Exhaled Breath Condensate in Mechanically Ventilated Brain-injured Patients with No Lung Injury or Sepsis

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ABSTRACT

Background: The inflammatory influence of prolonged mechanical ventilation in uninjured lungs remains a matter of controversy and largely unexplored in humans. The authors investigated pulmonary inflammation by using exhaled breath condensate (EBC) in mechanically ventilated, brain-injured patients in the absence of acute lung injury or sepsis and explored the potential influence of positive end-expiratory pressure (PEEP).

Methods: Inflammatory EBC markers were assessed in 27 mechanically ventilated, brain-injured patients with neither acute lung injury nor sepsis and in 12 healthy and 8 brain-injured control subjects. Patients were ventilated with 8 ml/kg during zero end-expiratory pressure (ZEEP group, n = 12) or 8 cm H_2O PEEP (PEEP group, n = 15). EBC was collected on days 1, 3, and 5 of mechanical ventilation to measure pH; interleukins (IL)-10, 1β, 6, 8, and 12p70; and tumor necrosis factor-α.

Results: EBC pH was lower, whereas IL-1β and tumor necrosis factor-α were greater in both patient groups compared with either control group; IL-6 was higher, whereas IL-10 and IL-12p70 were sporadically higher than in healthy control subjects; no differences were noted between the two patient groups, except for IL-10, which decreased by day 5 during PEEP. Leukocytes, soluble IL-6, and soluble triggering receptor expressed on myeloid cells-1 in blood were constantly higher during zero end-expiratory pressure; EBC cytokines appeared mostly related to soluble IL-8 and inversely related to soluble triggering receptor expressed on myeloid cells-1.

Conclusions: In brain-injured, mechanically ventilated patients with neither acute lung injury nor sepsis, EBC markers appear to indicate the presence of subtle pulmonary inflammation that is mostly unaffected by PEEP. There is evidence for a systemic inflammatory response, especially in patients during zero end-expiratory pressure.

What We Already Know about This Topic

- Mechanical ventilation can cause inflammation in normal animal lungs.

What This Article Tells Us That Is New

- Exhaled breath condensate (EBC) pH and inflammatory mediators in ventilated, brain-injured patients with no apparent lung disease were measured. These patients had EBCs that were more acidic and contained higher tumor necrosis factor-α and interleukin-1β concentrations compared with both healthy and brain-injured control subjects.

MANY brain-injured patients require admission to intensive care units (ICUs) and support with mechanical ventilation (MV) for coma management and neuroprotection. Although these patients may not have initial evidence of clinically relevant lung injury, pulmonary complications are the leading cause of nonneurologic morbidity. Mechanical ventilation itself may contribute to lung injury in this population, either by causing structural and functional lung damage alone or by affecting the subclinical initial predisposing factor. Indeed, experimental[1] and clinical trials[2,3] have shown that MV may lead to damage in both previously healthy and diseased lungs, a process known as ventilator-induced lung injury.[4,5] One of the mechanisms of

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ventilator-induced lung injury is increased pulmonary inflammation in response to repeated mechanical stimuli, a phenomenon called biotrauma,[6,7] which is mediated at least in part by proinflammatory cytokines.

A profound local and systemic inflammatory response associated with brain damage may contribute to pulmonary injury. The local (*i.e.*, brain) inflammatory response is characterized by cellular infiltration and the release of several immune mediators, including expression of adhesion molecules, cytokines, and growth factors, resulting in neurodegeneration or cell death.[8–10] However, the intracerebral events frequently have systemic effects, including those on lung tissue,[11] as evidenced by ultrastructural damage to type II pneumocytes.[12] Traumatic brain injury in animals enhances lung damage caused by subsequent injurious insults, such as mechanical stretch or ischemia–reperfusion injury.[13]

We have recently obtained clinical and biochemical evidence for subtle lung injury in brain-injured patients, including abnormal respiratory mechanics[14] and reduced pulmonary endothelial-bound angiotensin-converting enzyme activity (S.E.O., unpublished data, 2009, on the presence of pulmonary endothelial functional alteration in the mechanically ventilated human lung in the absence of acute lung injury or sepsis and the protective effect of moderate positive end-expiratory pressure application), suggestive of endothelial dysfunction.[15,16] Both mechanical and endothelial alterations could be attenuated by moderate levels of positive end-expiratory pressure (PEEP), indicating the potential role of ventilation strategy and providing rationale for the current study.

Whereas the deleterious MV effects on injured lung in the setting of acute lung injury (ALI) are well established, there is greater controversy regarding the inflammatory aspects of ventilation on healthy or uninjured lungs, both in the experimental and human setting. We reasoned that this issue could be explored in brain-injured patients with no clinical signs of lung injury but requiring prolonged MV for nonpulmonary reasons.

In search of mechanisms underlying respiratory mechanical and microvascular dysfunction, this study explored pulmonary and systemic inflammation in brain-injured, mechanically ventilated patients with neither ALI[17] nor sepsis.[18] Based on previous ALI studies and recent recommendations from the American Thoracic Society/European Respiratory Society task force on exhaled breath condensate (EBC), pulmonary inflammation was monitored by measuring EBC pH and cytokine concentrations.[19,20] Our primary aim was to define kinetics of EBC pH and cytokines during the first 5 days of MV. In addition, we evaluated the effect of PEEP on pulmonary inflammation, as reflected by EBC parameters. Finally, we explored the effect of PEEP on measures of systemic inflammation and potential relationships of the latter with EBC markers. Our study hypotheses were: (1) subclinical lung inflammation is present in brain-injured mechanically ventilated patients without manifest ALI or sepsis and may be detected by means of EBC-obtained indices; and (2) this preclinical inflammation is attenuated by moderate PEEP application.

