

Exhaled breath condensate pH measurement in children with asthma, allergic rhinitis and atopic dermatitis

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Recent studies have shown that the pH of exhaled breath condensate (EBC) could be predictive of asthma exacerbation. Moreover, it has been documented that both allergic rhinitis and atopic dermatitis constitute risk factors for the occurrence of asthma in a progression of disease known as atopic march. The aim of our study was to establish if condensate pH could be used as a valuable mean of monitoring of asthma in atopic children. We studied 34 atopic children with acute asthma, 70 with stable asthma, 35 children with allergic rhinitis, and 17 with atopic dermatitis. Thirty healthy children were used as controls. All children underwent skin prick tests and lung function tests. Exhaled breath condensate samples were collected with a condensing device and de-aerated with argon. The pH of EBC was measured using a pH meter. Children with acute asthma were treated with inhaled steroids and bronchodilators. We found that the pH of condensate in patients with acute asthma was lower than that of patients with stable asthma, rhinitis, and controls (7.25 vs. 7.32, $p < 0.05$; 7.25 vs. 7.48, $p < 0.02$; 7.25 vs. 7.78, $p < 0.0001$, respectively). Patients with stable asthma, rhinitis, and eczema had also lower pH than that of controls (7.32, 7.48, and 7.44 vs. 7.78; $p < 0.0001$, $p < 0.006$, $p < 0.04$, respectively). Patients with acute asthma normalized their pH after treatment (7.82 vs. 7.25; $p < 0.0001$). Finally, patients with acute asthma showed a positive correlation between pH and lung functional parameters (forced expiratory volume in 1 s; $r = 0.39$, $p = 0.04$). Our study shows that EBC pH measurement may be a promising marker for assessing airway inflammation and monitoring response to anti-inflammatory treatment in asthmatic children. Furthermore, we report the first evidence of airways acidification in children with allergic rhinitis and atopic dermatitis. Therefore, EBC pH assessment may be useful in the evaluation of progression of the atopic march toward the development of asthma later in life. Further studies are recommended in order to confirm this indication.

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Exhaled breath condensate (EBC) is a source of a variety of markers and mediators that reflects changes in the composition of the bronchoalveolar extra cellular lining fluid caused by oxidative damage and inflammation and the effect of treatment both in adults and children (1–4).

The interest in using EBC as a mean of monitoring lung inflammation is increased after the observation that this easily sampled fluid has measurable characteristics that differ from health (2–4). Acidification of EBC is a characteristic of acute asthma and the measure of its pH in

patients with asthma before and after therapy has been evaluated (2, 4).

Furthermore, a WHO workshop report on allergic rhinitis (AR) and an international randomized controlled study on asthma preventing measures in children with atopic dermatitis (AD), recognize that both conditions are risk factors for the development of asthma (7–9).

Kostikas et al. (3) suggest that among markers of airway inflammation, EBC pH may well correlate with induced sputum eosinophilia and neutrophilia; however, sputum induction is only possible in a proportion of children and the hypertonic saline used to induce sputum may cause bronchospasm (10).

Moreover, Carraro et al. (4) found respectively a negative and a positive correlation between fractional exhaled nitric oxide (FE_{NO}) levels and exhaled ammonia concentrations with EBC pH in asthmatic children, supporting the hypothesis that airway acidification may have a role in the pathobiology of asthma.

Recently, Vaughan et al. (5) report that EBC pH measurements, after de-aeration with argon, are robust, highly reproducible over-time, not affected by degree of ventilation, temperature or duration of specimen collection. Furthermore, longitudinal data in subjects suggest that EBC pH is a useful assay in individual patients (5).

The apparent simplicity of the EBC method must not be overstated. Comparison and interpretation of data on this rapidly growing field of research is mainly hampered by the lack of standardization. Therefore, when using this technique or when interpreting research data, one should be fully aware of the possible methodological limits (1, 6).

The aim of this study was to assess, for the first time the EBC pH in atopic children with intermittent acute exacerbation of asthma pre- and post-therapy, AR, and atopic eczema/dermatitis syndrome (AEDS).

Materials and methods

Participants

Between April and July 2003 a total of 104 consecutive children with intermittent asthma, defined according to the Global Initiative for Asthma 2004 Guidelines (11), who came for the outpatient-scheduled appraisal at the Department of Paediatrics, University of Bari, were enrolled. During the same period, outpatients with AR and AEDS were offered to enter the study. Allergic rhinitis or AEDS were diagnosed according to the allergic rhinitis and its impact on asthma (ARIA) report classification guidelines (7) and the European Academy for Allergology and Clinical Immunology (EAACI) definition (12), in presence of typical features, respectively.

