The effects of lung recruitment maneuvers on exhaled breath condensate pH

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Keywords: exhaled breath condensate, airway pH, lung recruitment

Abstract
Exhaled breath condensate (EBC) pH serves as a surrogate marker of airway lining fluid (ALF) pH and can be used to evaluate airway acidification (AA). AA is known to be present in acute respiratory distress syndrome (ARDS) and can be evaluated via continuous EBC pH measurement during mechanical ventilation. Lung recruitment maneuvers (LRMs) are utilized in the treatment of ARDS, however, their impact on EBC pH has never been explored. Here we described the acute effects of two commonly used LRMs on EBC pH. In a prospective, non-randomized, serial exposure study, 10 intubated pediatric subjects with acute respiratory distress syndrome sequentially underwent: a period of baseline ventilation, sustained inflation (SI) maneuver of 40 cm H2O for 40 s, open lung ventilation, staircase recruitment strategy (SRS) (which involves a systematic ramping of plateau pressures in 5 cm H2O increments, starting at 30 cm H2O), and PEEP titration. Maximum lung recruitment during the SRS is defined as a PaO2 + PaCO2 of >400 mmHg. Following lung recruitment, PEEP titration was conducted from 20 cm H2O in 2 cm H2O decrements until a PaO2 + PaCO2 was <380 and then increased by 2 cm H2O. EBC pH, arterial blood gases, lung mechanics, hemodynamics, and function residual capacity were obtained following each phase of the LRM and observational period. Seven out of 10 patients were able to reach maximum lung recruitment. Baseline EBC pH (6.38 ± 0.37) did not correlate with disease severity defined by PaO2/FiO2 ratio or oxygenation index (OI). Average EBC pH differed between phases and decreased after LRM (p < 0.001). EBC pH is affected by LRMs. EBC acidification following LRMs may represent a washout effect of opening acidic lung units and ventilating them or acute AA resulting from LRM.

Introduction
Acute respiratory distress syndrome (ARDS) is a diffuse, inflammatory lung response marked by increased pulmonary vascular permeability, increased lung water, and alveolar collapse [1, 2]. The clinical hallmarks of the disease are intrapulmonary shunting, hypoxemia, increased physiological dead space, worsening lung compliance and bilateral radiographic opacities [3]. Its histologic features include diffuse alveolar damage that appears as edema, inflammation, hyaline membrane, or hemorrhage occurring in response to local and systemic disease [4].

The acid-base chemistry of the airway lining fluid (ALF) may contribute to the pathophysiology of ARDS. Airway acidification (AA) may result from factors both intrinsic and extrinsic to the lung, and can result in one or more of the following: ciliary dysfunction, epithelial dysfunction, augmented oxidative injury, abnormal fluid transport, neutrophilic and eosinophilic inflammation, bronchospasm, bronchial hyper-reactivity, inhibition of transport of cationic drugs such as albuterol, and alteration of apoptosis [5–13]. Measuring ALF pH directly requires insertion of a pH probe within the airway and has not routinely been explored. Samples measured by this technique directly reflect only the pH of the local environment sampled which makes it impossible to evaluate the ALF pH of the lung unit as a whole, especially in heterogeneous disease such as ARDS14. Investigators have overcome this shortcoming by quantification of the pH of exhaled breath condensate (EBC) pH. To collect EBC, exhaled body-temperature, pressure saturated breath is condensed, collected and analyzed. This method serves as a surrogate marker for ALF pH and allows exploration without additional risks [15].
Over the past 15 years, acidification of EBC pH has been studied as a biomarker in a variety of diseases including asthma, [6, 16, 17] cystic fibrosis, [18, 19] respiratory syncytial virus, [14] bronchopulmonary dysplasia, [20] and ARDS [21, 22]. It is possible that the ability of the airway epithelium to acidify in response to various insults can be reflected through EBC pH [23–25]. Although airway acidification (AA) may be enhanced by Th1 cytokines [26], it also serves as an active and rapid innate immune response against invading pathogens by chemically attracting and activating relevant immunologic components of the expanded innate immune system including neutrophils [27–29]. Evidence suggests that AA can originate from aspiration of gastric content or from intrinsic alteration in acid base balance in the lung [30].