Materials and Methods

Subjects

Thirty-five critically ill, brain-injured patients (29 men) who were mechanically ventilated for less than 24 h were enrolled. The study protocol was approved by the Hospital Ethics Committee (Evangelismos Hospital, Athens, Greece). Informed written consent was obtained from the patients' next of kin. Brain injury (either head trauma or cerebral hemorrhage/stroke) was diagnosed by clinical history, neurologic examination, and brain computed tomography. All patients had no history of chronic lung disease and no lung injury on entry day (partial arterial oxygen pressure to inspired oxygen fraction ratio [Pa_{O_2}/Fi_{O_2}] $\geq$300 mmHg, no evidence of pathology on chest radiograph, and consequently no ALI[17]). Exclusion criteria were: age less than 16 yr, pregnancy, hemodynamic instability, and history of cardiopulmonary disease or kidney or liver failure. Two age-matched control groups also were studied: 12 spontaneously breathing healthy volunteers (healthy controls; 7 men, 4 smokers, 21–37 yr old); and 8 patients undergoing neurosurgery for recent brain injury (brain-injured controls; 6 men, 3 smokers, 24–40 yr old) not in need of MV before surgery. Subjects in these control groups had no history of or active lung disease.

Clinical Management

Mechanical ventilation was initiated in all patients in the hospital emergency department. Upon transfer to the ICU, all patients were sedated using midazolam or propofol plus fentanyl citrate intravenous infusions and placed in supine position with an approximately 30-degree head tilt. All patients were ventilated by volume control modality (Servo 900C or 900E; Siemens-Elema, Solna, Sweden), with a tidal volume of 8 ml/kg actual body weigh (following our ICU protocol for ventilating noninjured lungs), and FiO_2 ~0.35; immediately after, patients were randomly assigned to receive either zero end-expiratory pressure (ZEEP; ZEEP group) or 8 cm H_2O of PEEP (PEEP group) following a predesigned chart of randomization. The length of MV before randomization (*i.e.*, MV length before ICU admission) was similar between the two groups. Breath frequency was left to the discretion of the treating physician. Ventilation settings were kept constant for 5 days unless the clinical condition of the patient at any time warranted changes; in such cases, patients were withdrawn from the study.

During the first ICU day, 24 h ICU trauma score was recorded.[21] Disease and lung injury severity were estimated by assessing Acute Physiology and Chronic Health Evaluation (APACHE) II[22] and Lung Injury (LIS)[23] scores daily for 5 days; the presence of sepsis also was recorded.[18] Survival was subsequently documented at ICU discharge.

In all patients, EBC was collected on the first, third, and fifth days of the assigned MV modality. The following also were recorded or collected on these days: full hematologic-biochemical profile, chest radiographs, vital signs, intracranial pressure (CaminoV-420; Camino Medical Products,

San Diego, CA) and central nervous status assessment (Glasgow Coma Scale).[24] Immediately before each measurement, heart rate, mean systemic arterial pressure, intracranial pressure, and ventilator mode/settings were recorded. During EBC collection, arterial blood was withdrawn for arterial blood gases estimations and subsequent analysis of cytokines and other inflammatory markers in plasma/serum.

Exhaled Breath Condensate: Collection in Patients

EBC was collected from intubated patients with the RTube device (Respiratory Research Inc., Charlottesville, VA), which was positioned in the expiratory limb of the ventilator circuit. The humidifying filter (Humid-Vent Filter Compact S; Teleflex Medical, Athlone, Ireland) was omitted from the ventilatory circuit before collection. During each measurement, all patients received the aforementioned tidal volume (8 ml/kg) and FiO_2 (0.35), a standardized respiratory rate of 15/min, and the already assigned PEEP (*i.e.*, 0 or 8 cmH_2O). The RTube is a portable device that uses an exhalation valve that also serves as a syringe-style plunger to pool fluid off the condenser walls. Cooling is achieved by placing an aluminum cooling (at −20°C) sleeve over the disposable polypropylene condensation chamber.[20] Collecting time for EBC was 20 min, producing approximately a 1-ml condensate sample. Samples were aliquoted to three to four vials and stored at −80°C; the remaining sample was used for pH measurement.

Exhaled Breath Condensate: Collection in Control Subjects

In the healthy controls, EBC was collected during tidal breathing using the same condenser device used in the patient groups. Individuals breathed through a mouthpiece connected to the condenser while wearing a nose clip. Ambient air was inhaled through a one-way valve at the bottom of the device. Exhaled air was channeled through another one-way valve incorporated within the condenser chamber and the mouthpiece. Subjects were instructed to refrain from food intake and smoking beginning 2 h before sample collection. Amylase was assessed and not detected in any obtained samples, thus excluding the possibility of oropharyngeal contamination.

In the brain-injured controls, EBC was collected in the operating room immediately after intubation and MV initiation. Collection technique and MV settings were similar to those used for the patient groups. ZEEP was applied to all subjects during EBC collection.

EBC pH Measurement

pH was not assessed immediately after EBC collection to avoid production of unstable values. To achieve stability, deaeration with helium gas (*i.e.*, a carbon dioxide–free gas) was performed for 10 min.[19] Immediately after, EBC pH was measured in a gas analyzer as described previously.[25]

Blood Samples

Arterial blood was drawn and immediately placed on ice before centrifugation at 3,000 rpm (4°C, 10 min). Sera and plasma were then divided in 0.5-ml aliquots and stored at −80°C until processed.

Assays of EBC and Blood Markers

Proinflammatory and antiinflammatory cytokines were measured in EBC and blood by flow cytometry using Cytometric Bead Array technology.[26] A human inflammation cytometric bead array kit (BD Biosciences, San Jose, CA) was used to measure interleukins (IL)-8, IL-1β, IL-6, IL-10, and IL-12p70 and tumor necrosis factor-α (TNF-α). The kit sensitivity is comparable with that of conventional enzyme-linked immunosorbent assays.[27] Corresponding detection limits were 2.5, 2.1, 1.7, 1.8, 1.3, and 1.1 pg/ml, respectively. Samples were analyzed using a BD FACSCalibur (BD Biosciences) flow cytometer. Blood sample assays were performed according to the manufacturer's instructions; slight modifications were applied for EBC measurements.

