Lung protective mechanical ventilation

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Chapter 10

Lung Epithelial Injury Markers Are not Influenced by Use of Lower Tidal Volumes during Elective Surgery in Patients without Preexisting Lung Injury

Rogier M. Determann, Esther K. Wolthuis, Goda Choi, Paul Bresser, Alfred Bernard, Rene Lutter and Marcus J. Schultz

Abstract

**Background:** Clara cell protein levels are elevated in plasma of individuals with mild or subclinical lung injury.

**Methods:** 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 ($V_t$) of 12 ml/kg predicted bodyweight (PBW) without positive end-expiratory pressure (PEEP) or low $V_t$ of 6 ml/kg PBW and 10 cmH$_2$O PEEP. Plasma and bronchoalveolar lavage fluid (BALF) was collected directly after intubation and after 5 hours of mechanical ventilation.

**Results:** While systemic levels of SP–A and SP–D remained unchanged, systemic levels of CC16 and sRAGE increased significantly in both groups after 5 hours ($p < 0.001$ 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.

**Conclusion:** Systemic levels of CC16 and sRAGE rise after 5 hours in patients receiving mechanical ventilation for elective surgery. Mechanical ventilation with lower tidal volumes and PEEP did not have a different effect on levels of biomarkers of lung epithelial injury as compared to conventional mechanical ventilation.
Introduction
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) [1,2]. Data from two clinical trials confirm the existence of ventilator-associated lung injury as the clinical counterpart of VILI by showing reduced morbidity and mortality with the use of lower VT in patients with acute lung injury (ALI) or its more severe form, the acute respiratory distress syndrome (ARDS) [3,4]. Based on these studies, guidelines now strongly support the use of lower VT in patients with ALI/ARDS [5]. 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 pre–existing lung injury [6,7]. 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 [8-11]. While a recent paper has reported increased systemic cytokine levels in surgical patients ventilated with conventional VT as compared with patients ventilated at lower VT [9], three earlier comparable studies were negative [8,10,11].

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 [12-17]. The postulated mechanism for these increases is leakage to the circulation [18,19]. 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 [18,19]. 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 pre–existing lung injury we evaluated plasma samples saved from participants of a previously published study [20]. We studied whether levels of biomarkers would be affected differently by a “lower VT” strategy as compared to a conventional mechanical ventilation strategy (i.e., with the use of conventional VT).
Methods

Subjects

Patients planned for elective surgery with an estimated duration of 5 hours or more were eligible for the study as described previously [20]. 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 [20]. However, for reasons of clarity it is summarized in short. Anesthesia consisted of 2–3 mg/kg of propofol (thereafter 6–12 mg/kg per hour), fentanyl 2–3 µg/kg and rocuronium administered intravenously together with epidurally administered bupivacaine (0.125%)–fentanyl (2.5 µg/ml). 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 predicted body weight [PBW]) without PEEP (HV/ZEEP) or ventilation with lower $V_T$ (6 ml/kg PBW) with 10 cmH2O PEEP (LV/PEEP). PBW was calculated as described before [3]. FiO2 was set at 40%, inspiratory to expiratory ratio at 1:2 and the respiratory rate was adjusted to maintain normocapnia. Shortly after the start of mechanical ventilation, patients underwent bronchoscopy–guided bronchoalveolar lavage (BAL) according to the guidelines of the American Thoracic Society in one lung. Five hours thereafter, a second BAL was performed in the contra–lateral lung. Prior to each BAL, blood samples were drawn into vacutainers containing ethylenediamine tetraacetic acid (EDTA) from an indwelling arterial sheath. Blood gas analysis was performed every hour. All study related protocols were carefully evaluated for safety aspects by the investigators. The protocol was approved by the Medical Ethical Committee of the Academic Medical Center and informed consent was obtained from every patient before entry in the study.

