Breath Condensate pH in Children With Cystic Fibrosis and Asthma*
A New Noninvasive Marker of Airway Inflammation?

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Study objectives: The noninvasive assessment and monitoring of airway inflammation could be important in respiratory disease. The pH of exhaled breath condensate (EBC) is a promising marker. Although pH has been measured in the EBC of adults with inflammatory airway diseases, no study has measured this in children.

Design: This study aimed to assess whether there is a change in pH in the EBC of children with cystic fibrosis (CF) and asthma, and to try to determine whether pH could be used as a marker of airway inflammation. Furthermore, the relationships among EBC pH, severity of disease, and oxidative stress were studied.

Patients and methods: We studied 20 children with CF (mean [± SEM] age, 7 ± 3 years), 20 children with asthma (mean age, 7 ± 2 years), and 15 age-matched healthy children (mean age, 7 ± 2 years). The pH of EBC was measured using a pH meter.

Measurements and results: Lower pH values were observed in the EBC of children with CF and asthma compared to control subjects (mean pH, 7.23 ± 0.03 and 7.42 ± 0.01 vs 7.85 ± 0.02, respectively). Furthermore, relationships among EBC pH, severity of asthma, and the presence of an infective exacerbation of CF was found. There was a negative correlation between exhaled pH and exhaled leukotriene B4 concentrations (r = −0.5; p < 0.005).

Conclusion: We conclude that the measurement of EBC pH may be useful in the evaluation of airway inflammation in children with asthma and CF.


Key words: airway inflammation; asthma; cystic fibrosis; 8-isoprostane; exhaled breath condensate; leukotriene B4; pH

Abbreviations: CF = cystic fibrosis; EBC = exhaled breath condensate; IL = interleukin; LT = leukotriene

Airway inflammation plays a key role in the pathogenesis of asthma and cystic fibrosis (CF). In asthma, airway inflammation is thought to be the fundamental abnormality, which results in variable airway obstruction and bronchial hyperreactivity.1 In CF, chronic airway inflammation is the result of a combination of repeated bacterial lung infection and an exaggerated host response to this process.2 Since airway inflammation is important in many respiratory diseases, and because anti-inflammatory therapy with corticosteroids and leukotriene (LT) receptor antagonists is widely prescribed for its treatment, many groups have focused their research on finding a noninvasive method for assessment and monitoring.

The measurement of inflammatory markers in the exhaled breath condensate (EBC) of adults and children has added a new perspective to the study of airway inflammation.3–9 The markers that have been studied include interleukin (IL)-1β,10 IL-8,11 IL-10,12 IL-6, tumor necrosis factor-α,10 and LTB4.8 EBC pH is simple to measure, noninvasive, inexpensive, and reproducible,13 and also may be a marker of airway inflammation. Abnormally low (ie, acidic) pH levels already have been measured in the breath condensates of adults with asthma, CF, COPD, and bronchiectasis. However, no study has measured EBC pH in children.

The aim of the present study was to assess the pH of the EBC in children with CF and asthma, and to compare it with the concentrations of another known *From the Department of Thoracic Medicine, National Heart & Lung Institute, Imperial College, London, UK. Manuscript received July 10, 2003; revision accepted February 20, 2004. Reproduction of this article is prohibited without written permission from the American College of Chest Physicians (e-mail: permissions@chestnet.org). Correspondence to: Sergei A. Kharitonov, MD, PhD, Department of Thoracic Medicine, National Heart & Lung Institute, Imperial College, Dovehouse St, London SW3 6LY, UK; email: s.kharitonov@imperial.ac.uk

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inflammatory marker that is measurable in EBC, namely, LTB4. Furthermore, the relationships among EBC pH, severity of the diseases, and oxidative stress also were explored.

**Materials and Methods**

**Patients**

We studied 20 children with CF (11 male children; mean [± SEM] age, 7 ± 3 years; mean FEV1, 61 ± 2.4%; mean FVC, 73 ± 2.2%), 20 children with asthma (10 male children; mean age, 7 ± 2 years; mean FEV1, 69.3 ± 4.2%; and mean FVC, 78.4 ± 2.7%), and 15 healthy children (6 male children; mean age, 7 ± 2 years; mean FEV1, 102.2 ± 1.8%; mean FVC, 104.1 ± 2.3%) without any history of acute or chronic respiratory symptoms. All of the patients underwent a full history and physical examination. Both patients and control subjects were white and were recruited by the Royal Brompton Hospital, and all were able to perform spirometry reproducibly. Informed consent was obtained both from all the subjects enrolled and their caregivers, and the Ethics Committee of the Royal Brompton and Harefield National Health Service Trust approved the study.