Further analysis of blood constituents focused on markers of brain injury and systemic inflammation, including endothelium-related markers. Endothelial and brain-injury markers included soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1), measured using Luminex's xMAP technology (LINCO Research, Inc, St. Charles, MO); von Willebrand factor antigen, estimated by immunoturbidimetric assay (STA Liatest; Diagnostica Stago, Paris, France); S-100B protein in serum, determined with a luminometric immunoassay (Liaison Sangtec 100; DiaSorin, Vercelli, Italy). Systemic inflammatory markers included: soluble triggering receptor expressed on myeloid cells (sTREM-1), estimated by a commercially available developmental immunoabsorbent assay (R&D Inc, Minneapolis, MN; lowest detection limit 15.1 pg/ml); C-reactive protein, measured using an immunoturbidimetric assay (Tina-Quant CRP; Roche Diagnostics GmbH, Basel, Switzerland); procalcitonin, measured by means of a immunoluminometric assay (Liaison Brahms PCT; BRAHMS, Hennigsdorf, Germany; and DiaSorin).

Statistical Analysis

All indices and markers studied were part of the initial hypothesis and study design. Data are presented as percentages of total, mean ± SD, or median (interquartile range) when data distribution was skewed. Cytokine concentrations less than detection thresholds were considered as the corresponding detection limits.

Comparisons among all three groups within each time point were made using one-way analysis of variance (one-way ANOVA). Two-way ANOVA for repeated measures was performed to examine differences between the PEEP and ZEEP groups (factor 1) and among days 1, 3, and 5 (factor 2). The Tukey–Kramer test was used to correct for multiple comparisons. Logarithmic transformation of skewed values was performed to achieve normality. When appropriate,

Table 1. Baseline Data of the Two Patient Groups upon ICU Entry (before Randomization) and Outcome Measures

	ZEEP (n = 12)	PEEP (n = 15)	*P* Value
Age (yr)	23.5 (20–26.5)	23 (21–57)	0.328*
Male gender, n (%)	10 (83)	12 (80)	1.000†
Diagnosis, n (%)			
Head trauma	10 (83)	11 (73)	0.662†
Hemorrhage/stroke	2 (17)	4 (27)	
Undergoing neurosurgery, n (%)	6 (50)	4 (27)	0.257†
Other injuries, n (%)	1 (8)	4 (27)	0.342†
ICU outcome—death, n (%)	4 (33)	3 (20)	0.662†
ICU days (mean ± SD)	14.4 ± 8.44	17.2 ± 10.10	0.452‡
V_T upon ICU entry (ml) (mean ± SD)	610.4 ± 46.34	578.0 ± 59.79	0.136‡
Fio_2 upon ICU entry (%) (mean ± SD)	35.3 ± 4.03	37.0 ± 7.75	0.485‡
ICP upon ICU entry (mmHg) (mean ± SD)	17.9 ± 6.19	14.8 ± 1.82	0.226‡
GCS upon ICU entry (mean ± SD)	7.0 ± 2.98	6.7 ± 2.28	0.794‡

Data are presented as: means ± SD, median (interquartile range), and percentages of total (%).

Comparisons between groups were performed by * Mann–Whitney U rank test, † Fisher exact test, and ‡ Student *t* test, as appropriate.

Fio_2 = fraction of inspired oxygen; GCS = Glasgow Coma Scale; ICP = intracranial pressure; ICU = intensive care unit; PEEP = positive end-expiratory pressure; V_T = tidal volume; ZEEP = zero end-expiratory pressure.

Fisher exact test was used to examine the relationships of qualitative variables, and Student *t* test and Mann–Whitney U rank test for two group comparisons.

The relationships between EBC indices (*i.e.*, pH and cytokines) and clinical outcome or blood markers were examined by mixed-effects models to correct for patients' variability (multiple cases per patient); thus, the patient effect was random (random intercept), and estimation was done by the restricted maximum likelihood method. The covariance structure was compound symmetry. Cytokines that were logarithmically transformed were included as such in this statistical model. Simple mixed effects models were fitted for EBC variables with each independent variable separately; independent variables with $P < 0.1$ were further examined in a multiple analysis.

Statistical analyses were conducted with the statistical program SAS (SAS Institute Inc., Cary, NC). All *P* values are two-sided. Differences were considered significant at $P < 0.05$.

Results

Patient Characteristics and Clinical Outcomes

Ultimately, data from 27 patients (10 smokers) were included in the analysis. Eight patients (7 men; 4 under ZEEP) were withdrawn early from the study because of the need for protocol violation (alterations of ventilation settings) or ALI development. From the included patients, 12 were randomly assigned to the ZEEP group and 15 to the PEEP group.

Baseline group characteristics upon ICU admission, before randomization, are summarized in table 1. Upon entry, the two groups were well balanced with no significant differences between them.

The time course of measures of disease severity, neurologic condition, and lung injury during the study period are shown in table 2. APACHE II score, LIS, and Glasgow Coma Scale and intracranial pressure values showed no significant changes on MV days 1, 3, and 5 either between or within the groups (table 2).

Two patients were extubated on MV day 3 (one from each group) and one on day 5 (from the PEEP group). One patient of the PEEP group was removed from the analysis after day 3 because of MV changes; corresponding data before removal were included in the analysis. None of the final cohort experienced ALI[17]; however, both groups had a significant decrease in Pao_2/Fio_2 ratio ($P < 0.05$) at day 5 compared with day 1, with no differences in between (table 2). Four patients experienced mild lung injury as defined by the LIS [23]; the aforementioned mild lung injury was not associated with significant changes in EBC indices.

No patient experienced sepsis; in this respect no one had pneumonia, ventilator-associated pneumonia, or any other infection. All patients experienced systemic inflammatory response syndrome (SIRS)[18] at some time during the study. Nearly two thirds of the patients met SIRS criteria on day 1, one third by day 3, and one half by day 5 with no difference between the two groups (table 2).

Airway Acidification as Measured by EBC pH in Control Subjects and Patients

EBC pH in healthy controls was 7.47 ± 0.11. EBC pH was significantly lower in both ZEEP and PEEP patients on day 1 (7.34 ± 0.1 and 7.33 ± 0.07, respectively, $P < 0.05$; fig. 1) and remained lower than that of control subjects throughout the study period. Similar patterns were observed among EBC pH in brain-injured controls and the two patient groups (table 3). There were no significant changes in EBC pH values, either between the patients' groups or in time (fig. 1 and table 3).