Collection and processing of samples

All patients were pre–oxygenated with FiO2 = 1.0 for 5 minutes. BAL was performed using 7 separate 20–ml aliquots of 0.9% sterile saline instilled into the right middle lobe or lingula. The first aliquot was discarded, the second aliquot was used for bacteriological studies and the third aliquot was used for measurement of biological markers. The average recovery was 5 ± 3 for the first, 8 ± 2 and 10 ± 3 ml for the third aliquot. BAL–fluid (BALF) and blood samples were immediately stored on ice until processing. BALF was centrifuged at 800 x g for 10 minutes and blood at 1500 x g for 10 minutes. Supernatants were collected and stored at −80°C.
Levels of albumin were determined by an immunoturbidimetric assay according to the instructions of the manufacturer (BN ProSpec, Dade Behring, Germany), levels of α2–macroglobulin (α2M) were determined by an ELISA as described previously [21]. CC16 was measured by an automated latex immunoassay [22] of which the accuracy was confirmed with an ELISA [23]. SP–A was measured by the method of Shimizu et al. [24].

SP–D was measured by an ELISA developed in our laboratory. In short, 96–well EIA plates (Corning Incorporated, NY, USA) were coated overnight at 4°C with 100 ng mouse anti–human SP–D antibody (Antibodyshop, Copenhagen, Denmark). After washing three times with 0.05% Tween 20 / phosphate buffered saline (PBS), 200 μL of 0.5%BSA/PBS was added as blocking agent for 2 hours. Samples and standards were diluted as appropriate in buffer containing 10 mM Hepes (Sigma-Aldrich, Zwijndrecht, the Netherlands), 150 mM NaCl, 0.5% Triton-X and 1% BSA, and added to the wells. The plates were incubated for 2 hours, washed 3 times and 50 ng of biotinylated mouse anti–human SP–D antibody (Antibodyshop) was added to each well. After incubation for 2 hours, the plates were incubated with streptavidin poly-HRP (Sanquin, Amsterdam, the Netherlands) for 30 minutes. After washing 3 times, sodium-acetate buffer (pH 5.5) containing 100 μg/ml tetramethylbenzidine (sigma-Aldrich) and 0.003% H2O2 was added and the color reaction was stopped by 2 M H2SO4. The spike recovery in plasma was 95-105%, the detection limit of the assay was 2 ng/ml and all measurements were made in duplicate.

Levels of sRAGE were determined by an ELISA developed in our laboratory. In short, 96–well plates pre–coated with rabbit anti–mouse immunoglobulin (Dakopatts, Copenhagen, Denmark) were coated overnight with 400 ng mouse anti–human RAGE antibody (R&D Systems, Minneapolis, Minnesota, USA). Calibrator (R&D Systems), controls and samples diluted as appropriate were added and incubated for 2 hours. Next, 40 ng of biotinylated goat anti-human RAGE antibody (R&D Systems) was added and incubated for another 2 hours. Streptavidin poly-HRP was added for 30 minutes. Finally, sodium-acetate buffer (pH 5.5) containing 100 μg/ml tetramethylbenzidine and 0.003% H2O2 was added and the color reaction was stopped by 2 M H2SO4. The spike recovery in plasma was 92-98% and the detection limit of the assay was 20 pg/ml. 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 [25] we choose to use α2M concentrations as a correction parameter using the correction factor [α2M]t=0 hours/[α2M]t=5 hours. 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 = Q_{a2M} / Q_{alb}) [26].

Statistical analysis

The power calculation was based on previous results from a study on systemic CC16 levels after lipopolysaccharide inhalation in healthy volunteers [27]. In this study, inhalation of lipopolysaccharide induced a systemic increase of CC16 levels from a mean of 7.9 ng/ml to 10.7 ng/ml. In volunteers who were treated with glucocorticosteroids prior to 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 as compared to 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, Chi-square test and Fisher exact test where appropriate. Peri-operative 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 non-normal 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 Inc, Chicago, Illinois, USA).

