Lung epithelial injury markers are not influenced by use of lower tidal volumes during elective surgery in patients without preexisting lung injury

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Departments of 1Intensive Care Medicine, 3Anesthesiology, 4Pulmonology, and 6Experimental Immunology, Academic Medical Center, and 2Laboratory of Experimental Intensive Care and Anesthesiology (LEICA), Amsterdam, The Netherlands; 5Unit of Industrial Toxicology and Occupational Medicine, Department of Public Health, Catholic University of Louvain, Brussels, Belgium

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Determann RM, Wolthuis EK, Choi G, Bresser P, Bernard A, Lutter R, Schultz MJ. Lung epithelial injury markers are not influenced by use of lower tidal volumes during elective surgery in patients without preexisting lung injury. Am J Physiol Lung Cell Mol Physiol 294: L344–L350, 2008. First published December 14, 2007; doi:10.1152/ajplung.00268.2007.—Clara cell protein levels are elevated in plasma of individuals with mild or subclinical lung injury. We studied the influence of two mechanical ventilation strategies on local and systemic levels of Clara cell protein (CC16) and compared them with levels of soluble receptor for advanced glycation end products (sRAGE) and surfactant proteins (SP)-A and -D in patients undergoing elective surgery. Saved samples from a previously reported investigation were used for the study. Forty patients planned for elective surgery were randomized to mechanical ventilation with either a conventional tidal volume (VT) of 12 ml/kg without positive end-expiratory pressure (PEEP) or low VT of 6 ml/kg and 10 cmH2O PEEP. Plasma and bronchoalveolar lavage fluid (BALF) was collected directly after intubation and after 5 h of mechanical ventilation. While systemic levels of SP-A and SP-D remained unchanged, systemic levels of CC16 and sRAGE increased significantly in both groups after 5 h (< 0.001 for both). BALF levels of SP-A, SP-D, CC16, and sRAGE remained unaffected. No differences were found between the two mechanical ventilation strategies regarding any of the measured biological markers. In conclusion, systemic levels of CC16 and sRAGE are considerably smaller proteins than SP-A and SP-D, and sRAGE may be elevated even with short-term mechanical ventilation. Mechanical ventilation with lower tidal volumes and PEEP did not have a different effect on levels of biomarkers of lung epithelial injury compared with conventional mechanical ventilation.

Clara cell protein; receptor for advanced glycation end products; pulmonary inflammation

MECHANICAL VENTILATION has the potential to aggravate or even initiate lung injury. Indeed, results from numerous animal studies illustrate that mechanical ventilation strategies with conventional tidal volumes (VT) can cause pulmonary injury, a concept referred to as ventilator-induced lung injury (VILI; Refs. 7, 29). Data from two clinical trials confirm the existence of VILI by showing reduced morbidity and mortality with the use of lower VT in patients with ALI/ARDS (12). In contrast, there is little evidence supporting the use of lower VT in patients without ALI/ARDS partly because of a paucity of randomized controlled trial evidence on the best way to ventilate these patients. However, two retrospective studies suggest that long-term mechanical ventilation (i.e., for several days) with conventional VT contributes to development of ALI/ARDS in patients without preexisting lung injury (16, 17). Studies investigating the role of mechanical ventilation settings during short-term mechanical ventilation (i.e., for several hours, during surgery) in patients without ALI/ARDS have shown different results (22, 26, 36, 37). Although a recent paper has reported increased systemic cytokine levels in surgical patients ventilated with conventional VT compared with patients ventilated at lower VT (26), three earlier comparable studies were negative (22, 36, 37).

It can be hypothesized that mechanical ventilation affects the epithelial integrity of the lungs even with short-term mechanical ventilation. Several biological markers such as Clara cell protein (CC16), soluble receptor for advanced glycation end products (sRAGE), surfactant protein (SP)-A, and SP-D have been shown to be increased in sera of patients with ALI/ARDS (8, 14, 18, 19, 24, 35). The postulated mechanism for these increases is leakage to the circulation (13, 21). CC16 and sRAGE are considerably smaller proteins than SP-A and SP-D, and these can therefore be expected to enter the circulation more easily (13, 21). In this way, systemic levels of CC16 and sRAGE may be elevated even with short-term mechanical ventilation and thus serve as a biological marker of lung injury.