Mechanical ventilation is standard of care for the treatment of ARDS. It has been associated with adverse events such as ventilator-induced lung injury, sedative dependency, cardiovascular instability, and nosocomial infections of the respiratory tract. Duration of mechanical ventilation as well as plasma IL-6 and plasma IL-8 levels have correlated with EBC pH [31]. Bastin, et al reported a rapid (within 30 min) reduction in EBC pH following lung resection and one lung ventilation [31]. There is also evidence that invasive positive pressure ventilation alone (without lung disease or sepsis) results in a lower EBC pH when compared to controls [32].

It is a standard of practice to use increased fraction of inspired oxygen (FiO₂), as well as increasing airway pressure to recruit lung regions that suffer from collapse in ARDS in order to improve gas exchange. Sequela of heterogeneous lung disease and surfactant dysfunction is that compliant/healthy lung units receive more ventilation than diseased units and therefore are more susceptible to injury when aggressive ventilator settings are used to improve gas exchange. Maneuvers designed to open these less compliant units, known as lung recruitment maneuvers (LRM), apply sufficient pressure over time to open (recruit) and ventilate the diseased lung.

LRMs have been used to reverse episodes of profound hypoxemia, applied empirically or utilized as part of a ventilation strategy [33–41]. LRM may have adverse effects. Ventilating atelectatic regions of the lung may expose healthier lung units to organisms or pathogens that had been trapped by atelectasis. Also, overdistention of the healthier lung units is required to open the less compliant lung units and may result in volu/barotrauma and vascular compression locally as well as decreasing venous return to the thorax. We report the effects of two different lung recruitment maneuvers on EBC pH.

Materials and methods

This study was performed as part of a prospective study evaluating two types of LRM in patients with ARDS. It was approved by the institutional review board of Boston Children’s Hospital and registered at http://clinicaltrials.gov—NCT 00830284.

Subjects and monitoring

Patients admitted to the medical/surgical ICU at Boston Children’s Hospital over a two year period, were screened for this study. Inclusion criteria included: 44 week post conceptual age to 17 years old; diagnosis of ARDS according to the Berlin Definition [42] (with the exception of age), demonstrated apnea due to neuromuscular blockade or deep sedation; presence of an indwelling arterial line; presence of a cuffed endotracheal tube, conventional mechanical ventilation; and written informed parental consent. We conformed to the accepted standards of the Berlin definition of ARDS. Exclusion criteria were: diagnosis of ARDS according to the Berlin Definition for >72 h; active hemodynamic instability; history of prematurity; clinically recognized airway disease; known congenital heart disease; congenital diaphragmatic hernia; recent history of thoracic instrumentation; known restrictive lung disease, cystic fibrosis, or active treatment with inhaled nitric oxide; severe brain injury; or the use of extracorporeal life support.

Outcome measures included heart rate, oxygen saturation, invasive arterial blood pressure and EBC pH, all which were continuously monitored. Vital signs, EBC pH, arterial blood gases, FRC by multiple-breath nitrogen washout (Engstrom Carestation, GE Healthcare, Madison, WI), static compliance, and tidal volume (VT) were recorded between each phase of the protocol. Dead space ventilation ratio and elimination of carbon dioxide were also measured at those time points.

EBC collection from the mechanical ventilator and analysis of EBC pH occurred continuously using the airway lining fluid analyzer (ALFA Monitor, Respiratory Research, Inc., Austin, TX). See figure 1. This device condenses exhaled breath from the expiratory limb of the mechanical ventilator by exposing it to a cooling chamber, gas-standardizes the condensate with 100% oxygen to remove carbon dioxide, and collects the condensate in a chamber where a pH probe resides and measures pH in real-time [22]. This yields a continuous and responsive measurement.