EBC Cytokine Measurements

All cytokines were measurable in patients' EBC, with values decreasing in the linear portion of corresponding standard curves, with a few exceptions. In contrast, most cytokines were detectable only in a portion of EBC samples from either control group (fig. 2 and table 3). Exhaled

Table 2. Markers of Disease Severity and Brain and Lung Injury on Days 1, 3, and 5 of Mechanical Ventilation under ZEEP and PEEP

Markers	Day 1	Day 3	Day 5
Disease severity			
APACHE II score			
ZEEP	13.7 ± 5.05	14.5 ± 3.30	16.3 ± 7.04
PEEP	12.7 ± 4.80	14.9 ± 5.42	14.5 ± 5.16
24 h ICU Trauma score			
ZEEP	2 (2–3.5)		
PEEP	2 (2–2.75)		
Presence of SIRS			
ZEEP	75%	18%*	46%
PEEP	67%	31%	50%
MAP (mmHg)			
ZEEP	90.7 ± 8.85	88 ± 7.52	89.3 ± 7.65
PEEP	87.8 ± 7.45	90.5 ± 6.52	95.6 ± 8.06
Brain injury			
ICP (mmHg)			
ZEEP	17.6 ± 5.62	18.1 ± 6.09	18.9 ± 8.58
PEEP	17.1 ± 4.98	16 ± 4.85	17.8 ± 5.31
CPP (mmHg)			
ZEEP	72.2 ± 8.42	69.9 ± 7.27	70.4 ± 13.82
PEEP	70.7 ± 7.58	74.5 ± 7.46	77.8 ± 11.72
GCS			
ZEEP	6.4 ± 3.23	4.6 ± 2.46	5.2 ± 3.19
PEEP	6.8 ± 2.24	5.9 ± 2.22	5.8 ± 2.41
S-100B protein (μg/l)			
ZEEP	0.7 (0.51–1.44)	0.4 (0.21–1.75)*	0.2 (0.14–0.81)*†
PEEP	0.6 (0.37–0.96)	0.3 (0.18–0.60)*	0.2 (0.09–0.36)*†
Pentothal administration			
ZEEP	25%	46%	55%
PEEP	13%	23%	33%
Mannitol (g/day)			
ZEEP	15 ± 35.29	15.5 ± 22.96	1.8 ± 6.03‡
PEEP	14.7 ± 14.57	8.5 ± 15.19	21.7 ± 25.88
Lung injury			
LIS			
ZEEP	0 ± 0	0.03 ± 0.1	0.03 ± 0.10
PEEP	0.33 ± 0	0.33 ± 0	0.39 ± 0.20
Pao_2/Fio_2 (mmHg)			
ZEEP	442 ± 79.58	420 ± 72.65	407 ± 70.51*
PEEP	481 ± 90.11	498 ± 75.08	441 ± 97.32*

Data are presented as: mean ± SD, median (interquartile range), and percentages (%) of total.

* $P < 0.05$ and † $P < 0.05$ as compared with day 1 and day 3, respectively, within each group by one-way ANOVA for repeated measures and Tukey-Kramer test; ‡ $P < 0.05$ between the two groups at specific days by Student *t* test.

APACHE = Acute Physiology and Chronic Health evaluation; CPP = cerebral perfusion pressure; Fio_2 = fraction of inspired oxygen; GCS = Glasgow Coma Scale; ICP = intracranial pressure; ICU = intensive care unit; LIS = Lung Injury Score; MAP = mean arterial pressure; Pao_2 = partial arterial oxygen pressure; PEEP = positive end-expiratory pressure; SIRS = systemic inflammatory response syndrome; ZEEP = zero end-expiratory pressure.

IL-1β and TNF-α were the only significantly higher cytokines in both ZEEP and PEEP groups compared with both control groups, whereas EBC IL-6 depicted a similar pattern only in relation to the healthy controls (fig. 2 and table 3). Patients' EBC IL-10 and IL-12p70 values were higher than those of healthy controls, reaching significance at specific time points.

The time course of EBC cytokines in the two patient groups is given in figure 2. IL-10 tended to increase during ZEEP, whereas it decreased significantly during PEEP ($P < 0.05$). There were no significant differences between the two groups regarding all other cytokines. In addition, sTREM-1 was not detectable in any of the tested EBC samples.

Markers of Systemic Inflammation in Blood

Table 4 depicts indices and markers of systemic inflammation, markers of endothelial activation and injury, and proinflammatory and antiinflammatory cytokines and their ratios. Time-dependent changes in several parameters occurred in both groups. Significant differences between the two patient groups were observed in leukocyte counts, sTREM-1, and sIL-6. All three parameters were constantly higher on

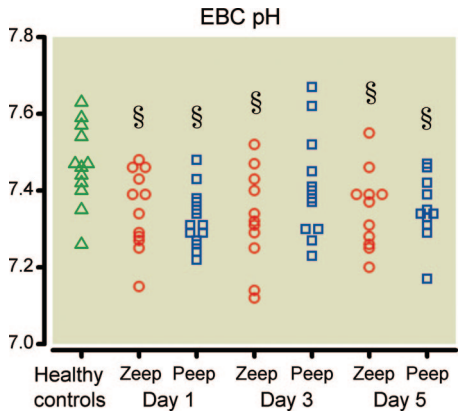

Fig. 1. Exhaled breath condensate (EBC) pH values in healthy control subjects and on days 1, 3, and 5 of mechanical ventilation under zero end-expiratory pressure (ZEEP) or positive end-expiratory pressure (PEEP). Data are presented as individual values. § $P < 0.05$ from the control group by one-way ANOVA and Tukey–Kramer test.

ZEEP ($P < 0.05$; two-way ANOVA for repeated measures). Both leukocyte number and sTREM-1 decreased with time in both groups. A trend for higher sTNF-α/sTREM-1 was noted during PEEP ($P = 0.06$).

