itself could not be excluded, but are equal to 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 hours. 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, mechanical ventilation for 5 h with lower tidal volumes and PEEP may limit pulmonary pro-inflammatory changes in patients with non–injured lungs during major surgery. Even during a relatively brief period of mechanical ventilation, patients will most likely benefit from lower tidal volumes and PEEP. The specific contribution of both lower tidal volumes and PEEP on the protective effects of the lung should be further investigated.
References

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