**Asthma Group**

Asthma diagnosis and severity were established according to the criteria of the American Thoracic Society.14 Atopic status was confirmed in by skin-prick test for four common aeroallergens (ie, house dust mite, grass pollen, Aspergillus fumigatus, and cat fur) [ALK Abello; Horsholm, Denmark]. The severity of asthma was classified according to the National Heart, Lung, and Blood Institute guidelines.15 Children with mild intermittent asthma (five children) had symptoms less often than weekly and were not receiving any medication on a regular basis, but they did use an inhaled β2-agonist, as needed, for symptom relief. Children with mild persistent asthma (four children) had more frequent but not daily symptoms and were given therapy with inhaled corticosteroids (budesonide, 0.2 to 0.4 mg; fluticasone propionate, 0.1 to 0.2 mg) regularly (starting 2 to 4 months before entering the study). Children with moderate-to-severe persistent asthma (11 children) had daily symptoms and were taking high-dose inhaled steroids regularly (budesonide, > 0.4 mg/d; fluticasone propionate, > 0.2 mg/d).

**CF Group**

Children with CF received diagnoses on the basis of the typical symptoms of the condition, two mutations in the CF gene, and an abnormal sweat test result (ie, sweat chloride concentration, > 60 mmol/L).16 Ten children were studied during an exacerbation of their lung disease, which had been diagnosed using conventional criteria,17 and the other 10 children were stable. All children were chronically infected with Pseudomonas aeruginosa, Staphylococcus aureus, or both. Additional exclusion criteria were the concurrent diagnosis of asthma, current oral steroid therapy, and a sputum culture positive for Burkholderia cepacia.

**Lung Function**

Spirometry (Erich Jaeger; Market Harborough, UK) was performed within 1 day of EBC collection. The best value of three maneuvers was expressed as a percentage of the predicted normal value.

**EBC**

EBC was collected using a condenser that allowed for the noninvasive collection of the nongaseous components of the expired air (EcoScreen; Jaeger; Wurzburg, Germany), as a previously described.18

**Assays**

A specific enzyme immunoassay (Cayman Chemical; Ann Arbor, MI) was used to measure LTB4 in the EBC. Intra-assay and interassay variability was < 10%. The specificity was 100%, and the detection limit of the assay was 3 pg/mL. A specific enzyme immunoassay kit (Cayman Chemical) was used to measure 8-isoprostane concentrations in EBC. Intraassay and interassay variability was ± 5% and 6%, respectively. The detection limit of the assay was 4 pg/mL. The reproducibility of repeated LTB4 and 8-isoprostane measurements was assessed by the Bland-Altman method and by the variation coefficient.19

**pH Measurement**

A stable pH was achieved in all cases after deaeration/decarbonation of the EBC specimens by bubbling them with argon (350 mL/min) for 10 min, as previously reported.20 pH then was measured by means of a pH meter (Jenway-350; Jenway; Gransmore Green, UK) with a 0.00 to 14.00 pH range and a mean resolution/accuracy on the order of 0.01 ± 0.02 pH. The reproducibility of the repeated measurements of pH was confirmed by the Bland-Altman test and by the variation coefficient.

**Statistical Analysis**

Data were expressed as the mean ± SEM. Mann-Whitney tests were used to compare groups, and correlations between variables were performed using the Spearman rank correlation test. Significance was defined as p < 0.05.

**Results**

**EBC pH**

EBC pH was lower in CF children than in healthy control subjects (7.23 ± 0.03 vs 7.85 ± 0.02, respectively; p < 0.0001) [Fig 1, top, A]. The EBC pH of CF patients with an exacerbation was significantly lower than that of stable patients with CF (7.12 ± 0.02 vs 7.31 ± 0.01, respectively; p < 0.0001) [Fig 1, middle, B]. EBC pH was lower in children with asthma compared to healthy control subjects (7.42 ± 0.01 vs 7.85 ± 0.02, respectively; p < 0.0001) [Fig 1, top, A]. A lower pH was observed in children with severe and moderate asthma compared with those with mild asthma (7.36 ± 0.02 vs 7.49 ± 0.01, respectively; p < 0.0005) [Fig 1, bottom, C]. No correlations were observed among exhaled pH, FEV1, and FVC. The reproducibility of EBC pH measurements was assessed in 10 nonsmoking healthy adults (six men; mean age, 35 ± 7 years). The mean difference between the two measurements was −0.01 ± 0.4. The coefficient of variation for EBC pH was 0.4% [Fig 2, top, A].

