Safety and success of exhaled breath condensate collection in asthma

E Baraldi, L Ghiro, V Piovan, S Carraro, F Zacchello, S Zanconato

Background: Exhaled breath condensate (EBC) is a rapidly expanding area of research to study airway inflammation through the detection of volatile and non-volatile substances in the airways.

Aims: To determine the safety and feasibility of EBC procedure in a group of children with asthma of varying severity.

Methods: In a cross-sectional study of children aged 4–17 years, 18 healthy and 91 asthmatic children (69 in stable condition and 22 with asthma exacerbation) underwent the EBC procedure. Outcomes assessed included completion of the procedure, decrease in FEV1, change in fractional exhaled nitric oxide (FENO), and adverse effects. No pretreatment with β2 agonists was given. All children were able to successfully complete the EBC procedure.

Results: Median fall in FEV1 after the procedure was −1% (IQR −3.5, 1.8) in asthmatics and was comparable to that observed in healthy children. In only one asthmatic child did the drop in FEV1 exceed 12%. No significant changes in FENO were observed after EBC.

Conclusion: This study suggests that EBC is a simple and well-tolerated method for evaluating biological samples from the lower airway. The procedure was safe in children with asthma exacerbation, and the success rate was 100% in children aged 4 years and above.

E xhaled breath analysis is a rapidly expanding area of research to study airway inflammation and lung oxidative stress, allowing the detection of volatile and non-volatile substances from the airways. The most studied volatile marker is exhaled nitric oxide (FENO), which is considered a good marker of airway inflammation. Induced sputum is a valid and well-established method for analysing both cellular and biochemical markers of airway inflammation. However, this procedure is not easily performed in children, and the inhalation of hypertonic saline solution may induce coughing, bronchoconstriction, and a temporary inflammatory response.

An alternative and simple means for detecting non-volatile substances from the lungs is the method of exhaled breath condensate (EBC), which enables the collection of volatile and non-volatile substances in the airways. EBC samples were collected in a condensing device formed by two glass chambers. The inner glass chamber was cooled by means of ice and suspended in a larger glass chamber. The children, without nose clips, were instructed to tidally breathe through a saliva valve served as a saliva trap and children were asked to periodically swallow their saliva. None of the children had previously performed EBC collection.

MATERIALS AND METHODS

Subjects
A total of 91 asthmatic children (median age 10.5 years, range 4–17) with variable disease severity (forced expiratory volume

ORIGINAL ARTICLE

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Exhaled breath condensate (EBC) collection

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Abbreviations: EBC, exhaled breath condensate; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; IQR, interquartile range

See end of article for authors’ affiliations

Correspondence to:
Dr E Baraldi, Department of Pediatrics, Via Giustinianni 3, 35128 Padova, Italy; eugi@pediatria.unipd.it

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E Baraldi, L Ghiro, V Piovan, S Carraro, F Zacchello, S Zanconato

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### Pulmonary function

Pulmonary function parameters (FEV₁, FVC) were measured by means of a 10 litre bell spirometer (Biomedin, Padova, Italy). In children with stable asthma, short acting β₂ agonists were withheld for eight hours and long acting β₂ agonists for 15 hours before the EBC procedure. In children with asthma exacerbation, β₂ agonists were stopped three hours before the procedure.

### Fractional exhaled nitric oxide measurement

FENO was measured by means of the NIOX system (Aerocrine, Stockholm, Sweden) with a single breath on-line method according to ATS guidelines. Children inhaled NO free air and exhaled through a dynamic flow restrictor with a target flow of 50 ml/s for at least 6–7 seconds.

The hospital ethics committee reviewed and approved the protocol, and all parents gave their informed consent.

### Statistical analysis

Data are expressed as median [interquartile range (IQR)]. The comparison of FEV₁ and FENO measurements before and after condensate collection was performed by applying the Wilcoxon signed rank test. Differences between experimental and control groups were analysed using the Mann-Whitney test. Correlations were evaluated by Spearman's rank test. Results were considered significant at a value of p < 0.05.

### RESULTS

All of the children were able to successfully complete the EBC procedure, which resulted in it being well accepted (table 1). The mean amount of collected condensate was 1.5 ml (range 0.5–3 ml) and was weakly but significantly correlated with the age of children (r = 0.35, p < 0.001)

Median FEV₁ in the whole group of asthmatic children was 90% (IQR 81, 100) before EBC collection and 89% (81, 98) after the procedure (p = 0.06). In asthmatic children the median decrease in FEV₁ after the procedure was −1% (−3.5, 1.8) and was not different (p = 0.28) from that observed in healthy children (table 1). The largest decrease in FEV₁ after EBC was 12.8%.

