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Exhaled breath condensate acidification in acute lung injury

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Summary Lung injury in ventilated lungs may occur due to local or systemic disease and is usually caused by or accompanied by inflammatory processes. Recently, acidification of exhaled breath condensate pH (EBC-pH) has been suggested as marker of inflammation in airway disease. We investigated pH, ammonia, lactate, pCO_2, HCO_3^-, IL-6 and IL-8 in EBC of 35 ventilated patients (AECC-classification: ARDS: 15, ALI: 12, no lung injury: 8).

EBC-pH was decreased in ventilated patients compared to volunteers (5.85 ± 0.32 vs. 7.46 ± 0.48; $P < 0.0001$). NH_4^+, lactate, HCO_3^-, pCO_2, IL-6 and IL-8 were analyzed in EBC and correlated with EBC-pH. We observed correlations of EBC-pH with markers of local (EBC IL-6: $r = -0.71$, $P < 0.0001$, EBC IL-8: $r = -0.68$, $P < 0.0001$) but not of systemic inflammation (serum IL-6, serum IL-8) and with indices of severity of lung injury (Murray's Lung Injury Severity Score; $r = -0.73$, $P < 0.0001$, paO_2/FiO_2; $r = 0.54$, $P < 0.001$). Among factors potentially contributing to pH of EBC, EBC-lactate and EBC-NH_4^+ were found to correlate with EBC-pH.

Inflammation-induced disturbances of regulatory mechanisms, such as glutaminase systems may result in EBC acidification. EBC-pH is suggested to represent a marker of acute lung injury caused by or accompanied by pulmonary inflammation.

Introduction

Exhaled breath condensate (EBC) is a novel technique of sampling the lining fluid of the lung.[1] EBC is obtained during exhalation into a cold trap. Commercial devices are available and the method can be adapted to mechanical ventilation by inserting an appropriate conduit into the expiratory limb of the ventilator tubing. Although mostly water vapor, EBC contains other constituents such as small molecules, proteins and even DNA.[1] The mechanisms that contribute to the presence of non-volatile molecules in EBC have not yet been elucidated but the formation of an aerosol during reopening of alveoli or at the branching of small airways is a likely mechanism. We have found that the amount of breath condensate depends almost exclusively on the expired volume.[2] EBC has been used for monitoring inflammatory airway diseases such as asthma and COPD but also interstitial lung disease and the acute respiratory distress syndrome (ARDS).[3] Investigations of EBC have focused on H_2O_2,[4–6] 8-isoprostanes,[7–9] eicosanoids,[10] and cytokines.[11]

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Recently, a reduced pH of EBC (EBC-pH) was demonstrated in acute asthma and this effect was normalized following steroid therapy.[12] The authors proposed increased inflammation particularly due to eosinophil mediators to be the cause of this acidification of EBC. In a subsequent study they suggested a pH regulatory role of glutaminase in the human airway epithelium. Glutaminase might liberate ammonia thereby buffering pH.[13]

EBC-pH was investigated in another group of patients with airway diseases such as COPD, bronchiectasis and asthma.[14] The greatest decrease in EBC-pH was observed in patients with bronchiectasis not currently using inhalative corticosteroids.

Several investigations have thus demonstrated that EBC acidification may reflect airway inflammation. We hypothesized that EBC acidification may be a more general indicator of pulmonary inflammation and/or injury and investigated the usefulness of EBC-pH in mechanically ventilated patients with acute inflammatory lung disease.

Methods

Study subjects and clinical scores

EBC was collected from 35 medical ICU patients. Patients characteristics are depicted in Table 1. Twelve of the 35 patients with pneumonia suffered from a pre-existing chronic obstructive pulmonary disease (COPD). Because we did not find significant differences between patients with pneumonia with and without COPD, data were not presented for these subgroups. Patients were included after 24–72 h of ventilation. All patients were ventilated using a Servo 300 (Siemens, Germany). Lung injury in ventilated patients was estimated using the criteria stated at the American–European Consensus Conference on ARDS (AECC) [15] and the Murray's Lung Injury Severity Score (LISS),[16] (Table 2). Lung injury was estimated at the time of EBC collection. General (in contrast to pulmonary) disease severity was described by the APACHE II score.[17]

Table 1 Patients characteristics.