Experimental protocol

All subjects were in the supine position, sedated, and received an FiO₂ of 1.0, except where specified. All subjects were assessed for intravascular volume status and underwent pressure volume curve determinations to obtain the upper and lower inflection points via the AVEA maneuvers (Carefusion, Yorba Linda, CA). Complete lung opening was defined as the sum of PaO₂ and PaCO₂ exceeding 400 mm Hg as proposed by Borges et al to be associated with less than 5% atelectasis in adults [43, 44].
The protocol consisted of five phases (figure 2): baseline ventilation (phase 1); sustained inflation (SI) followed by open lung ventilation (phase 2: 40 cm H₂O for 40 s, PEEP set 2 above LIP, 6 mL Kg⁻¹ VT, and rate per baseline minute ventilation); staircase recruitment strategy (SRS) (phase 3: PC ventilation of 15 cm H₂O above PEEP of 15 cm H₂O and increasing both by 5 cm H₂O every 5 min until PaO₂ + PaCO₂ was <400 or an inspiratory pressure of 50 cm H₂O was reached); PEEP titration (phase 4-following SRS, PEEP was adjusted to 20 cm H₂O and decreased by 2 cm H₂O every 5 min until PaO₂ + PaCO₂ < 380, which was then defined as closing PEEP); and observation (phase 5-PEEP was maintained 2 cm H₂O above closing PEEP for 60 min).

**Stopping criteria**
Criteria for aborting the study at any step included arterial pH below 7.00 regardless of cause, decrease in mean arterial blood pressure by more than 20%, increase in vasoactive support by >50%, an arterial lactate >2 mg dL⁻¹, peak inspiratory pressure above upper inflection point of the PV curve, or an oxygen saturation below 80%.
Data management
All ventilator data, arterial blood gas values, pulmonary mechanics, and EBC pH were recorded on a data collection form and subsequently entered into a database. SPSS was utilized for analysis (Version 21, IBM, Armonk, NY). One-way repeated measures ANOVA with Bonferroni correction was used to detect differences in EBC pH during and after each phase of the study, as compared to baseline values. Correlations were calculated using Spearman’s rho. Statistical significance was considered with a P value of $\leq 0.05$.

Results
During the study period, 58 patients with the diagnosis of ARDS were assessed for eligibility. Of these patients, 46 were excluded and 12 were enrolled. The primary reason for exclusion was for spontaneous breathing. Others included prematurity, known restrictive lung disease, severe reactive airway disease, age >18 years, air leak or presence of a thoracostomy tube, research team member unavailability, hemodynamic criteria, unilateral disease, pericardial effusion, PEEP >15 cm H2O, primary pulmonary hypertension, and tracheomalacia. Of the 12 enrolled, one was excluded due to heart block during baseline observation, and a second was excluded because of a small pneumothorax discovered on most recent chest x-ray during the timeout period prior to start of protocol. Table 1 outlines the subject demographics, EBC pH, and ventilator parameters prior to initiation of the protocol.

Table 1. Patient demographics and baseline findings.

| Subject | Gender | Diagnosis                          | Age (yr) | IBW (kg) | EBC pH | FiO2 | P/F Ratio$^a$ | PEEP (cm H2O) | Crs | OI$^b$ |
|---------|--------|------------------------------------|----------|----------|--------|------|---------------|---------------|-----|------|
| 1       | Female | Parainfluenza pneumonia            | 5.9      | 20       | 6.1    | 0.7  | 87            | 12            | 0.7 | 16   |
| 2       | Male   | Kawasaki syndrome                  | 4.2      | 16       | 6.2    | 1.0  | 65            | 12            | 0.4 | 28   |
| 3       | Female | Sepsis                             | 10.2     | 14       | 6.6    | 0.6  | 192           | 12            | 0.5 | 9    |
| 4       | Female | Sepsis                             | 5.6      | 34       | 5.9    | 0.5  | 224           | 8             | 0.4 | 7    |
| 5       | Female | Necrotizing pneumonia              | 10.4     | 39       | 6.8    | 0.6  | 123           | 14            | 0.2 | 17   |
| 6       | Female | Sepsis                             | 13.9     | 55       | 7.0    | 0.6  | 123           | 14            | 0.3 | 23   |
| 7       | Female | Macrophage activation syndrome     | 17.3     | 73       | 6.4    | 0.7  | 201           | 10            | 0.5 | 7    |
| 8       | Female | Respiratory syncytial virus pneumonitis | 4.9  | 18       | 6.0    | 1.0  | 60            | 15            | 0.5 | 30   |
| 9       | Male   | Sepsis                             | 11.7     | 43       | 6.1    | 0.4  | 268           | 12            | 0.5 | 6    |
| 10      | Female | Trauma                             | 9.4      | 39       | 6.7    | 0.4  | 188           | 10            | 0.4 | 8    |