To investigate the effects of short-term mechanical ventilation on markers of lung epithelial injury in patients without preexisting lung injury, we evaluated plasma samples saved from participants of a previously published study (9). We studied whether levels of biomarkers would be affected differently by a lower VT strategy compared with a conventional mechanical ventilation strategy (i.e., with the use of conventional VT).

MATERIALS AND METHODS

Subjects. Patients planned for elective surgery with an estimated duration of 5 h or more were eligible for the study as described previously (9). Exclusion criteria consisted of any form of severe lung disease (chronic obstructive pulmonary disease requiring medication, lung fibrosis, pneumonia, ALI/ARDS, pulmonary thromboembolism,
previous pneumonectomy or lobectomy), use of immunosuppressive agents, and pregnancy.

**Study protocol.** All patients received anesthesia and were ventilated as described previously (9). However, for reasons of clarity, the protocol is summarized in short. Anesthesia consisted of 2–3 mg/kg of propofol (thereafter 6–12 mg·kg⁻¹·h⁻¹), 2–3 µg/kg fentanyl, and rocuronium administered intravenously together with 2.5 µg/ml epidurally administered bupivacaine (0.125%)-fentanyl. Immediately after endotracheal intubation, mechanical ventilation was initiated in the volume-controlled mode in all patients. Patients were randomized to ventilation with conventional 

\[ V_T \] (12 ml/kg ideal body weight (IBW)) without positive end-expiratory pressure (PEEP) (HVT/ZEEP) or ventilation with lower 

\[ V_T \] (6 ml/kg IBW) with 10 cmH₂O PEEP (LVT/PEEP). IBW was calculated as described before (1). Fraction of inspired oxygen (FIO₂) was set at 40%, inspiratory-to-expiratory ratio of 1.0 for 5 min. BAL was performed using 7 separate aliquots. Sampling was made in duplicate.

The detection limit of the assay was 2 ng/ml, and all measurements were made in duplicate.

As all patients received considerable amounts of fluids during surgery, we chose to study changes in plasma proteins with and without correction for hemodilution. Since α2M concentrations are hardly influenced during the acute phase (15), we chose to use α2M concentrations as a correction parameter using the correction factor α2M₂M /α2M₀. To correct the influx of albumin and α2M into the pulmonary compartment for the variation of these proteins in the systemic circulation, we calculated the quotient (Q) of BALF to plasma levels. To assess size selectivity, we calculated the relative coefficient of excretion [RCE = Qα2M/quotient albumin (Qalb); Ref. 5].

**Statistical analysis.** The power calculation was based on previous results from a study on systemic CC16 levels after lipopolysaccharide inhalation in healthy volunteers (25). In this study, inhalation of lipopolysaccharide induced a systemic increase of CC16 levels from a mean of 7.9 to 10.7 ng/ml. In volunteers who were treated with glucocorticosteroids before inhalation of lipopolysaccharide, this increase was significantly attenuated. In the present study, we expected the CC16 levels to increase in both groups. However, we hypothesized that mechanical ventilation with lower tidal volumes would attenuate this increase compared with conventional mechanical ventilation.

To detect a difference in increase of 2.5 ng/ml with a two-sided significance level of 0.05 and a power of 80%, 17 patients had to be included in each group.

Data are presented as medians with interquartile range (IQR) unless otherwise stated. Comparisons in baseline data between groups were made by the Mann-Whitney U test, χ² test, and Fisher exact test where appropriate. Perioperative ventilation parameters were analyzed by repeated-measures analysis of variance. Repeated-measures analysis of variance was also used to study changes in lung and plasma proteins over time. The effect of either mechanical ventilation strategy was analyzed by entering randomization group as a factor in the model. To overcome the nonnormal distribution of protein levels, the analysis was performed on log-transformed data. A two-tailed P value <0.05 was considered as statistically significant. Data were analyzed using SPSS version 12.02 (SPSS, Chicago, IL).