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Clinical Investigations
Exhaled LTB₄

LTB₄ was detectable in the EBC of all subjects. LTB₄ concentrations were significantly increased in children with CF compared to the healthy control subjects (35.9 ± 4.1 vs 7.4 ± 0.3 pg/mL, respectively; p < 0.0001) [Fig 3, top, A]. A significant elevation of LTB₄ levels was observed in children with an acute exacerbation of CF (47.8 ± 5.9 pg/mL) compared with the children with stable CF (24.2 ± 1.8 pg/mL; p < 0.0005). The asthmatic children had higher levels of LTB₄ compared to the healthy control subjects (18.8 ± 2.4 vs 7.4 ± 0.3 pg/mL; p < 0.0001) [Fig 3, middle, B]. The degree of reproducibility between two successive measurements of pH (top, A), LTB₄ concentration (middle, B), and 8-isoprostane concentration (bottom, C) in the breath condensate of healthy subjects using the Bland-Altman method.

**Figure 1.** Top, A: EBC pH in children with CF, children with asthma, and healthy control subjects. Middle, B: EBC pH of CF children with respiratory exacerbation of their disease and in stable conditions. Bottom, C: EBC pH of children with moderate-to-severe asthma and children with mild asthma.

**Figure 2.** The degree of reproducibility between two successive measurements of pH (top, A), LTB₄ concentration (middle, B), and 8-isoprostane concentration (bottom, C) in the breath condensate of healthy subjects using the Bland-Altman method.

**Exhaled LTB₄**

LTB₄ was detectable in the EBC of all subjects. LTB₄ concentrations were significantly increased in children with CF compared to the healthy control subjects (35.9 ± 4.1 vs 7.4 ± 0.3 pg/mL, respectively; p < 0.0001) [Fig 3, top, A]. A significant elevation of LTB₄ levels was observed in children with an acute exacerbation of CF (47.8 ± 5.9 pg/mL) compared with the children with stable CF (24.2 ± 1.8 pg/mL; p < 0.0005). The asthmatic children had higher levels of LTB₄ compared to the healthy control subjects (18.8 ± 2.4 vs 7.4 ± 0.3 pg/mL; p < 0.0001).
Compared with the healthy children, higher levels of LTB4 were observed in children with severe and moderate asthma, but not in the children with mild asthma (27.2 ± 2.1 and 8.4 ± 0.4 pg/mL, respectively; p < 0.0005). There was a negative correlation between the EBC pH and LTB4 values in the healthy children, CF children, and asthmatic children (r = −0.5; p < 0.0005) [Fig 3, bottom, B]. The reproducibility of the exhaled LTB4 measurements was assessed in 10 nonsmoking healthy subjects (six men; mean age, 35 ± 7 years). The mean difference between the two measurements was −0.04 ± 0.18 pg/mL. The coefficient of variation for LTB4 measured was 2.0% (Fig 2, middle, B).

Exhaled 8-Isoprostane

8-Isoprostane was detectable in the EBC of all subjects, and 8-isoprostane levels were significantly increased in children with CF and asthma compared with those in the healthy control subjects (19.5 ± 0.7 and 13.8 ± 0.6 vs 4.6 ± 0.5 pg/mL, respectively; p < 0.0001 and p < 0.0001) [Fig 4]. No correlation was observed between exhaled pH and 8-isoprostane levels in the EBC. The reproducibility of the 8-isoprostane measurements was assessed in 10 nonsmoking healthy subjects. The mean difference between the two measurements was −0.14 ± 0.32 pg/mL. The coefficient of variation for 8-isoprostane measured was 4.4% (Fig 2, bottom, C).

**Discussion**

The main findings of this study were the existence of a lower EBC pH in children with CF compared with that in asthmatic children. Both groups had a lower EBC pH than did control subjects. EBC LTB4 and 8-isoprostane concentrations were higher in CF and asthmatic children compared with healthy control subjects. Furthermore, EBC pH and LTB4 levels were related to the severity of asthma and the presence of an exacerbation of CF. There was no correlation among EBC pH, 8-isoprostane level, and lung function.

The pH values observed in our control group of children were slightly higher than those reported in adults by Kostikas et al13 and Tate et al,20 which may be an effect of age or may be due to the use of different collection devices. It is also possible that adults had a lower pH because of long-term exposure to air pollution and/or cigarette smoke, which might result in chronic low-grade airway inflammation reflected by the acidity of their EBC.