Relationships of EBC Parameters with Systemic Indices

Relationships of EBC pH and cytokines (dependent variables) with all of the estimated systemic parameters (independent variables) shown in tables 2 and 4 also were examined (data not shown). Variables with $P < 0.1$ were explored further in a multiple analysis using the stepwise backward procedure. This analysis is exploratory because of the data-driven approach and the limitation of the large number of examined relationships compared with the sample size of our study. EBC pH was not significantly related to any parameter. Although IL-10 was the only EBC measure depicting differences between the two groups, after adjusting for PEEP and time, no significant relationships were found. EBC cytokines appeared mostly related to sIL-8 and inversely related to sTREM-1 (and sTNF-α/sTREM-1 for IL-6).

Discussion

The principal aim of the study was to obtain further insights into inflammatory MV mechanisms in a unique patient population with apparently normal lungs who require prolonged MV not because of respiratory failure but because of brain trauma. We have shown previously that such patients have altered respiratory mechanics[14] and pulmonary endothelial function (S.E.O., unpublished data, 2009), providing evidence for the existence of subtle lung injury. The current study extended these observations into the airway and alveolar compartment by studying inflammatory mechanisms using EBC. Furthermore, the study aimed at evaluating the effect of PEEP, an important protective MV component, and the relationship between systemic and airway inflammation in our patients. The choice of 8 ml/kg tidal volume followed our ICU protocol for providing MV to patients with no ALI.

The major findings of our study were: (1) patients' EBCs were more acidic and contained higher TNF-α and IL-1β concentrations than did those of healthy and brain-injured controls; all other cytokines, except IL-8, were constantly or intermittently higher only in relation to the healthy controls; (2) no differences were observed in EBC markers between

Table 3. EBC pH and Cytokine Measurements in Brain-injured Controls and in Patients on Days 1, 3, and 5 of Mechanical Ventilation under ZEEP and PEEP

	Brain-injured Controls		Day 1	Day 3	Day 5
EBC pH	7.454 ± 0.07	ZEEP	7.342 ± 0.103*	7.326 ± 0.128†	7.351 ± 0.102*
		PEEP	7.325 ± 0.072*	7.408 ± 0.137	7.351 ± 0.084*
EBC IL-10 (pg/ml)	3.9 ± 1.85	ZEEP	3.68 ± 2.25	4.68 ± 2.16	4.96 ± 3.54
		PEEP	4.97 ± 1.53	4.52 ± 1.68	3.83 ± 1.81‡
EBC IL-1β (pg/ml)	5.6 ± 4.91	ZEEP	20.47 ± 21.62	33.68 ± 28.32*	32.92 ± 24.47*
		PEEP	32.80 ± 18.63*	35.01 ± 14.31*	34.92 ± 21.08*
EBC TNF-α (pg/ml)	1.7 (1.1–2.6)	ZEEP	3.9 (3.4–5.5)*	5.6 (4.1–7.9)§	5 (3.3–10.3)†
		PEEP	4.3 (3.5–5)*	4.7 (4.3–5.6)§	4.6 (3.7–6.5)*
EBC IL-6 (pg/ml)	4.5 (1.7–5.6)	ZEEP	4.3 (2.6–5.6)	5.3 (3.1–6.8)	5.9 (3.9–7.1)
		PEEP	3.8 (3.4–5.2)	4.5 (3.5–5.0)	4.4 (3.3–4.9)
EBC IL-8 (pg/ml)	2.5 (2.5–6.5)	ZEEP	3.5 (2.6–6.0)	4.2 (3.2–5.4)	4.5 (2.5–11.4)
		PEEP	5.0 (3.5–5.6)	4.5 (3.8–7.5)	5.1 (3.2–8.9)
EBC IL-12p70 (pg/ml)	1.3 (1.3–4.1)	ZEEP	3.0 (1.7–7.5)	4.3 (1.3–12.2)	4.3 (1.3–11.7)
		PEEP	5.1 (3.8–6.2)	5.6 (4.1–6)	5.7 (4–12.2)*

Data are presented as mean ± SD (normal distribution) and median (interquartile range; skewed distribution).

* $P < 0.05$ as compared with corresponding brain-injured control values by one-way ANOVA and Tukey-Kramer test. † $P < 0.05$ as compared with brain-injured corresponding control values by Student *t* test. ‡ $P < 0.05$ as compared with day 1 corresponding values within the group by one-way ANOVA for repeated measures and Tukey-Kramer test. § $P < 0.001$ as compared with corresponding brain-injured control values by one-way ANOVA and Tukey-Kramer test.

EBC = exhaled breath condensate; IL = interleukin; PEEP = positive end-expiratory pressure; TNF-α = tumor necrosis factor-α; ZEEP = zero end-expiratory pressure.