Patients characteristics	
Total number	35
Male	17
Female	18
Age (years)	61 ± 15
Reason for intubation	
(A) severe pneumonia (without COPD-patients)	23
(B) COPD exacerbation (with pneumonia)	12
Pre-existing chronic lung diseases	
COPD (smokers*)	12 (8)
Non (smokers*)	23 (2)

*Smoking was defined as current smokers or ex-smokers that discontinued smoking no longer than twelve months.

For comparison with the physiological situation, EBC was also collected from twelve spontaneously breathing, healthy, non-smoking volunteers (5 male, 7 female, age: 57 ± 10).

Approval for this investigation was gained from the ethics committee of the University of Leipzig.

EBC collection

EBC was collected by inserting a special conduit (FILT Lung and Chest Diagnostics Ltd., Germany) for the EcoScreen® breath condensate collecting device (Jaeger-Toennies, Germany) into the expiratory limb of the ventilator tubing directly after the Y-shaped connecting piece. EBC was collected for a 30 min time period. Humidification of inspiratory gas was achieved using identical heat humidifiers.

EBC was similarly collected for 30 min from spontaneously breathing volunteers with the EcoScreen® system as previously described.[2]

pH measurement

EBC-pH was measured at 10°C immediately after completion of the 30 min collection period in a blood gas analyzer (ABL 505, Radiometer, Denmark). pH measurement was repeated following dearation of the condensate with argon for 10 min as described by Hunt et al.[12] In addition to pH, pCO_2, HCO_3^-, sodium and potassium were analyzed in EBC.

In 19 of the 35 patients pH was also measured in the lingual bronchus using a small diameter pH probe (Flexilog 2000, antimon single channel catheter, Schwa-Medico, Germany). The probe was inserted through the working channel of a bronchoscope and positioned as closely to the bronchial wall as possible in order to achieve good contact with the airway lining fluid. Measurements were taken before other interventions such as bronchial lavages were performed.

Mediators and serum markers

All EBC samples were examined for amylase activity (alpha-Amylase ESP1491300 kit; detection limit

Table 2 Ventilatory parameters in patients classified according to AECC and LISS definitions.

Criteria	Diagnosis (A/B)	Number of patients	PEEP (mbar)	PIP (mbar)	BF (/min)	VT (ml/kgBW)	EMV (l/min)	EMV/BW (ml/min kgBW)
Classification of ALI and ARDS of the American–European Consensus Conference								
No lung injury	(5/3)	$n = 8$	9.0±4.6	24.0±8.3	22.5±4.5	6.7±2.3	11.1±1.4	149±54
ALI criteria	(9/3)	$n = 12$	8.6±3.5	20.9±3.7	21.5±3.3	6.3±1.0	9.8±2.3	137±39
ARDS criteria	(9/6)	$n = 15$	11.5±3.5	25.5±8.3	24.1±6.4	6.8±1.6	11.1±3.1	163±62
Murray's Lung Injury Severity Score								
No lung injury (score: 0)	(1/1)	$n = 2$	5.0±0.0	24.0±4.2	19.0±1.4	6.6±3.0	8.8±1.5	129±67
Mild-to moderate lung injury (score: 0.1–2.5)	(14/6)	$n = 20$	8.5±3.4	20.6±6.3	22.9±3.9	6.6±1.6	10.3±2.0	151±46
Severe lung injury (score > 2.5)	(8/5)	$n = 13$	12.8±3.0	28.1±6.8	23.2±6.8	6.6±1.5	11.0±2.6	154±64

0.05 μmol/l s; Boehringer Mannheim, Germany) in order to exclude contamination by saliva.

Five milliliter aliquots of EBC were lyophilized, reconstituted in 500 μl and used in IL-6, IL-8, and TNF-α ELISA assays (Quantikine HS human IL-6, QuantiGlo human IL-8, QuantiGlo human TNF-α, R&D Systems, USA). Ammonia concentrations were determined from 300 μl aliquots of native EBC by ammonia test kit (No. 1877984, Roche Diagnostics GmbH, Germany). EBC lactate was measured by spectrometry using a routine lactate test kit (No. 735, Sigma, Germany) at 540 nm.