Thirty non-atopic, healthy, age and gender matched, subjects enrolled among children of the staff of our Department, with no clinical history of allergy, asthma or AD, and no symptoms of airway inflammation, were used as controls. Patients with: (i) chronic disease other than asthma, AEDS or AR, (ii) clinical evidence of pneumonia, (iii) respiratory distress requiring hospitalization, and (iv) severe respiratory or cardiac co-morbidities, were considered not eligible. According to global initiative for asthma guidelines (GINA) guidelines (11), our patients with intermittent asthma did not undergo long-term steroid treatment before study enrolment. The characteristics of the patients are summarized in Table 1.

Procedures

At enrolment, all children underwent: (i) skin prick tests to common aero- (cypress, olive tree, grass, parietaria, dermatophagoides pteronyssinus, dermatophagoides farinae, alternaria, dog, and cat dandruff) and food- (cereals, egg, fish, meat, fruits, legumes, solancee, milk, and soy) allergens, (ii) lung function tests (Master Screen

Table 1. Demographic characteristics of the study population and FEV₁ values at entry

	No.	M/F	Median age* (range)	Atopy (%)	Median value (%) FEV ₁ (range)
Intermittent asthma	104	72/32	9.9 (4.7–15.7)		
Acute asthma	34	23/11	10.6 (6–14.7)	100	68.1 (57.6–78.6)
Stable asthma	70	49/21	9.2 (4.7–15.7)		104.1 (84.7–143.6)
Atopic eczema/dermatitis	17	8/9	7.6 (4.9–13.2)	66.6	100.7 (86.9–121.4)
Allergic rhinitis	35	14/21	9.4 (4.5–10.7)	100	102.9 (72.2–132.6)
Controls	30	11/19	7 (4.3–17)	0	101.5 (83.1–133.4)

FEV₁, forced expiratory volume in 1 s; M, males; F, females.

*Age in years at referral in our unit.

Pneumo TM Jaeger spirometer), and (iii) EBC pH measurement.

Children were defined 'atopic' in presence of positive skin prick tests and/or elevated immunoglobulin (Ig)E (12).

According with GINA Guidelines (11), acute asthma was diagnosed in presence of a forced expiratory volume in 1 s (FEV_1) $\leq$ 80% of predicted, and characteristic symptoms (cough and wheezing), and stable asthma in presence of a $FEV_1 \geq$ 80%, and children clinically stable. Among children with acute exacerbation of asthma, two of them, who required hospitalization, were excluded. Patients with moderate acute asthma were treated with inhaled bronchodilators (salbutamol, 400–800 mcg every 4 h) (13) and with inhaled steroids (budesonide, 200–400 mcg daily) for a week, then lung function tests were repeated. A significant reversibility of airflow obstruction was indicated by a 12% increase of pre-treatment FEV_1 (11). All children had a complete recovery of symptoms within 4 h, and they did not required hospitalization (13).

Exhaled breath condensate pH measurement. For condensate collection, all children performed quiet tidal breathing for 15 min, through a non-rebreathing valve, while wearing nose clips. The exhaled air was introduced in a steel condensing conduit (diameter: 13 mm), surrounded by a Teflon coolant containing dry ice (diameter: 44 mm), and was collected as condensate in a sampling container located at the end of the steel tube (made in house). Saliva was trapped in a 0.3 μ m particle filter covering the tube. All condensate samples were tested for amylase activity (3). pH was measured after specimens de-aeration by bubbling with argon (350 ml/min for 10 min) (2), using a pH meter (Radiometer Analytical S.A., Italy) with a 0.00–14.00 pH range, and a mean accuracy on the order of 0.01 ± 0.02 . After therapy all children with acute asthma underwent a second EBC sample collection for pH measurement. The reproducibility of repeated pH assessments was confirmed by the coefficient of variation.

The study had been approved by the local Ethical Committee and a written informed consent was obtained in all patients from both parents.

Statistical analysis

Data are expressed as median with a range. The statistical analysis was performed using SPSS program (Version 11.5). Non-parametric Mann–Whitney test was used for comparison of continuous data. Sperman's rho correlation test was

used to investigate the relationship between continuous variables. A $p < 0.05$ was considered significant.

Results

Of the 104 asthmatic children, 34 (32.7%) had evidence of acute asthma, while 70 (67.3%) had stable asthma. Out of a total of 47 children with AR and 21 AEDS, 35 (74.5%), and 17 (80.9%), respectively, consented to enter the study (Table 1). According with the EAACI task force recommendations, children with AEDS were divided in AD [nine; five males, median age 7.2 yr (range 4.9–10.7)] and intrinsic dermatitis (ID) [eight; three males, median age 8.1 yr (range 6–13)] subgroups (12).