| Mean    | 9.3    | 35.1 | 6.38 | 0.65 | 153.1 | 11.9 | .44 | 15.1 |

$^a$ PaO2/FiO2 upon meeting inclusion criteria.
$^b$ Oxygenation index = FiO2 X mean airway pressure/PaO2.
IBW = ideal body weight.
Crs = compliance of the respiratory system.

EBC pH correlations at baseline
Baseline EBC pH (mean ± SD) was 6.38 ± 0.37 (n = 10). There was a negative EBC pH correlation with respiratory system compliance ($r = −0.63$, n = 55). EBC pH had a positive correlation with baseline CO2 elimination ($r = 0.53$, n = 55). No other correlations at baseline were significant.

EBC pH correlation among all samples
ABG pH correlated negatively with EBC pH when testing all intervals ($r = −0.31$, n = 55 measurements). There was a negative EBC pH correlation with respiratory system compliance at all testing intervals ($r = −0.34$, n = 55). EBC pH had a negative correlation with FRC when compared among the testing intervals ($r = −0.34$, n = 55). (See figure 3. No other correlations were observed.

Effects of lung recruitment and PEEP titration on EBCpH
A one-way repeated measures ANOVA was conducted to compare EBC pH in patients undergoing lung recruitment at the end of each of the following phases: baseline, sustained inflation, open lung ventilation, maximal recruitment, optimal PEEP, and observation. Results indicate that EBC pH differed over phases, F(df 5, mean sq 0.172) = 5.32, p = 0.001. Table 2 describes the mean, standard deviation and number of patients tested. Pairwise comparisons among means did not significantly differ between individual phases. See figure 4.
Discussion

New discoveries are being reported yearly primarily due to the fact that EBC can be safely collected during mechanical ventilation and analyzed for a variety of markers [14, 15, 31, 32, 45, 46]. EBC pH can not only be collected safely during mechanical ventilation, but also can be collected and analyzed continuously throughout the course of mechanical ventilation [22]. This allows researchers to detect changes in EBC pH in near real-time. Here, we have shown that EBC pH decreases following lung opening in a series of 10 pediatric patients with ARDS undergoing serial LRMs. EBC pH in all patients was acidic at baseline, compared to the normal cutoff of 7.4 [14] and the EBC pH continued to fall after reversal of atelectasis. This leads us to several possible hypotheses: 1) The diseased or collapsed alveoli are acidic and are ‘flushed out’ by restoration of ventilation and subsequently detected within EBC; 2) LRM produce an acidic response within the lung that is detected within EBC; 3) A combination of 1 and 2 that lead to the increase in acidity measured; or 4) some level of contamination from the ALFA system is responsible for the observation (although it is unlikely that this contamination would be found in all subjects).

AA as a component of ARDS

EBC has been found to be acidic in adults with ARDS [21]. Many clinical findings associated with ARDS are too quickly attributed to the syndrome generally instead of considering them direct effects of specific pathologic abnormalities such as AA. It has been suggested that airway acidity may contribute to extensive and diverse dysfunction at the chemical, cellular, physiologic, and symptom levels. ARDS has often been broken into two types, primary and secondary. Primary ARDS has been associated with injury that has occurred within the lung. Secondary ARDS has been associated with injury occurring outside the lung such as in sepsis, trauma, or other organ derangements. It has been speculated that the type of ARDS should influence your management of these patient, however to our knowledge no one has described EBC pH difference between these type of ARDS.