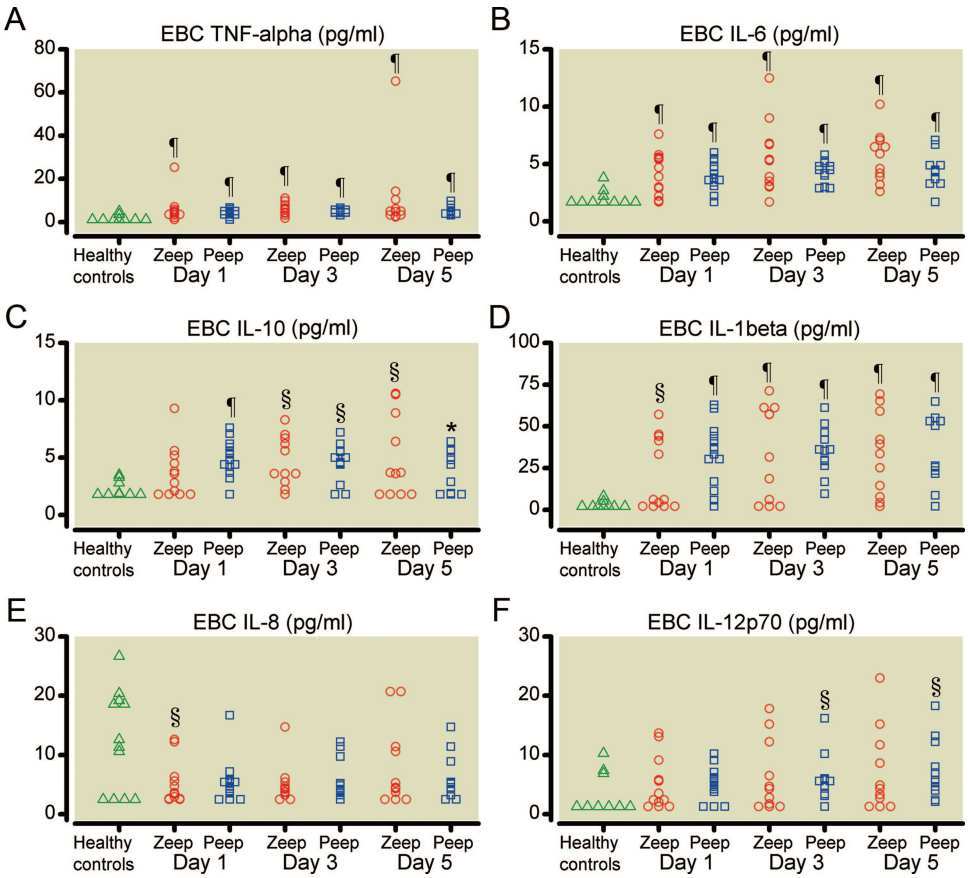

Fig. 2. Exhaled breath condensate (EBC) cytokine concentrations in healthy control subjects and on days 1, 3, and 5 of mechanical ventilation under zero end-expiratory pressure (ZEEP) or positive end-expiratory pressure (PEEP). Data are presented as individual values. Statistical analyses of skewed values, which was the case for the four cytokines shown in panels *A, B, E,* and *F,* were performed after logarithmic transformation to achieve normality. Values of cytokines shown in panels *C* and *D* were normally distributed and were analyzed as such. Patterns of all EBC cytokines were analyzed by two-way ANOVA for repeated measures (ANOVARM); interleukin (IL)-10 was the sole cytokine to show significant interaction between time and PEEP ($P < 0.05$). *$P < 0.05$ as compared with day 1 corresponding values within the group by one-way ANOVARM and Tukey–Kramer test. §$P < 0.05$. ¶$P < 0.001$ as compared with corresponding control values by one-way ANOVA and Tukey–Kramer test. TNF = tumor necrosis factor.

patients during ZEEP or PEEP, with the exception of IL-10; and (3) during ZEEP, patients had significant early and sustained increases of the systemic inflammatory indices leukocytes, sIL-6, and sTREM-1.

Two control populations were used in this study: a healthy control group, consisting of spontaneously breathing healthy volunteers, and a brain-injured control group, consisting of patients with recent brain injury and healthy lungs who were not in need of MV and whose EBC samples were collected immediately after MV initiation in the operating room and before surgery. Both groups have their limitations: the former, although used as control for mechanically ventilated subjects in several studies, may not entirely allow distinction of phenomena related to MV *versus* brain injury; the latter was subjected to a very short period of MV as part of anesthetic induction for neurosurgery. The appropriate control group would be patients without brain and lung injury who require prolonged MV, such as patients experiencing drug overdose. However, no such admissions occurred during a prolonged period of time in our ICU, so the formation of such a control group was not possible. Nevertheless, the three EBC indices that differ among the mechanically ventilated patients (*i.e.*, acidic pH and higher TNF-α and IL-1β) and both control groups should mostly reflect the impact of MV in our subjects.

All patients received the appropriate medications as assessed by the treating physicians. To avoid the possibility that administered drugs might have influenced the obtained results, agents such as mannitol or pentothal that might modify patients' inflammatory and redox status have been included in our univariate and multivariate analyses. Only pentothal administration appeared to have an effect on the obtained EBC IL-1β values, as revealed by our exploratory multiple analysis.

EBC pH has received considerable attention as a potential noninvasive marker of airway inflammation. EBC pH has been found to be low in lung inflammatory diseases, such as asthma and chronic obstructive pulmonary disease, and showed strong association with inflammatory cell popula-

tions measured in induced sputum.[20,28] Therefore, the decreased pH observed in our mechanically ventilated, brain-injured patients may also indicate the presence of airway inflammation. Using the same EBC collector, subjects with no apparent lung pathology undergoing elective surgical procedures had alkalotic EBC pH values during MV.[29] The reasons for this apparent discrepancy with our study are not known but may be explained by both brain-injured controls and mechanically ventilated patients having brain damage; the latter also had SIRS and were subjected to a longer period of MV before EBC collection. Factors that might have potentially influenced EBC pH include systemic pH, atelectasis, suction trauma, or endotracheal tube irritation. Plasma pH values concomitantly assessed in our patients were slightly alkalotic, for neuroprotection, and not related to the EBC values. Atelectasis might have contributed to the observed acidic EBC pH; such a pathology might explain why on day 3, PEEP patients did not differ from the two control groups. However, patients' chest radiographs revealed no signs of atelectasis, and arterial Pa_{O_2}/Fi_{O_2} values on day 3 were not different from those of day 1, making this possibility less likely. Suctioning was never performed close to EBC collection, although this possibility cannot be entirely excluded. Endotracheal tube irritation should be present, although for a short time, in our brain-injured controls, thus minimizing the related confounding impact.

Other studies performed in mechanically ventilated patients after coronary artery bypass graft or those with pneumonia[25,30] revealed acidic EBC pH values, whereas continuous EBC monitoring in mechanically ventilated patients revealed that pH became more acidic during clinical deterioration and normalized with recovery.[31] These studies further support the value of EBC acidification as marker of lung inflammation. Thus, a degree of inflammation may be present in our patients, although this may be less intense than in the studies mentioned, in which lower EBC pH values accompanied more severe lung pathologies. It should be noted that in our patients who were withdrawn from the study because of developing ALI, the latter was associated with EBC acidosis ($n = 4$; $pH = 7.29 \pm 0.05$).