Results

Patients

Forty-six patients were included in the study. Reasons for surgery were radical prostatectomy (n = 14), 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 hours). Of the remaining 40 patients, 19 patients were randomized to LV{sub}T/PEEP mechanical ventilation and 21 patients to HV{sub}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.
Lung epithelial injury markers are not influenced by use of lower tidal volumes

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristics</th>
<th>HV_/ZEEP (n = 19)</th>
<th>LV_/PEEP (n = 21)</th>
<th>p–value</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, number (%)</td>
<td>14 (74)</td>
<td>14 (67)</td>
<td>0.63</td>
</tr>
<tr>
<td>History of smoking, number (%)</td>
<td>6 (32)</td>
<td>9 (43)</td>
<td>0.46</td>
</tr>
<tr>
<td>Predicted body weight, mean ± SD, kg</td>
<td>69 ± 10.6</td>
<td>70 ± 9.5</td>
<td>0.67</td>
</tr>
<tr>
<td>Surgical procedure, n</td>
<td></td>
<td></td>
<td>0.24</td>
</tr>
<tr>
<td>Whipple procedure</td>
<td>8</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Laparoscopic radical prostatectomy</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Hemihepatectomy</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Retroperitoneal tumor resection</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total pancreatectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon conduit</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open prostatectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HV_/ZEEP: tidal volume of 12 ml/kg, 0 cmH2O positive end expiratory pressure; LV_/PEEP: tidal volume of 6 ml/kg and 10 cmH2O positive end expiratory pressure

Peri–operative characteristics

After intubation mechanical ventilation was initiated and all patients were mechanically ventilated in a volume–controlled mode. Patients in the HV_/ZEEP group were ventilated with 11.8 ± 0.6 ml/kg PBW, patients in the LV_/PEEP group with 5.9 ± 0.7 ml/kg PBW. Respiratory rate increased over time in the LV_/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_/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.

<table>
<thead>
<tr>
<th>Table 2 Pre- and perioperative fluid status characteristics</th>
<th>HV_/ZEEP (n = 19)</th>
<th>LV_/PEEP (n = 21)</th>
<th>p–value</th>
</tr>
</thead>
<tbody>
<tr>
<td>t = 0 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>77 ± 12</td>
<td>69 ± 14</td>
<td>0.04</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>78 ± 14</td>
<td>69 ± 13</td>
<td>0.29</td>
</tr>
<tr>
<td>CVD (mmHg)</td>
<td>10 ± 3</td>
<td>12 ± 5</td>
<td>0.28</td>
</tr>
<tr>
<td>t = 5 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crystalloids (ml)</td>
<td>4000 ± 1500</td>
<td>4500 ± 2200</td>
<td>0.33</td>
</tr>
<tr>
<td>Colloids (ml)</td>
<td>700 ± 500</td>
<td>1000 ± 600</td>
<td>0.09</td>
</tr>
</tbody>
</table>

HV_/ZEEP: tidal volume of 12 ml/kg, 0 cmH2O positive end expiratory pressure; LV_/PEEP: tidal volume of 6 ml/kg and 10 cmH2O positive end expiratory pressure; CVD: central venous pressure