It must be acknowledged that the interpretation of EBC pH is controversial, and the level of airway pH in patients with CF also has been debated. Effros et al21 suggested that EBC pH was determined by...
measurement in the oral cavity, on the basis of measurements made in patients who have received a tracheostomy. It is difficult to understand how changes in the oral cavity could account for the results, at least in the children with different severities of asthma. Although low airway pH has been found in children with asthma and CF, direct measurements of nasal and airway pH in children with CF using a gold electrode were identical to the results in control subjects. Previous contradictory findings were attributed to artifacts caused by changes in the transepithelial potential difference, which were detected using the antimony electrode. However, all of these measurements were made at relatively few sites in the peripheral airway generations, whereas it is likely that EBC may reflect more distal airways, where there is a much larger total epithelial surface area.

It is more likely that lower pH in the airways of adult and children with CF and asthma is due to airway inflammation, for example, to the presence of neutrophils. Myeloperoxidase, which is released into the airway lumen from neutrophil granules, catalyzes a reaction between hydrogen peroxide and chloride ions to form hypochlorous acid. This highly volatile acid has been thought to be responsible for the acidification of EBC in stable patients with COPD and bronchiectasis.

Other mechanisms may be also important. The vesicles of macrophages and eosinophils contain vacuolar hydrogen-adenosine triphosphatase, which is responsible for an increased $H^+$ release, and eosinophilic granules contain eosinophil peroxidase, which, in the presence of $H_2O_2$, is able to oxidize halides to form highly reactive hypohalous acids. These mechanisms could account for the relationship between disease severity and pH that we observed. However, a longitudinal study is required in children to provide more evidence of this link. The possible link between EBC pH and airway inflammation also is supported by the study of Hunt et al., who observed a normalization of EBC pH values in asthmatic subjects after anti-inflammatory treatment.

We have observed significantly higher values of the neutrophil chemoattractant $LTB_4$ both in children with moderate-to-severe asthma and CF than in healthy subjects. By contrast, normal levels of this marker are found in children with mild asthma. This result reflects, perhaps, different patterns of inflammation in patients with asthma of different severities, although since we did not look at airway cytology with induced sputum, this remains speculative. The presence of neutrophilic inflammation has been well-described in persons with CF, and this may account for the presence of high EBC $LTB_4$ levels.

We speculate that the correlation found between the values of EBC $LTB_4$ and EBC pH in the control subjects, children with CF, and children with moderate and severe asthma may suggest that EBC pH is a marker of neutrophilic inflammation. However, further studies in which sputum differential cell count and measurements of other neutrophil chemoattractants, such as IL-8, are performed and correlated with EBC pH are needed to confirm this hypothesis. The fact that a correlation was observed in control subjects but not in children with mild asthma is unexplained.

We also have determined whether there was a link between an oxidative stress, measured by 8-isoprostanate, and EBC pH. Consistent with other studies, we have observed higher concentrations of EBC 8-isoprostanate in children with CF and asthma compared with those in healthy control subjects, confirming the presence of airway oxidative stress. However, in contrast with other studies, we did not observe a correlation between 8-isoprostanate levels and pH values. We cannot account for this discrepancy. Our findings suggest, perhaps, that these markers reflect different, pathogenetic mechanisms, underscoring the fact that one single “inflammmometer” is unlikely ever to give a true picture of airway inflammatory pathology.

We also have found no correlation between the pH and $LTB_4$ values and the FEV$_1$ and FVC values, confirming the generally poor value of lung function as a predictor of active inflammation. It is likely that EBC pH and $LTB_4$ levels reflect the intensity of ongoing inflammation, while lung function tests are at least in part related to the long-standing lung damage.

In conclusion, we believe that EBC pH may be a useful marker of airway inflammation in children with CF and asthma. The fact that this marker can be measured in a simple, noninvasive, inexpensive, and reproducible manner is advantageous. Furthermore, the speed of this measurement (ie, values can be obtained within 10 min of the EBC measurement) compared to other tests taking 2 to 3 days, can allow us to encourage its future use in the monitoring of respiratory disease. Further studies, however, are needed for the following reasons: (1) to validate the EBC pH measurement; (2) to expand EBC pH measurement to different patient populations and to different clinical diseases; and finally (3) to determine the place of the measurement of EBC pH in clinical practice for the diagnosis and monitoring of inflammatory respiratory diseases.

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