The increased concentrations of the proinflammatory IL-1β, TNF-α, and IL-6 (the last compared with healthy controls) are in agreement with results of previous studies in which cytokines in bronchoalveolar lavage fluid were increased in mechanically ventilated patients at risk for acute respiratory distress syndrome or undergoing coronary artery bypass graft,[32,33] in addition to patients with various chronic pulmonary inflammatory diseases, such as chronic obstructive pulmonary disease and cystic fibrosis.[34,35]

IL-8 was the sole cytokine that tended to be higher in healthy controls than in mechanically ventilated patients. This finding, although unexpected, is similar to previous reports showing higher EBC IL-8 concentrations in spontaneously breathing controls than in patients with emphysema or chronic bronchitis.[36,37] Furthermore, concentrations of IL-8 in our healthy controls were similar to those reported by Cunningham *et al.*[35]

The short- and long-term findings of inflammatory EBC indices in our patient population are intriguing. These patients did not have ALI,[17] any other apparent lung pathology, or sepsis[18] on admission, and they remained free of ALI and sepsis during the 5-day follow-up period (only four subjects experienced a very mild lung injury, as defined by Murray *et al.*[23]). Consequently, these data are interpreted as evidence of subclinical pulmonary inflammation in brain-injured patients with apparently healthy lungs and may be explained either by the underlying trauma-induced SIRS or MV *per se via* ventilator-induced, lung-injury–related pathways. In an effort to distinguish these mechanisms, we further evaluated the effect of PEEP.

There may be concerns regarding the application of PEEP in such patient populations, especially for the potential increase in intracranial pressure, reduction in cerebral perfusion pressures,[38] and the requirements for mild hypocapnia. However, in agreement with previous reports by us and others,[14,39–41] the moderate PEEP applied in our patients induced changes to neither intracranial pressure nor cerebral perfusion pressures compared with baseline and corresponding values during ZEEP (tables 1 and 2). Cerebral perfusion pressure values during PEEP had been more than 60 mmHg throughout the study. Contrary to our previous report on lung mechanics,[14] PEEP application made no difference over time to any proinflammatory EBC marker. One possible explanation of this difference might be the higher sensitivity of the previously studied parameters to such a protective MV modality than the indices related to the airway/alveolar compartment as measured by EBC. However, although the presence of patients' EBC indices that differ from both control groups points to a MV-associated effect, the fact that cytokine differences between patients receiving PEEP and those receiving ZEEP are minimal might reflect a phenomenon related to brain injury.

EBC concentrations of the antiinflammatory cytokine IL-10 decreased over time during PEEP and tended to increase in the ZEEP group. This observation might be explained by the complex response of proinflammatory and antiinflammatory mediators during inflammation and trauma[42]; such a response might also be triggered by the addition of the ventilation insult. This notion is further supported by the study of McRae *et al.*, who found that EBC IL-10 concentrations peaked after ischemia–reperfusion injury in experimental lung transplantation.[43]

In our population, ZEEP application was associated with higher systemic inflammatory markers, such as sTREM-1, sIL-6, and leukocytes. Protective ventilation with high PEEP has been shown to reverse systemic cytokine release in patients with ALI,[44] whereas high PEEP and low tidal volume has minimized the inflammatory response in acute respiratory distress syndrome.[6,45] We now provide evidence that moderate PEEP attenuates systemic inflammation in brain-

Table 4. Systemic Inflammatory Markers on Days 1, 3, and 5 of Mechanical Ventilation under ZEEP and PEEP

Markers	Day 1	Day 3	Day 5
General indices of SIRS/sepsis			
Temperature (°C)			
ZEEP	37.7 ± 1.08	37.2 ± 0.85	37.5 ± 1.38
PEEP	37.6 ± 0.76	37.8 ± 0.71	38 ± 1.01
Leukocytes, total (/μl)			
ZEEP	14,739 ± 3,738*	9,406 ± 2,409*†	8,436 ± 3,411*†
PEEP	11,457 ± 3,861	7,899 ± 2,547†	6,960 ± 2,318†
Neutrophils (% of total)			
ZEEP	80.3 ± 8.13	81.1 ± 5.82	78.6 ± 6.91
PEEP	80.3 ± 9.89	74.1 ± 11.18	75.9 ± 7.83
Albumin (g/dl)			
ZEEP	3.8 ± 0.65	3.1 ± 0.52†	3.2 ± 0.55†
PEEP	3.4 ± 0.51	3.1 ± 0.44†	3.0 ± 0.42†
sTREM-1 (pg/ml)			
ZEEP	330 (30–1,748)*	377 (85–1,952)*	418 (106–2,030)*
PEEP	35 (4–95)	40 (4–279)	72 (4–425)
Acute-phase proteins			
CRP (mg/dl)			
ZEEP	8.4 ± 4.46	14.6 ± 4.19†	13.9 ± 6.74†
PEEP	12.6 ± 7.68	11.7 ± 4.89	9.3 ± 5.76
PCT (ng/ml)			
ZEEP	0.4 (0.15–0.71)	0.2 (0.11–0.62)†	0.3 (0.1–0.7)
PEEP	0.2 (0.1–0.65)	0.1 (0.1–0.16)†	0.1 (0.1–0.16)
Endothelial markers			
sICAM-1 (ng/ml)			
ZEEP	222.1 ± 85.78	297.9 ± 151.68	337.7 ± 179.14†
PEEP	210.1 ± 64.45	221.8 ± 59.58	248.5 ± 102.49†
sVCAM-1 (ng/ml)			
ZEEP	799.4 ± 393.6	944.2 ± 392.84	804.8 ± 363.62
PEEP	800.8 ± 253.36	851.2 ± 313.65	804 ± 462.03
vWF:Ag (%)			
ZEEP	165.1 ± 52.19	165.9 ± 50.00	212.4 ± 107.90†
PEEP	185.9 ± 29.72	192.5 ± 34.93	210.9 ± 75.93†
Antiinflammatory cytokines			
sIL-10 (pg/ml)			
ZEEP	3.5 (1.8–17.3)	4.6 (2.5–6.7)	4.0 (1.8–11.9)
PEEP	2.9 (1.8–5.5)	1.8 (1.8–2.9)	2.3 (1.8–8.5)
Proinflammatory cytokines			
sIL-1β (pg/ml)			
ZEEP	22.7 (2.1–244.9)	22.7 (2.1–75.3)	15.3 (2.1–261.5)
PEEP	34.0 (7.9–186.2)	28.5 (6.9–151.3)	59.1 (2.1–157.7)
sTNF-α (pg/ml)			
ZEEP	1.6 (1.1–6.9)	2.3 (1.1–4.5)	1.3 (1.1–5.3)
PEEP	1.1 (1.1–3.0)	1.1 (1.1–1.5)	2.1 (1.1–3.6)
sIL-6 (pg/ml)			
ZEEP	483.8 (125.3–721.0)*	125.5 (61.6–280.9)*†	179.8 (98.7–295.2)*†
PEEP	113.3 (66.6–210.9)	73.9 (44.4–144.8)†	102.6 (41.8–144.8)†
sIL-8 (pg/ml)			
ZEEP	104.5 (16.2–516.7)	50.8 (18.3–660.6)	82.8 (27–293)
PEEP	29.6 (18.6–131.6)	33.7 (12.5–179.6)	34.9 (15.1–373.2)
sIL-12p70 (pg/ml)			
ZEEP	14 (1.3–34.5)	6.9 (1.4–11.3)	4.1 (1.3–37.6)
PEEP	8.3 (1.6–27.7)	5.3 (1.4–22.7)	11.8 (1.3–23)
Cytokine balance			
sTNF-α/sTREM-1 (10^{-3})			
ZEEP	9.0 (3.25–204.93)	4.9 (1.91–27.06)	11.1 (2.63–12.26)
PEEP	82.5 (11.58–275.00)	43.0 (5.21–275.00)	54.2 (4.00–525.00)