In addition, IL-6, IL-8, TNF-α (Immulite, DPC Biermann, Germany), and procalcitonin (LUMItest PCT, BRAHMS Diagnostica, Germany) were measured from serum concomitantly.

Statistical analysis

Statistical analysis was performed with the SPSS software package (SPSS Inc., Chicago, USA). Linear regression analysis was performed to analyze the correlation of EBC-pH with EBC markers, serum markers, and clinical scores. Comparison of groups were performed by ANOVA testing and post hoc analysis (Bonferroni). Statistical significance was accepted at the 5% level. Results are mean ± SD.

Results

Amylase measurement and EBC diluting factor

None of the samples of ventilated or spontaneously breathing individuals exhibited amylase activity.

Mean values for EBC-sodium (8.54 mmol/l ± 0.9) and EBC-potassium (0.13 mmol/l ± 0.06) did not differ among spontaneously breathing healthy volunteers and patients with ARDS, ALI, or without lung injury. The dilution of ELF in EBC was estimated using the equation described by Effros et al.[18] The resulting dilution factor ranged from 12.8 to 21.1 with a mean of 16.7 ± 1.8. There were no differences in the dilution factor among AECC as well as LISS subgroups. In addition dilution factors did not differ between healthy volunteers and ventilated patients (healthy volunteers: 16.1 ± 1.8, ventilated patients: 16.7 ± 1.8; $P = 0.3$).

Methodical aspects of pH measurement

EBC-pH measured directly in ventilated patients (5.85 ± 0.32) and measured after argon dearation (5.98 ± 0.36) were closely correlated ($r = 0.89$,

$P<0.0001$). The standard deviation of repeated pH measurements were within the error of the pH probe (<10%).

EBC-pH, EBC-NH_4^+, and EBC-lactate in spontaneously breathing volunteers and ventilated patients

EBC-pH and EBC-NH_4^+ in mechanically ventilated patients were significantly decreased compared to spontaneously breathing individuals (EBC-pH: 5.85 ± 0.32 vs. 7.46 ± 0.48; $P<0.0001$; EBC-NH_4^+; EBC-NH_4^+: 26.5 μmol/l ± 16.4 vs. 222.8 μmol/l ± 112.9; $P<0.0001$).

In contrast, EBC-lactate was significantly higher in ventilated patients (0.45 μmol/l ± 0.3) compared to spontaneously breathing volunteers (0.13 μmol/l ± 0.09; $P<0.001$).

EBC-pH, EBC-NH_4^+, and EBC-lactate and severity of lung injury

Figure 1 summarizes EBC-pH, EBC-NH_4^+, and EBC-lactate in ventilated patients grouped according to both AECC as well as LISS criteria. EBC-pH and EBC-NH_4^+ were found to be decreased in patients with more injured lungs while EBC-lactate was increased.

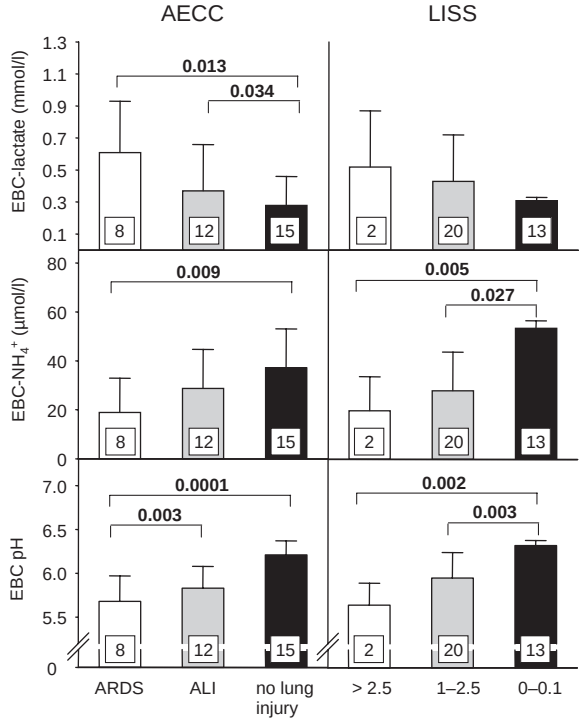

Figure 1 EBC-pH, EBC-NH_4^+, and EBC-lactate concentrations in lung disease severity subgroups according to the AECC and LISS classifications (Table 2).