Table 2. Descriptive statistics for EBC pH from baseline to observation phase of the protocol.

|             | Mean  | Std. Deviation | N  |
|-------------|-------|----------------|----|
| Baseline    | 6.40  | .39            | 10 |
| SI          | 6.43  | .41            | 10 |
| Open lung vent | 6.38 | .31            | 10 |
| Max recruit | 6.28  | .46            | 9  |
| Optimal PEEP | 6.14 | .41            | 9  |
| Observation | 6.11  | .37            | 10 |
The EBC pH values reported in this study, while acidic, are not as profoundly acidic as we expected. Previously, we have reported EBC pH values below 6 during acute asthma exacerbations and other inflammatory pulmonary disorders. While the values reported here and their response to the LRM interventions are consistent with our hypothesis, it is surprising that the EBC pH reported in these critically ill subjects is not lower. A possible explanation for this is that these patients had been stabilized in the ICU prior to enrollment in the study, and that their pH at the onset could be lower than observed at the time of enrollment.

Observed correlations with EBC pH and their possible relationship

The negative correlation between EBC pH, respiratory system compliance, and FRC are interesting discoveries. Conceivably the alveolar space is more acidic than the airways. It is possible that the more compliant or the higher the FRC there is, the more alveolar contribution of volatile acids there will be to the system. These hypotheses would explain the decrease in EBC pH after ‘opening’ the alveolar units via LRM. The negative correlation between EBC pH and ABG pH is also interesting. It would stand to reason that systemic acidosis would contribute to airway acidification or at least inflammation, but, this data contradicts that. One possible explanation could be related to ARDS disease severity. As ARDS worsens, ABG pH may decrease due to an inability to properly ventilate a subject and remove CO2 from the blood. In conjunction with this, as ARDS severity increases, alveolar ventilation becomes more difficult, which could lead to more ‘trapping’ of acidic compounds in poorly ventilated units. In turn, as these acids are trapped in poorly ventilated units, they would not contribute to EBC pH, leading to an increase in EBC pH as ABG pH (and potentially alveolar ALF pH) decrease. This observation could also explain the witnessed decrease in EBC pH following LRM, as these acidic units are ‘flushed’ of their volatile acids by ventilation.

AA as a result of LRM-related injury

The presence of ventilator-associated lung injury related to the protocol cannot be completely ruled out, though the absence of air leak was reassuring. In fact, positive pressure ventilation alone without lung disease has been associated with a lower EBC pH therefore the AA at baseline in our patient population with significant lung injury is not surprising [46]. As subjects approached the critical opening pressure during SRS, VT decreased, respiratory system compliance (CRS) decreased, and carbon dioxide elimination decreased. The pressures required to recruit densely consolidated lung units concurrently overdistant more compliant/healthier units. During this process, LRM may injure the healthiest lung units and be the catalyst for additional inflammation. Pugin and colleagues demonstrated that cyclic stretch of human lung cells induces an acidification and promotes bacterial growth [47]. The lower EBC pH could be the result of the stretching or overdistantion the lung may undergo during LRM.

LRMs can transiently improve oxygenation and pulmonary mechanics, but may also injure airway epithelium and endothelium and may increase alveolar-capillary permeability [48–51]. It has been recently demonstrated that high flow, rapid rise to pressure used with SI may worsen markers of airway epithelial cell damage when compared to lower flow, slower rise to pressure maneuvers in animals [52]. In our study we applied SI to all subjects. This high flow rapid rise to pressure may injure the lung and lead to additional AA seen at the end of the study. While this was never our intent, it cannot be ruled out as a complication of the recruitment maneuver sequence we used.