**Table 1. Baseline characteristics**

<table>
<thead>
<tr>
<th></th>
<th>HV₅/ZEEP (n = 19)</th>
<th>LVT/PEEP (n = 21)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD</td>
<td>61±9.5</td>
<td>62±9.8</td>
<td>0.60</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>14 (74)</td>
<td>14 (67)</td>
<td>0.63</td>
</tr>
<tr>
<td>History of smoking, n (%)</td>
<td>6 (32)</td>
<td>9 (43)</td>
<td>0.46</td>
</tr>
<tr>
<td>Ideal body weight, mean ± SD</td>
<td>69±10.6kg</td>
<td>70±9.5kg</td>
<td>0.67</td>
</tr>
<tr>
<td>Surgical procedure, n</td>
<td>8</td>
<td>5</td>
<td>0.24</td>
</tr>
<tr>
<td>Whipple procedure</td>
<td>8</td>
<td>0</td>
<td>0.91</td>
</tr>
<tr>
<td>Laparoscopic radical prostatectomy</td>
<td>3</td>
<td>6</td>
<td>0.38</td>
</tr>
<tr>
<td>Hemiepatectomy</td>
<td>3</td>
<td>6</td>
<td>0.38</td>
</tr>
<tr>
<td>Retropertioneal tumor resection</td>
<td>2</td>
<td>0</td>
<td>0.91</td>
</tr>
<tr>
<td>Total pancreatectomy</td>
<td>2</td>
<td>0</td>
<td>0.91</td>
</tr>
<tr>
<td>Colon conduit</td>
<td>1</td>
<td>0</td>
<td>0.91</td>
</tr>
<tr>
<td>Open prostatectomy</td>
<td>1</td>
<td>0</td>
<td>0.91</td>
</tr>
</tbody>
</table>

**HV₅/ZEEP, tidal volume of 12 ml/kg, 0 cmH₂O positive end-expiratory pressure (PEEP); LVT/PEEP, tidal volume of 6 ml/kg and 10 cmH₂O PEEP.**
RESULTS

Patients. Forty-six patients were included in the study. Reasons for surgery were radical prostatectomy \( (n = 11) \), Whipple procedure \( (n = 16) \), partial liver resection \( (n = 10) \), and other abdominal surgery \( (n = 6) \). Six patients did not finish the study protocol due to unforeseeable early ending of surgery (i.e., surgery did not last long enough to reach \( t = 5 \) h). Of the remaining 40 patients, 19 patients were randomized to LV\(_T\)/PEEP mechanical ventilation and 21 patients to HV\(_T\)/ZEEP mechanical ventilation. The baseline characteristics were as presented before (9) but, for reasons of clarity, are shown in Table 1. Smoking history was comparable between the groups. None of the patients had chronic alcoholism.

Perioperative characteristics. After intubation, mechanical ventilation was initiated, and all patients were mechanically ventilated in a volume-controlled mode. Patients in the HV\(_T\)/ZEEP group were ventilated with 11.8 ± 0.6 ml/kg IBW, and patients in the LV\(_T\)/PEEP group with 5.9 ± 0.7 ml/kg IBW. Respiratory rate increased over time in the LV\(_T\)/PEEP group from 13 ± 1 to 17 ± 2 breaths/min. Peak inspiratory pressure did not change significantly over time and was not different between the two groups. During the operating procedure, the partial carbon dioxide pressure and pH were slightly higher and lower, respectively, in the LV\(_T\)/PEEP group as mentioned previously (9). The partial oxygen pressure was similar between both study groups. The pre- and postoperative fluid status is given in Table 2. During the operation, 12 patients (5

Table 2. Pre- and perioperative fluid status characteristics

<table>
<thead>
<tr>
<th></th>
<th>HV(_T)/ZEEP ( (n = 19) )</th>
<th>LV(_T)/PEEP ( (n = 21) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t = 0 ) h</td>
<td>Mean arterial pressure, mmHg</td>
<td>77±12</td>
<td>69±14</td>
</tr>
<tr>
<td></td>
<td>Heart rate, beats/min</td>
<td>78±14</td>
<td>69±13</td>
</tr>
<tr>
<td></td>
<td>CVD, mmHg</td>
<td>10±3</td>
<td>12±5</td>
</tr>
<tr>
<td>( t = 5 ) h</td>
<td>Crystalloids, ml</td>
<td>4,000±1,500</td>
<td>4,500±2,200</td>
</tr>
<tr>
<td></td>
<td>Colloids, ml</td>
<td>700±500</td>
<td>1,000±600</td>
</tr>
</tbody>
</table>

CVD, central venous pressure.