A total of 22 patients were evaluated during asthma exacerbation. Their median variation in FEV₁ after EBC was 0% (−4.1, 2.8) and was comparable (p = 0.19) to that observed in children with stable asthma. There was no correlation between the percentage of reduction of FEV₁ and baseline FEV₁ (p = 0.89).

After the procedure only one asthmatic girl presented clinical signs of intolerance—cough and mild dyspnoea, which required the use of inhaled albuterol. Her FEV₁ decreased by 8.4%. The asthmatic child with a 12.8% reduction in FEV₁ did not have symptoms during and after the procedure. With the exception of three children with mild cough, there were no other side effects. No child required interruption of the procedure because of adverse effects.

Median FEV₁ did not change after the procedure (table 1). No correlation was found between baseline FEV₁ and the decrease in FEV₁ after the procedure (p = 0.64).

### DISCUSSION

Safety is an important factor to consider before applying a diagnostic test in clinical research. Although breath condensate has a long tradition in medicine, it has only recently been applied in patients with lung diseases. There is limited information concerning its use in children, and no studies have investigated the safety of EBC.

The present study shows that the procedure of EBC collection is well accepted, safe, and feasible in asthmatic children aged 4 years and above. The success rate was 100%. No significant adverse effects were found in either clinically stable children or in those with asthma exacerbation, and no relevant reduction in airway calibre was found after the procedure. None of the subjects had to stop the procedure because of side effects, and only one had a more than 12% reduction of FEV₁, but without respiratory symptoms. In addition we have found that EBC collection, unlike other laboratory procedures, does not affect FENO, suggesting that it can be performed before or after NO measurement, when several investigations are planned. The finding of no significant changes in FENO confirms the safety of the EBC procedure and shows that the 15–20 minute ventilatory pattern with mouth breathing required for the test does not evoke thermal disregulation of the bronchial circulation with related increase in FENO, as recently reported for thermally induced asthma.

Several studies have investigated breath condensate as a means to analyse some substances from airways of patients with pulmonary diseases. The exhaled aerosol droplets of breath condensate are believed to reflect the composition of extracellular fluid lining the bronchoalveolar tract, but the proportional contribution of these compartments has yet to be determined. Successful collection of EBC utilising different devices has been reported. However, no standardised methods have been established for EBC collection, and formal recommendations are required to ensure uniformity of measurement. Recently, Griese et al have described a method to collect EBC from the nose starting from 4 weeks of age.

A variety of inflammatory markers have been detected in the EBC of asthmatic patients, and new macromolecules...
continue to be added to this list. Increase in hydrogen peroxide (H$_2$O$_2$) levels, a marker of oxidative stress, has been detected in EBC of asthmatic children. Increased concentrations of cysteinyl leukotrienes, lipid mediators derived from arachidonic acid, have been reported in the EBC of another group of asthmatic patients; they remained increased irrespective of treatment with inhaled corticosteroids.

Isoprostanes, stable markers of lipid peroxidation, have been found increased in asthmatic patients despite treatment with inhaled corticosteroids, suggesting that these drugs may not be fully effective in reducing oxidative stress. Nitrotyrosine is a stable compound expressing involvement of NO derived oxidants in the lung. It is increased in the breath condensates of asthmatic subjects and associated with worsening of asthma symptoms. Recently, Hunt and coworkers measured the pH of EBC in patients with acute asthma, and found low values that normalised with corticosteroid therapy suggesting that airway acidification may explain increased levels of FENO during asthma exacerbation. In addition they found that pH values of EBC are identical to samples taken directly from the lower airway. Taken together this evidence suggests a potential role of EBC in the monitoring of airway inflammation that could provide new insights into the pathobiology of lung diseases. However, long term prospective studies correlating EBC findings with measures of disease control and established measures of lung pathology (BAL analysis, biopsy histology) are necessary to validate and address the clinical relevance of this method.

In conclusion, this study suggests that EBC is a simple and well tolerated method to evaluate biological samples from the lower airway. The procedure was safe, even for children with asthma exacerbation, and the success rate was 100% in children aged 4 years and above.

Authors’ affiliations
E Baraldi, L Ghiro, V Piovan, S Carraro, F Zacchello, S Zanconato, Department of Pediatrics, Unit of Allergy and Respiratory Medicine, University of Padua, Italy

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