EBC-pH was closely correlated with EBC-NH_4^+ ($r = 0.52$, $P<0.001$) and inversely correlated with EBC-lactate ($r = -0.53$; $P<0.001$).

Bicarbonate, pCO_2, and EBC-pH

A correlation of EBC-pH existed with EBC-pCO_2 ($r = -0.57$, $P<0.0001$) and EBC-HCO_3^- ($r = 0.54$, $P<0.001$). However, all three of these EBC parameters were not correlated with equivalent parameters in arterial blood.

Correlation of EBC-pH and pH of bronchial ELF

Bronchial ELF-pH determined in the lingual lobe bronchus in a subgroup of 19 patients did not exhibit a similar reduction in pH (bronchial pH: 7.04 ± 0.44; EBC-pH: 5.76 ± 0.29) and was only weakly and not significantly correlated with EBC-pH ($r = 0.4$, $P = 0.08$).

Pro-inflammatory cytokines and EBC-pH

IL-6 was detectable in EBC of 28 ventilated patients (85.1%). IL-6 was not detected in EBC from four patients without lung injury, two patient with ALI and one patient with ARDS according to the AECC classification. EBC IL-6 correlated inversely with EBC-pH ($r = -0.71$; $P<0.0001$; Fig. 2a).

IL-8 was detected in EBC of 23 ventilated patients (74.5%). IL-8 was not detected in EBC from seven patients without lung injury, four patients with ALI and one patients with ARDS according to AECC classification. Similarly to EBC IL-6, EBC IL-8 correlated inversely with EBC-pH ($r = -0.68$; $P<0.0001$; Fig. 2b).

In contrast, EBC-pH did not correlate with IL-6 or IL-8 in serum. TNF-α was not measurable in EBC. TNF-α was not measurable in EBC. The most likely reason for the non-detection of TNF-α in EBC was insufficient sensitivity (detection limit: 0.28 pg/ml) of the assay at low TNF-α levels. TNF-α levels, however, were measurable in serum. Both serum TNF-α and serum PCT were not correlated with EBC-pH.

EBC-pH and scores of lung injury and disease severity

EBC-pH was inversely correlated with specific lung injury scores determined at the time of breath condensate collection. Correlation of EBC-pH and paO_2/FiO_2 ratio was $r = 0.54$ ($P<0.001$; Fig. 3a) and of EBC-pH and LISS $r = -0.73$ ($P<0.0001$; Fig. 3b). In contrast to these lung specific scores, EBC-pH did

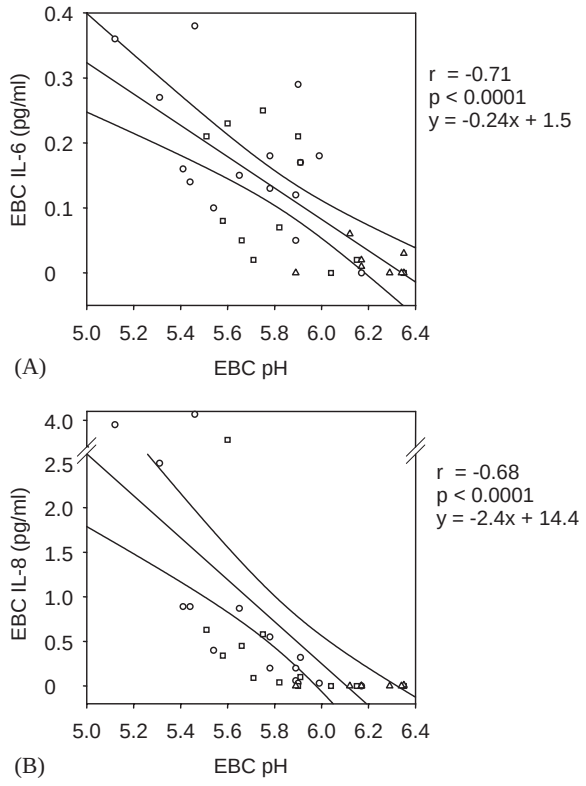

Figure 2 Correlation of (A) EBC IL-6 and (B) EBC IL-8 with EBC-pH in patients exhibiting ARDS criteria (circles), ALI criteria (squares), no signs of acute lung injury (triangles; AECC subgroups).