Fig. 1. Bronchoalveolar lavage fluid (top) and systemic (bottom) levels of Clara cell protein (CC16) and soluble receptor for advanced glycation end products (sRAGE) in both ventilator groups. Bars indicate median and interquartile range, open circles indicate outliers, and * indicates extreme outliers. Plasma levels on \( t = 5 \) h are corrected for hemodilution. HV\(_T\)/ZEEP, high tidal volume (VT)/zero end-expiratory pressure; LV\(_T\)/PEEP, low VT/positive end-expiratory pressure.
of the HV$_{1}$/ZEEP group and 7 of the LV$_{1}$/PEEP group; $P = 0.69$ received blood products (median 2, range 1–9) during surgery. The LV$_{1}$/PEEP group had a slightly lower mean arterial pressure on $t = 0$ h. The amount of colloids and crystalloids given during surgery was not different among both group ($P = 0.09$ and $P = 0.33$, respectively; Table 2).

**CC16 and sRAGE.** BALF CC16 and sRAGE levels did not change over time, and no differences were found between the two groups (Fig. 1). Systemic CC16 levels increased significantly over time ($P < 0.001$). The absolute values are shown in Fig. 1. The absolute increase was median 2.0 (IQR 0.3–4.7) ng/ml in the HV$_{1}$/ZEEP group, and 1.7 (0.5–3.7) ng/ml in the LV$_{1}$/PEEP group ($P = 0.87$ for the difference in increase between both groups). After correction for hemodilution, the increase was more pronounced [HV$_{1}$/ZEEP group, 2.8 (0.6–8.2) ng/ml; LV$_{1}$/PEEP, 4.8 (1.6–8.7) ng/ml; Fig. 1]. However, no differences were found in increases between the two groups ($P = 0.38$). After correction for hemodilution, systemic sRAGE levels also increased significantly after 5 h [HV$_{1}$/ZEEP, median 115 (IQR −85 to 456) pg/ml; LV$_{1}$/PEEP, 175 (91–670) pg/ml; $P < 0.001$; Fig. 1], but no significant differences were found between both study groups ($P = 0.14$).

**SP-A and SP-D.** Systemic SP-A and SP-D levels decreased significantly after 5 h of mechanical ventilation ($P < 0.001$ for SP-A and $P = 0.004$ for SP-D; data not shown). After correction for hemodilution, both SP-A [HV$_{1}$/ZEEP, median 43 (IQR 21–60) ng/ml vs. 28 (17–43) ng/ml; LV$_{1}$/PEEP, 30 (21–48) ng/ml vs. 29 (21–48) ng/ml; Fig. 2] and SP-D [HV$_{1}$/ZEEP, 2.4 (0.4–15.8) ng/ml vs. 4.2 (1.2–14.8) ng/ml; LV$_{1}$/PEEP, 5.2 (2.6–13.8) ng/ml vs. 6.6 (2.8–22.9) ng/ml; Fig. 2] were found to be unaltered over time, and no significant differences were found between the two mechanical ventilation groups.

**Albumin and α2M.** No differences were found in baseline plasma and BALF albumin levels between the study groups. Baseline systemic levels of α2M were significantly lower in the HV$_{1}$/ZEEP group [median 2.9 (IQR 2.5–3.1) mg/ml vs. 4.1 (3.3–5.8) mg/ml; $P < 0.001$]. Whereas BALF albumin and α2M levels did not change over the 5-h study period, both
systemic albumin and plasma α2M levels declined in both groups as a result of hemodilution (P < 0.001 for both; data not shown). The Qalb, Qu2M, and RCE increased over time, but these increases did not reach statistical significance, and no differences were observed between the two mechanical ventilation groups (Fig. 3).