Children in the different diagnostic group were homogeneous for gender and age ($p = ns$). The median amount of collected condensate was 1.2 ml (range 0.8–2.1 ml) with no difference in the four groups of children. pH measurements were highly stable and reproducible on consecutive evaluations. The mean intra-subject coefficient of variation for EBC pH was 0.5%.

All children were able to successfully complete the EBC procedure, which was well accepted. After the procedure none presented clinical signs of intolerance (cough or dyspnoea) or required the use of inhaled steroid/bronchodilators. No child required interruption of the procedure.

Fig. 1 shows EBC pH values of patients, according to diagnosis. Patients with acute asthma had a significantly lower pH than patient with stable asthma, AR or controls [7.25 (range 5.6–

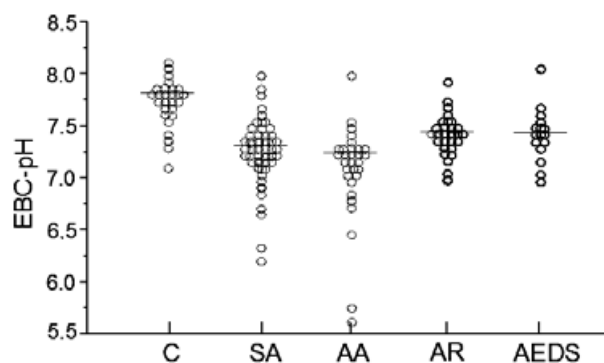


Fig. 1. Exhaled breath condensate (EBC) pH median values of patients according to diagnosis. Patients with acute asthma (AA) have a lower EBC pH than patients with stable asthma (SA), allergic rhinitis (AR), and controls (C). Patients with SA have also a lower pH than C. Similarly, the median pH of EBC of children with AR and atopic eczema/dermatitis syndrome (AEDS) is lower than that of control group. Acute asthma vs. SA, AR or C: $p < 0.05$, < 0.02 and < 0.0001 , respectively; SA, AR, AEDS vs. C: $p < 0.0001$, < 0.006 , and < 0.04 , respectively.

8.06) vs. 7.32 (range 6.2–8.06), 7.48 (range 6.90–7.98), and 7.78 (range 7.06–8.10); $p < 0.05$, $p < 0.02$, and $p < 0.0001$, respectively]. Similarly the median condensate pH of children with stable asthma was significantly lower than that of controls [7.32 (range 6.2–8.02) vs. 7.78 (range 7.06–8.10); $p < 0.0001$]. Patients with AR and AEDS had also a median pH value lower than that of control group [7.48 (6.90–7.98) and 7.44 (6.85–8.05) vs. 7.78 (7.06–8.10), $p < 0.006$, $p < 0.04$, respectively]. Moreover, children with AD had a lower EBC pH than that of children with ID [7.40 (7.00–7.97) vs. 7.60 (6.90–7.98); $p = \text{ns}$], although not reaching statistical significance.

The FEV₁ median values at enrolment in the different groups of children are reported in Table 1.

Patients with moderate acute exacerbation of asthma, 1 wk after treatment, had improved both clinically and according to lung function test [median FEV₁ 102.5% (range 84.2–136.4)]. Moreover, we found a significantly higher median EBC pH as compared with pre-treatment values and not different than that of controls [7.82 (7.0–8.05) vs. 7.25 (5.6–8.06) vs. 7.78 (7.06–8.10); $p < 0.0001$, $p = \text{ns}$, respectively] (Fig. 2).

Finally, among patients with asthma we found a weak positive correlation between pH and FEV₁ ($r = 0.39$, $p = 0.04$), as showed in Fig. 3.

Discussion

The research for non-invasive tests for children with chronic diseases is extremely actual and

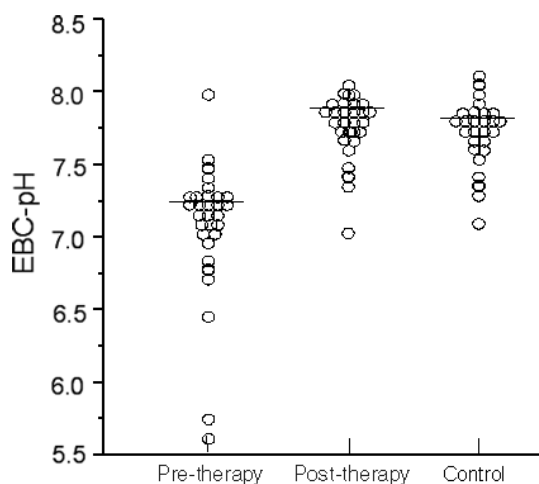


Fig. 2. Exhaled breath condensate (EBC) pH of patients with Acute Asthma (AA) pre- and post-therapy and controls. Patients with AA 1 wk after β_2 -agonist and budesonide treatment show higher median EBC pH values as compared with pre-treatment levels and not different values than those of controls. Post-therapy pH median value vs. pre-therapy or controls: $p < 0.0001$, $p = \text{ns}$, respectively.