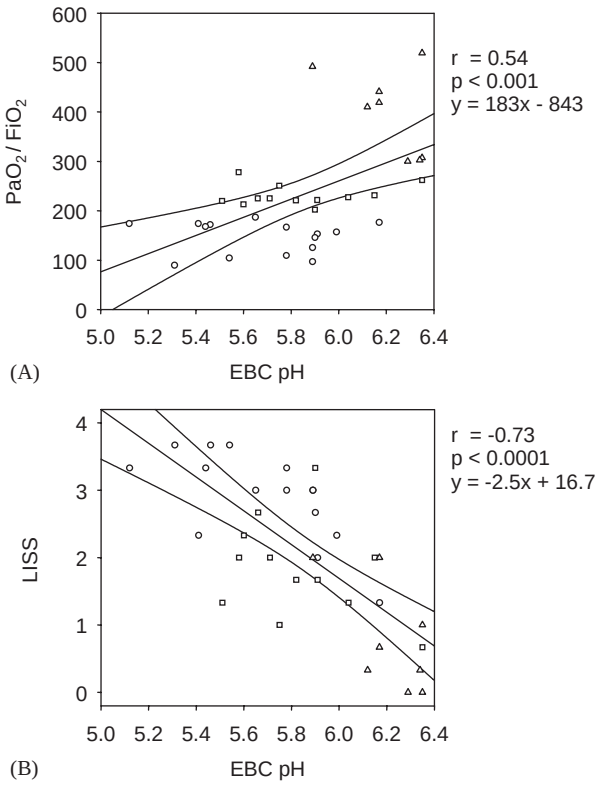

Figure 3 Correlation of (A) paO_2/FiO_2 and (B) LISS with EBC-pH in patients exhibiting ARDS criteria (circles), ALI criteria (squares), no signs of acute lung injury (triangles; AECC subgroups).

not correlate with a more generalized and widely used indicator of disease severity, the APACHE II score ($r = -0.14$; $P = 0.42$). Similarly, EBC-NH_4^+ was not correlated with the APACHE II score ($r = -0.2$; $P = 0.25$).

In contrast to EBC-pH, both EBC-NH_4^+ and EBC-lactate were not observed to correlate with paO_2/FiO_2 or LISS.

Discussion

In this study we report findings of an investigation of exhaled breath condensate pH (EBC-pH) in ventilated patients with and without signs of ALI or ARDS according to the AECC classification or LISS. The main findings were (i) a decreased EBC-pH, i.e. an acidification in EBC, accompanied by decreased ammonia and increased lactate in EBC in all ventilated patients in comparison with a control group of spontaneously breathing healthy volunteers, (ii) a close relation of exhaled breath condensate acidification and the extent of lung injury, (iii) an inverse correlation of EBC-pH with concentrations of pro-inflammatory cytokines in EBC but not in serum. The later findings suggest that a lower EBC-pH indicates inflammatory lung injury.

Amylase activity was not detectable in this study both in spontaneously breathing volunteers as well as in ventilated patients using an enzymatic assay. Although saliva contamination in spontaneously breathing individuals cannot be entirely excluded without the use of mass spectrometry, a relevant contamination may be ruled out. In fact, contamination is almost avoided due to intubation in ventilated patients. At the same time no differences in EBC sodium, potassium and dilution factors were found between spontaneously breathing volunteers and ventilated patients. This indicates that EBC of spontaneously breathing volunteers and ventilated patients may indeed be comparable. Differences between volunteers and ventilated patients therefore should not be due to saliva contamination or differences in dilution factors. This line of reasoning also argues against a significant influence of exogenous humidification on EBC parameters.