**DISCUSSION**

The concept of VILI has been proven to be an important clinical entity in patients with ALI/ARDS; guidelines now strongly support the use of mechanical ventilation with lower VT (12). Retrospective clinical studies suggest that the use of large VT mechanical ventilation favor the development of lung injury in critically ill patients without ALI/ARDS (16, 17). Randomized studies on short-term mechanical ventilation during surgery, however, have shown inconclusive results (30). The present study shows that in patients without preexisting lung injury receiving mechanical ventilation for elective surgery, systemic levels of CC16 and sRAGE increase. No differences were, however, found between patients ventilated with conventional or lower tidal volumes.

We (9) recently reported changes in alveolar fibrin turnover favoring the use of lower tidal volumes in this cohort of mechanically ventilated patients. Alveolar fibrin deposition is a hallmark of pulmonary inflammation, resulting from activation of coagulation and inhibition of fibrinolysis (31). In this same cohort of patients, we here show both plasma CC16 and sRAGE levels to increase over time. However, we did not find any significant differences between the two ventilation groups, i.e., the use of a lower VT ventilation strategy did not prevent a rise in the levels of these biological markers. CC16 is a small protein of 16 kDa and has been used to detect lung injury in studies on subclinical lung injury (such as seen after exposure to smoke and after experimental inhalation of ozone or lipopolysaccharide; Refs. 3, 6, 25). Based on our previous results on fibrin turnover, we expected to find higher CC16 levels in the HVT/ZEEP group. Our negative findings may have been due to a limited sensitivity of the measurement. However, CC16 levels from the study that we used for our power calculation were also measured in EDTA anticoagulated plasma and by the same technique as in the present study. Furthermore, based on our calculation, we included a sufficient number of patients. The absolute difference we found in increases of CC16 levels between the two ventilation groups was much smaller than we expected.

An explanation for these findings may be found in the institution of 10 cmH2O of PEEP in the LVT/PEEP group. We applied 10 cmH2O of PEEP to achieve similar peak pressures. In this way, if any overdistention of lung tissue occurred in our patients, the effect may have been comparable between groups. Repetitive alveolar closing and reexpansion has been recognized as a mechanism of VILI (28a), although it has been challenged in the past. A study by Taskar et al. (34) showed that unjured lungs can tolerate 1 h of mechanical ventilation with negative end-expiratory airway pressure. However, although only temporary, changes in permeability were seen. Therefore, it can be argued that during 5 h of mechanical ventilation, repetitive alveolar closing and opening can indeed cause injury. Whereas this may have played an important role in causing the disturbed alveolar hemostasis in our previous study, it may have had little impact on transpulmonary transfer of pulmonary proteins. Experimental studies have shown that the effect of PEEP on formation of ventilator-induced pulmonary edema is comparable to the effect of high tidal volume when the end-inspiratory pressure is similar. Moreover, increasing PEEP, while maintaining tidal volume constant, can lead to formation of pulmonary edema. In a study on mechanical ventilation in rats by Lesur et al. (23), a 1.5- to 7-fold increase in systemic CC16 levels was found after a 2-h period of mechanical ventilation. Interestingly, in this study, both high levels of PEEP and conventional tidal volume were shown to be the major causes of increased blood CC16 levels. From this we may conclude that the use of 10 cmH2O of PEEP in the low tidal volume group could have accounted for the lack of differences between the two study groups.

![Fig. 3. Quotient of bronchoalveolar lavage fluid levels to systemic levels of albumin and α2-macroglobulin (α2M) and the relative coefficient of excretion (RCE; quotient α2M divided by quotient albumin). Bars indicate median and interquartile range.](http://ajplung.physiology.org/ by 10.220.33.5 on October 13, 2017)
An additional explanation may be that activation of coagulation resulting from mechanical ventilation with high VT and ZEEP is a process that precedes increases in pulmonary permeability. We have previously shown that HV_{VT)/ZEEP ventilation leads to higher levels of bronchoalveolar thrombin-antithrombin complexes (9). We suggest that within the first hour of mechanical stress applied to endothelial-epithelial membrane, intraalveolar coagulation is a physiological response to injury of the endothelial-epithelial membrane. It may also be hypothesized that the increased local coagulation activity prevents further barrier dysfunction of the endothelium/epithelium and therefore prevents further increases in systemic CC16 or sRAGE levels. In patients who underwent longer periods of mechanical ventilation, the injury may be more severe, and intraalveolar coagulation may no longer be sufficient to hold the barrier function intact, resulting in higher rates of pulmonary protein leakage. In the present study, mechanical ventilation may have been too brief to demonstrate a sufficient difference in barrier disruption to cause differences in systemic levels of CC16 between groups.