(*continued*)

Table 4. Continued

Markers	Day 1	Day 3	Day 5
sTNF-α/sIL-10			
ZEEP	0.6 (0.48–1.17)	0.6 (0.24–1.21)	0.6 (0.2–0.82)
PEEP	0.5 (0.24–0.61)	0.6 (0.53–0.66)	0.6 (0.32–0.81)
sIL-6/sIL-10			
ZEEP	75.6 (33.56–159.68)	33.4 (15.62–66.24)	57.7 (12.03–86.89)
PEEP	19.81 (11.89–72.39)	29.8 (22.28–47.53)	23.2 (12.79–39.17)

Data are presented as mean ± SD (normal distribution) and median (interquartile range; skewed distribution).

* $P < 0.05$ between the two groups at specific days by Student *t* test. † $P < 0.05$ as compared with day 1 within each group by one-way ANOVA for repeated measures and Tukey-Kramer test.

CRP = C-reactive protein; PCT = procalcitonin; PEEP = positive end-expiratory pressure; sICAM-1 = soluble intercellular adhesion molecule-1; sIL = serum interleukin; SIRS = systemic inflammatory response syndrome; sTNF-α = serum tumor necrosis factor-α; sTREM-1 = soluble triggering receptor expressed on myeloid cells-1; sVCAM-1 = soluble vascular cell adhesion molecule-1; vWF:Ag = von Willebrand Factor antigen; ZEEP = zero end-expiratory pressure.

injured patients with no ALI/acute respiratory distress syndrome, suggesting that MV in the presence of brain injury augments the systemic inflammatory response. However, it should be noted that the average time between first EBC sampling and ICU admission was approximately 8 h. Future studies focusing on this early time period and including data obtained before MV initiation are needed to clarify this issue fully. In a previous report on patients with no lung pathology undergoing elective surgery, high tidal volumes and ZEEP did not result in higher serum cytokine concentrations compared with lung protective strategies.[46] However, those measurements were performed 1 h after MV initiation, whereas our initial measurements were performed 8 h or more after initiation of MV and mostly in the presence of SIRS. Patients undergoing coronary artery bypass graft and ventilated for at least 6 h[33] had inflammatory profiles similar to those of our patients, suggesting that MV may participate in the inflammatory response related to cardiac surgery.

Because all our patients had brain injury and SIRS, we sought to investigate whether EBC-obtained indices correlate with markers of disease severity, brain and lung injury, and systemic inflammation. Although several sporadic relationships were present, pointing to some link between EBC markers and all but lung injury-related indices, in our exploratory analysis the former appeared mostly related to markers of systemic inflammation, especially IL-8 and sTREM-1.

TREM-1 is expressed on neutrophils and monocytes. Its soluble form, sTREM-1, has been detected in plasma during experimental and clinical sepsis and has been advocated as a diagnostic marker of pneumonia-related infection and a prognostic marker in septic shock.[47,48] It has been recently proposed that sTREM-1 may possess antiinflammatory activity.[49] In the current study, the numerous relationships of EBC indices with plasma sTREM-1 shown by our exploratory analysis support the potential association of the EBC proinflammatory expression with systemic inflammation. sTREM-1 was not detected in the EBC samples of controls or mechanically ventilated patients. In a recent report, sTREM-1 was assessed in bronchoalveolar lavage fluid and EBC of patients with and without ventilation-associated pneumonia[50]; sTREM-1 was identified only in EBC from patients with ventilator-associated pneumonia. Thus, it is possible that patients (like our subjects) without pneumonia do not release detectable sTREM-1 into EBC.

Several limitations of our study are noted: First, our study is a single-center investigation with relatively low patient numbers. However, we previously observed statistical differences in a same population with a similar design regarding respiratory mechanics[14] and biochemistry (pulmonary endothelial-bound angiotensin-converting enzyme activity; S.E.O., unpublished data, 2009). Second, we did not measure total proteins or nitrites in EBC. The former would have allowed cytokine normalization, whereas the latter would have provided additional evidence for pulmonary mechanical stress.[51]

While this work was under revision, two recent publications provided relevant information: The study by Roca *et al.*[52] addressed inflammatory aspects of MV in the weaning phase of critically ill patients compared with spontaneously breathing healthy volunteers. This study supports our conclusion regarding the inflammatory impact of MV *per se*. The study by Fremont *et al.*[53] addresses systemic inflammation in trauma patients and similar to our results demonstrates a complex cytokine response to trauma and highlights the importance of cytokine biomarkers in preclinical lung injury.

In conclusion, using sensitive EBC markers, we provide evidence for lung inflammation in brain-injured, mechanically ventilated patients without ALI or sepsis. Moderate PEEP application did not induce detectable changes in most EBC mediators but attenuated the underlying systemic inflammatory response of our patients. EBC markers appear mostly related to the presence of systemic inflammation, especially to sTREM-1, rather than to indices of brain, lung, and endothelial injury.

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