During the operation, twelve patients (5 of the HV_/ZEEP group and 7 of the LV_/PEEP group, p = 0.69) received blood products (median 2, range 1–9) during surgery. The LV_/PEEP group had a slightly lower mean arterial pressure on t = 0 hours. 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. Systemic CC16 levels increased significantly over time (p < 0.001). The absolute values are shown in figure 1. The absolute increase was median 2.0
(IQR 0.3–4.7) ng/ml in the HVt/ZEEP group and 1.7 (0.5–3.7) in the LVt/PEEP group (p = 0.87 for the difference in increase between both groups). After correction for hemodilution the increase was more pronounced (HVt/ZEEP group, 2.8 (0.6–8.2) ng/ml; LVt/PEEP, 4.8 (1.6–8.7) ng/ml). 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 hours (HVt/ZEEP, median 115 (IQR -85 to 456) pg/ml; LVt/PEEP, 175 (91–670) pg/ml; p < 0.001, figure 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 hours 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 (HVt/ZEEP: median 43, IQR [21–60] ng/ml vs. 28 [17–43] ng/ml; LVt/PEEP: 30 [21–48] ng/ml vs. 29 [21–48] ng/ml; **figure 2**) and SP–D (HVt/ZEEP: median 2.4, IQR [0.4–15.8] ng/ml vs. 4.2 [1.2–14.8] ng/ml; LVt/PEEP: 5.2 [2.6–13.8] ng/ml vs. 6.6 [2.8–22.9] ng/ml; **figure 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 HVt/ZEEP group (median 2.9, IQR [2.5–3.1] mg/ml vs. 4.1 [3.3–5.8] mg/ml; p < 0.001). While BALF albumin and α2M levels did not change over the 5–hour 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, Qα2M and RCE increased over time, but these increases did not reach statistical significance and no differences were observed between the two mechanical ventilation groups (**figure 3**).
Figure 1 Bronchoalveolar lavage fluid (upper graphs) and systemic (lower graphs) 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. The open circles indicate outliers and the "*" indicates extreme outliers. Plasma levels on t = 5 hours are corrected for hemodilution. HVt/ZEEP, high tidal volume (VT)/zero end–expiratory pressure; LVt/PEEP, low VT/positive end–expiratory pressure.
*Figure 2* Bronchoalveolar lavage fluid (upper graphs) and systemic (lower graphs) levels of surfactant protein A (SP–A) and surfactant protein D (SP–D) in both ventilator groups. Bars indicate median and interquartile range. Plasma levels on t = 5 hours are corrected for hemodilution.

*Figure 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 / quotient albumin). Bars indicate median and interquartile range.
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 $V_T$ [5]. Retrospective clinical studies suggest that the use of large $V_T$ mechanical ventilation favor the development of lung injury in critically ill patients without ALI/ARDS [6,7]. Randomized studies on short–term mechanical ventilation during surgery, however, have shown inconclusive results [28]. The present study shows that in patients without pre–existing 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 recently reported changes in alveolar fibrin turnover favoring the use of lower tidal volumes in this cohort of mechanically ventilated patients [20]. Alveolar fibrin deposition is a hallmark of pulmonary inflammation, resulting from activation of coagulation and inhibition of fibrinolysis [29]. 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 study groups, i.e., the use of a lower $V_T$ 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 sub–clinical lung injury (such as seen after exposure to smoke, and after experimental inhalation of ozone or lipopolysaccharide) [27,30,31]. Based on our previous results on fibrin turnover we expected to find higher CC16 levels in the HV$_T$/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 anti–coagulated 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 cmH$_2$O of PEEP in the LV$_T$/PEEP group. We applied 10 cmH$_2$O 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 re–expansion has been recognized as a mechanism of VILI [32] although it has been challenged in the past. A study by Taskar et al. [33] showed that uninjured lungs can tolerate one hour 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 hours of mechanical ventilation, repetitive alveolar closing and opening can indeed cause injury. While 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. [34], a 1.5 to 7-fold increase in systemic CC16 levels was found after a 2 hour 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.

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 HVt/ZEEP ventilation leads to higher levels of bronchoalveolar thrombin–antithrombin complexes. We suggest that within the first hours of mechanical stress applied to endothelial–epithelial membrane, intra–alveolar 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 undergo longer periods of mechanical ventilation the injury may be more severe and intra–alveolar coagulation may not 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 LVt/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. [35] on hypercapnic acidosis in a VILI model, we conclude that if hypercapnic acidosis could have influenced our results, inflammation in the LVt/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 up–regulated 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. [34] showed a decrease in local CC16 which was ascribed to increased leakage to the circulation. In a study by Uchida et al. [17] increased sRAGE levels were observed in an acute lung injury rat model. The expression of
RAGE could have been up–regulated in our patients leading to higher local levels and consequently higher systemic levels. However, this hypothesis is not supported by our results of the measurements of sRAGE in BALF. While 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.

While 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 [36]. 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) [12-15,37]. Moreover, systemic SP–D levels are associated with VILI in ARDS–patients [13]. 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 [16]. 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.
References

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