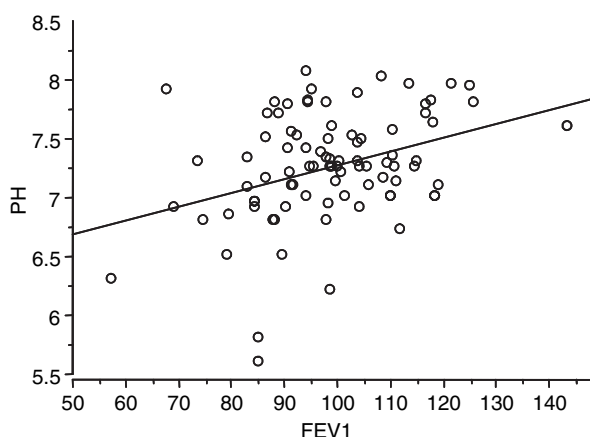


Fig. 3. Correlation between pH values of exhaled breath condensate (EBC) and forced expiratory volume in 1 s (FEV₁) in patients with asthma. Positive correlation between EBC pH of children with asthma and FEV₁ ($r = 0.31$; $p = 0.04$).

important mainly for conditions such as asthma that need constant monitoring of therapy and progression of disease.

In the present study we have shown that children with acute asthma have lower EBC pH than children with stable asthma and controls and that it reverses to normal after inhaled corticosteroid and bronchodilator therapy. This is consistent with the experience of Hunt and co-workers who showed that the mean pH of exhaled airway vapor condensate samples of subjects with acute asthma was significantly lower than controls and that it reversed to normal after systemic steroid treatment (2). We believe that the mean pH value of children in our study differs from that reported by Hunt because of the different severity of disease exacerbation (our children had a moderate acute episode of asthma not needing hospitalization), age (children have a shorter exposure to smoke or pollution) and different collecting devices used in the two series. However, we observed an intra-subject coefficient of variation of EBC pH measurements of 0.5%, confirming data on reproducibility reported by other independent research groups (5).

Recently, Rosias had demonstrated that EBC collection is applicable in children and that its acidity was significantly lower in asthmatic children than in controls although no correlation was found with any of the conventional asthma measures (14). In our experience, EBC pH can be useful to assess acute asthma exacerbation in children as the pH progressively decreases with worsening of lung function test performances (Fig. 3). Similarly to the work of Kostikas in

adults (3), we have found in children a positive correlation between EBC acidification and reduction of lung function test performance (Fig. 3); this may be indicative of a possible use of this parameter as a semi-quantitative assay for assessing lung disease activity although other investigators did not have replicated these findings (14). Thus, before the application of the method for individual monitoring, more studies need to be performed. Our main result is the evidence that EBC pH might be helpful to monitor the effect of therapy in asthmatics and that the alkalization of the EBC is parallel to clinical improvement (Fig. 2).

Epidemiological studies have consistently shown that dysfunction of the upper and lower airways frequently co-exists leading to the concept of a 'united airways' (15–17) and that both AR and AD are correlated to, and constitutes a risk factor for allergens sensitization and the occurrence of asthma (18–25) in a progression of disease known as 'atopic march' (10, 26–28). Based on the fact that lower airway appears to be the dominant determinant of EBC pH (5), we speculate that EBC pH measurements in children with AR and AEDS may be an early predictor of the progression of the atopic march toward the development of asthma later in life. In the present study we report the first evidence of an endogenous airways acidification in patients with AR and AEDS without any clinical sign of airway inflammation, demonstrating a significant lower EBC pH than that of controls. Interestingly, patients with AD have a lower (not significant) EBC pH than that of children with ID. However, our hypothesis needs to be confirmed in large prospective studies.

Exhaled breath condensate pH measurement is a safe, highly reproducible, inexpensive, easy-to-perform technique that deserves particular attention especially in children (5, 6). The strength of this new method is that it does not require forced expiration efforts, thus becoming particularly useful in patients with acute asthma. In our experience the technique was well accepted by all children at any age; all children, even those with acute exacerbation of asthma, were successful in completing the test without any problem or side effect as recently reported by Baraldi (29).

In conclusion we report that EBC pH measurement may be a simple way to monitor respiratory performances and response to treatment in children with acute intermittent asthma, and that it may open new field of research in the prospective evaluation of children with conditions such as AEDS, in whom the acidification of the EBC may represent the first sign in the

progression of disease and therefore the alert sign for a preventive therapy.

We are aware that this new approach to monitoring lung diseases is not standardized and that therefore our results should be considered as preliminary reports that need to be confirmed for the management of individual patients.

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