There was a small difference between the study groups with regard to arterial pH. Indeed, patients in the LV_{VT)/PEEP group had a slightly lower pH, which was caused by a higher partial carbon dioxide pressure. Based on results of an earlier publication by Sinclair et al. (33) on hypercapnic acidosis in a VILI model, we conclude that if hypercapnic acidosis could have influenced our results, inflammation in the LV_{VT)/PEEP group would have been attenuated. This would have resulted in less leakage and thus a larger difference between the two study groups. From this, we conclude that a possible difference between the two study groups was not masked by presence of hypercapnic acidosis.

Considering the results of experimental studies and earlier human studies, we hypothesize the production of CC16 was not upregulated during mechanical ventilation. It is even likely that alveolar CC16 levels may have dropped during the mechanical ventilation period. Indeed, animal studies by Lesur et al. (23) showed a decrease in local CC16, which was ascribed to increased leakage to the circulation. In a study by Uchida et al. (35), increased sRAGE levels were observed in an ALI rat model. The expression of RAGE could have been upregulated in our patients leading to higher local and consequently higher systemic levels. However, this hypothesis is not supported by our results of the measurements of sRAGE in BALF. Although we ascribe the increased CC16 and sRAGE levels to increased leakage to the circulation, we did not see an increase in pulmonary albumin or α2M levels. As CC16 is considerably smaller than albumin (16 vs. 69 kDa), and sRAGE is a little smaller (±48 kDa), these markers may pass the pulmonary barrier more easily, making them more sensitive for pulmonary injury than albumin or α2M.

Although we consider the increased CC16 and sRAGE levels an effect of injury to the alveolocapillary membrane, we could not determine whether this resulted from mechanical ventilation per se or from systemic inflammation due to surgery. Abdominal surgery is known to activate circulating neutrophils and to induce systemic inflammation (11). Moreover, transfusion of blood products may also have contributed to activation of systemic inflammatory cells and have caused subsequent lung injury. As such, these processes may have simply overshadowed any observable effects of differing mechanical ventilation strategies.

SP-A, SP-D, and α2M are relatively large proteins (650, 560, and 725 kDa, respectively). With developing lung injury, one may expect systemic levels of smaller proteins to increase earlier than those of larger proteins. Systemic levels of SP-A and SP-D are increased in patients with more advanced lung injury (such as seen in patients with ALI/ARDS or chronic lung fibrosis; Refs. 8, 14, 18, 19, 27). Moreover, systemic SP-D levels are associated with VILI in ARDS patients (14). As mentioned above, systemic levels of CC16 are increased in patients with milder forms of lung injury as well as in patients with ALI/ARDS (24). In our study, levels of SP-A and SP-D did not increase, and this is consistent with the clinical data as none of the patients developed clinical signs of ALI/ARDS.

The present study shows that elective surgery, blood transfusion, and mechanical ventilation, regardless of strategy, may all contribute to increased systemic levels of markers of lung epithelial injury. The use of lower VT does not necessarily attenuate these increases. It may well be that mechanical ventilation itself is potentially injurious in any scenario and that the goal needs to be to ventilate patients in the least injurious way possible. Prevention of overdistention by a too high PEEP may be just as important as prevention of overdistention by applying too high tidal volumes. Future studies may show us the effects of mechanical ventilation in patients without injured lungs who are put on a ventilator for longer periods of time and may reveal what settings of PEEP and VT are the least injurious. Critically ill patients needing mechanical ventilation on an intensive care unit may indeed benefit from lower VT mechanical ventilation. Therefore, clinical studies are warranted to study the effects of prolonged mechanical ventilation on pulmonary injury and patient outcome.

GRANTS

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REFERENCES


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LUNG INJURY MARKERS IN MECHANICAL VENTILATION