Limitations

This study has several limitations. Patients with ARDS, indwelling arterial line, and no spontaneous respirations are rare within our center and limited our sample size. This limited sample sizes did not allow us to develop cohorts of patients by type of ARDS injury or disease severity to further analyze. In addition, ARDS is a largely heterogeneous syndrome with complex and
variable treatment strategies and modalities. Ventilator settings, oxygenation strategies, and other pulmonary-specific interventions widely vary between subjects and could certainly confound our results. Future studies should be conducted that incorporate a larger sample size and more homogenous population. Also, we only monitored for an additional hour after our last change, which limits our ability to determine if EBC pH would return to baseline over time.

An acidic EBC pH can only occur as a result of an acidic airway lining fluid pH at some level. EBC pH measurement within a log scale represents a net proton concentration signal resulting from amounts and ratios of multiple acids and bases coming from multiple ventilated lung units. The normal EBC pH in the published literature of adults is between 7.7–8.2 [15, 53] and 7.7–8.1 in children [17, 18, 54–58]. However, all reports show a cutoff from normal at approximately 7.4 [14, 45]. While no healthy controls were used to compare our findings, there is sufficient evidence that the EBC pH values we report are markedly low.

This study incorporated EBC from the lower airways only, effectively excluding oro/nasopharyngeal contamination that can add ammonia, which can neutralize volatile exhaled acids and decrease the sensitivity of orally collected EBC for airway acidity assessment. Thus our system is relatively immune to critiques of poor sensitivity caused by the upper airway. It is also relatively, but not completely immune to concerns about gastric acid reflux and aspiration, which can certainly cause declines in airway pH. We attempted to reduce the likelihood of gastric micro-aspiration by only evaluating patients that had cuffed endotracheal tubes that were maximally inflated in order to maintain a seal throughout the protocol and by not adjusting head position nor endotracheal tubes during the study.

There are reasons to suspect that the effects of our recruitment maneuvers on actual airway pH may be more pronounced than our EBC pH data reveal. First, EBC pH is a marker of airway acidity, but as measured by the ALFA technology it is a moving average of the sample collected from the entire ventilated lower airway. The length of the moving average is shorter or longer depending on the minute ventilation of the patient, volatiles alkalines/acidcs and rate of EBC formation and collection. The absolute humidity level of the gases leaving the mechanical ventilator and the efficiency of the condenser also play a role in condensate formation. Newly collected EBC gradually replaced previously collected EBC in the ALFA’s gravity fed system over a period of 10 to 30 min prior to the continuous pH reading (Walsh, B unpublished bench study data).

Subsequent studies aimed at identifying whether the acidification of EBC pH demonstrated here results from the recruitment of atelectatic, acidified airways or from the an intrinsic acidification response to the LRM would further elucidate the results we report and are desired.

Conclusion

EBC pH at baseline is acidic in our sample of pediatric patients with mild to severe ARDS. EBC pH becomes increasingly acidic and declines an average of 0.29 pH units following LRM. This decline is attributed to either a washout effect of opening previously collapsed acidic lung units, acute AA as a direct result of the procedure of lung recruitment, or a combination of effects. There was a negative correlation between EBC pH, compliance, ABG pH and FRC. Future studies are needed to delineate if results differ by disease ARDS severity or type of initial injury and whether EBC pH could play a role in new treatments or outcomes of this critically ill patient population.

Acknowledgments

BKW contributed to the development of protocol, enrolling patients, statistical analysis and drafting the manuscript. MDD contributed to the drafting of the manuscript and analysis of EBC data. JFH contributed to the drafting of the manuscript and analysis of EBC data. JNK contributed to the development of protocol, enrolling patients, statistical analysis and editing the manuscript. CDS contributed to drafting the manuscript and assisting with IRB compliance. JHA contributed to the development of protocol, enrolling patients and editing the manuscript.

JFH is founder and director of Respiratory Research, Inc. JFH and BKW are co-inventors of IP relating to the Alfa collection device used in this study. Otherwise, there are no conflicts of interest to disclose.

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