Safety and success of exhaled breath condensate collection in asthma

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Arch Dis Child 2003;88:358–360

Exhaled 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 (FE\textsubscript{NO}), 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 components of the fluid lining the respiratory tract to be studied. Measurement of several endogenous substances can then be carried out in the condensate. EBC is considered safe and feasible, but limited information concerning its use in preschool and school age children is available and, to our knowledge, no studies have formally addressed the safety and the feasibility of this sampling procedure in asthmatic children, particularly during acute asthma exacerbation.

Although considered safe, EBC collection involves a prolonged oral breathing which, without the humidifying and warming action of the nose, could lead to airway mucosa dehydration and act as a bronchoprovocative stimulus in patients with airway hyperresponsiveness. Therefore, the aim of this study was to evaluate the safety and feasibility of the EBC procedure in a large group of asthmatic children aged 4–18 years during both stable and unstable clinical conditions in order to evaluate its general applicability.

In addition, as other laboratory procedures such as spirometry and sputum induction may affect exhaled nitric oxide (FENO), and adverse effects. At the end of each test, acceptability was assessed by asking the children or the parents to give a written answer to the following question: “Have you experienced any discomfort, or any unusual or unpleasant events during the test?” The physician recorded the severity of symptoms and/or signs, if present, their duration, and the treatment required.

Exhaled breath condensate (EBC) collection

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 by the mouth through a two-way non-rebreathing valve for 15–20 minutes. To minimise salivary contamination the two-way 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 in one second (FEV\textsubscript{1}) ranging between 53% and 124% of predicted values) were enrolled in the study. Of these, 69 were clinically stable and 22 were patients with asthma exacerbation. Eighteen healthy children (median age 9.2 years, range 6–14) without a history of asthma and other airway diseases were also recruited (table 1).

Study design

Clinical evaluation, FE\textsubscript{NO} measurement, and spirometric testing were performed immediately before and 8–10 minutes after the end of EBC collection. Clinical evaluation was also repeated after 20 minutes. The outcomes assessed were proportion of children completing the EBC procedure, acceptability, changes in lung function (% drop in FEV\textsubscript{1}), and FE\textsubscript{NO}, clinical tolerance of the patient to the procedure (cough, wheezing, dyspnoea), and adverse effects. At the end of each test, acceptability was assessed by asking the children or the parents to give a written answer to the following question: “Have you experienced any discomfort, or any unusual or unpleasant events during the test?” The physician recorded the severity of symptoms and/or signs, if present, their duration, and the treatment required.

Abbreviations: EBC, exhaled breath condensate; FE\textsubscript{NO}, fractional exhaled nitric oxide; FEV\textsubscript{1}, forced expiratory volume in one second; FVC, forced vital capacity; IQR, interquartile range
Safety of EBC in asthmatic children

### 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 and interquartile range (IQR). The comparison of FEV₁ and FEV₀ 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

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 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 FEV₀ did not change after the procedure (table 1). No correlation was found between baseline FEV₀ 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, but 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 FEV₀, suggesting that it can be performed before or after NO measurement, when several investigations are planned. The finding of no significant changes in FEV₀ 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 FEV₀, 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 condensate of asthmatic patients; they remained increased irrespective of treatment with inhaled corticosteroids.

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 FEn 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.

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This study was in part supported by the Italian Nitric Oxide Club (INOC). Dr L Ghio and Dr V Piovan were in part supported by a grant of Regione Veneto, Ricerche Sanitarie Finalizzate (no. 885/03/99).

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Arch Dis Child 2003 88: 358-360
doi: 10.1136/adc.88.4.358

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