An important and as of yet unexplained result of our study was the remarkable difference in EBC-pH

between spontaneously breathing volunteers (7.46 ± 0.48) and ventilated patients (5.87 ± 0.31) which exceeds the differences found in disease severity subgroups of ventilated patients. This difference may be explained by (i) differences between healthy and injured lungs, (ii) differences between spontaneous breathing and mechanical ventilation or (iii) both. Hunt et al.[12] reported a mean EBC-pH of 7.65 ± 0.20 in spontaneously breathing control subjects, which is comparable to our findings. In acute asthma they found a decreased mean EBC-pH of 5.23 ± 0.21. Thus the extent of EBC acidification in asthma in spontaneously breathing patients exceeded that observed in our study in ventilated patients with various degrees of inflammatory lung disease leading to acute lung injury. Although we cannot from our data estimate the effect of intubation and ventilation on EBC-pH the study of Hunt et al. demonstrates that the spectrum of EBC-pH in non-ventilated patients encomprises the spectrum observed in our study.

The subgroup analysis for lung injury severity revealed that the greatest changes of EBC-pH, EBC-lactate and EBC-NH_4^+ occurred in the group of patients with the greatest extent of lung injury. This observation is strengthened by the correlation of EBC-pH and clinical scores of lung injury (both LISS and AECC score). These findings support EBC-pH to be a marker of lung injury. However, lung injury may be caused by a variety of reasons and may be due to pulmonary inflammation or else occur in response to systemic disorders which are usually also inflammatory in a broader sense. Because EBC acidification correlates well with inflammatory cytokines in the very material which is used for pH measurement such as EBC-IL6 and EBC-IL8 we propose EBC-pH to be a marker of pulmonary inflammatory lung injury. A correlation of EBC-pH and serum IL-6 and IL8 as well as PCT in serum would in this case not be expected and indeed was not observed in this study. EBC-pH may therefore, similar to the situation in acute asthma, be the consequence of a local (pulmonary and/or airway) inflammation of such an extent, that lung injury occurs.

What are the mechanisms in acute inflammatory lung injury leading to EBC acidification? Several mechanisms may be envisioned that could disturb the local pH homeostasis: (i) The lung is highly dependent on glucose for both energy demand as well as phospholipid synthesis. Aerobic as well as anaerobic glucose utilization takes place in alveolar epithelial cells.[19] In hypoxia, continuing glucose utilization for energy demands will result in increased lactate production. However, lactate generation of the lung in acute lung injury is complex, but the lung has been recognized to be a major source of lactate production. Lactate flux was found to correlate with LISS in a study determining the arterial–venous lactate differences in acute lung injury.[20] In our ventilated patients lactate levels were generally elevated compared with those in healthy volunteers and were more increased in patients with higher lung injury scores (Fig. 1). Increased pulmonary lactate release is therefore likely to contribute to EBC acidification. (ii) A mechanism that may counteract airway acidification was recently suggested by Hunt et al.[13]: secretion of ammonia due to glutaminase activity generating glutamate from glutamine. Glutaminase has been demonstrated in bronchial epithelial cells as well as in the AT II cell derived A549 cell line. Inflammatory cytokines inhibit glutaminase resulting in the inhibition of alkalinization and promotion of acidification.[13] The authors reported low levels of EBC-ammonia in acute asthma (median 30 μM) versus controls (median 327 μM) comparable to the differences observed in acute lung injury versus volunteers in our study.[13] EBC-acidification may therefore be due to essentially two mechanisms: pulmonary lactate production and a reduced local buffer capacity (i.e. ammonia).

pH in the larger airways in our hands did not correlate with EBC-pH in a subset of 19 patients in whom both measurements were performed. The two measurements were performed by different devices, but both devices were calibrated before use and calibration solutions resulted in very similar results. Although the generation of an aerosol in distal airways or alveoli has been suggested to be a likely mechanisms,[1] breath condensate generation is not completely understood. The compartmentalization of lung injury may explain the differences in pH values of EBC (distal lung) and the lining fluid in central large airways.

We conclude that EBC-pH may reflect the extent of acute lung injury and because it correlates with proinflammatory cytokines locally—but not systemically—this injury is likely to be caused by or accompanied by pulmonary inflammatory processes. Because EBC-pH measurement is rapid and does not require extensive instrumentation it may be useful for both the estimation of the actual extent of acute lung injury as well as a repetitive monitoring parameter in ventilated patients.

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