**ORIGINAL ARTICLE**

Safety and success of exhaled breath condensate collection in asthma

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

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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.1 The most studied volatile marker is exhaled nitric oxide (FENO), which is considered a good marker of airway inflammation.1 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.1

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.1 2 Measurement of several endogenous substances can then be carried out in the condensate.1 2 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 application. In addition, as other laboratory procedures such as spirometry and sputum induction9 10 may affect exhaled NO values, we systematically evaluated the effect of EBC collection on exhaled NO production.

**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 (FEV1) 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, FENO 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 FEV1) and FE25–75 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.

**Exhaled breath condensate (EBC) collection**
EBC samples were collected in a condensing device formed by two glass chambers.11 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.

**Abbreviations:** EBC, exhaled breath condensate; FE25–75, fractional exhaled nitric oxide; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; IQR, interquartile range
Pulmonary function
Pulmonary function parameters (FEV\textsubscript{1}, FVC) were measured by means of a 10 litre bell spirometer (Biomedin, Padova, Italy). In children with stable asthma, short acting \beta\textsubscript{2} agonists were withheld for eight hours and long acting \beta\textsubscript{2} agonists for 15 hours before the EBC procedure. In children with asthma exacerbation, \beta\textsubscript{2} agonists were stopped three hours before the procedure.

Fractional exhaled nitric oxide measurement
FE\textsubscript{NO} was measured by means of the NIOX system (Aerocrine, Stockholm, Sweden) with a single breath on-line method according to ATS guidelines.\textsuperscript{12} 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 FEV\textsubscript{1} and FE\textsubscript{NO} 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 Wilcoxon 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 ($r = 0.35$, $p < 0.001$).

FE\textsubscript{NO} 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 FEV\textsubscript{1} 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 FEV\textsubscript{1} and baseline FE\textsubscript{NO} ($r = 0.35$, $p < 0.001$). However, no standardised methods have been established for EBC collection, and formal recommendations are required to ensure uniformity of measurement.\textsuperscript{13,14} Recently, Griese et al have described a method to collect EBC from the nose starting from 4 weeks of age.\textsuperscript{15}

A total of 22 patients were evaluated during asthma exacerbation. Their median variation in FEV\textsubscript{1} 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 FEV\textsubscript{1} and baseline FE\textsubscript{NO} ($p = 0.89$).

After the procedure only one asthmatic girl presented clinical signs of intolerance—cough and mild dyspnoea, which required the use of inhaled albuterol. Her FEV\textsubscript{1} decreased by 8.4%. The asthmatic child with a 12.8% reduction in FEV\textsubscript{1} 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 FE\textsubscript{NO} did not change after the procedure (table 1). No correlation was found between baseline FE\textsubscript{NO} and the decrease in FEV\textsubscript{1} 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,\textsuperscript{16} 17 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 FEV\textsubscript{1} but without respiratory symptoms. In addition we have found that EBC collection, unlike other laboratory procedures,\textsuperscript{10} does not affect FE\textsubscript{NO}, suggesting that it can be performed before or after NO measurement, when several investigations are planned. The finding of no significant changes in FE\textsubscript{NO} 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 disorganisation of the bronchial circulation with related increase in FE\textsubscript{NO}, as recently reported for thermally induced asthma.\textsuperscript{13}

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,\textsuperscript{1,3,5} but the proportional contribution of these compartments has yet to be determined. Successful collection of EBC utilising different devices has been reported.\textsuperscript{13,17} However, no standardised methods have been established for EBC collection, and formal recommendations are required to ensure uniformity of measurement.\textsuperscript{13} Recently, Griese et al have described a method to collect EBC from the nose starting from 4 weeks of age.\textsuperscript{15}

A variety of inflammatory markers have been detected in the EBC of asthmatic patients,\textsuperscript{1,3} and new macromolecules...
continue to be added to this list. Increase in hydrogen peroxide (H₂O₂) 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.¹⁷ Isoprostanes, stable markers of lipid peroxidation, have been found increased in asthmatic patients despite treatment with inhaled corticosteroids,¹⁴ suggesting that these drugs may not be fully effective in reducing oxidative stress.¹³ 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.

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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