EXHALED BREATH CONDENSATE IN CHILDREN

Silvia Carraro, Eugenio Baraldi

Department of Pediatrics, University of Padova, Padova, Italy

Corresponding author:
Silvia Carraro, MD
Unit of Respiratory Medicine and Allergy
Department of Pediatrics
University of Padova
Via Giustiniani, 3
35128 Padova (Italy)
Email:silvia.carraro.1@unipd.it
Exhaled breath condensate (EBC) is obtained when exhaled air is cooled and its composition is believed to reflect the physiological and pathological processes happening in the respiratory tract (1,2).

To collect EBC, subjects are asked to breath tidally through their mouth for 10-15 minutes into the condenser. The technique is safe and easy, requiring only a minimal cooperation and can be performed in children as young as 4 year old (3).

A joint ATS/ERS task force recently published a document containing recommendations for EBC collection and analysis (4).

**Exhaled breath condensate in children with asthma**

Several biomarkers of inflammation and oxidative stress have been assessed in the EBC of children with asthma to investigate the role of different inflammatory pathways in the pathogenesis of the disease.

**Oxidative stress products.** EBC levels of malondialdehyde were found to increase significantly during acute asthma exacerbation, dropping to concentrations no different from those detected in healthy children after a short course of oral steroids (5). In the same group of children a significant reduction in the antioxidant glutathione was demonstrated during acute asthma exacerbation (5). After the course of oral steroids, glutathione levels increased but they were still lower than in controls. A number of studies evaluated 8-isoprostane, a marker of oxidative stress derived from arachidonic acid peroxidation: 8-isoprostane was increased in EBC of stable asthmatic children, though no difference was detectable between children treated with inhaled steroids and steroid-naive children (6-8). 8-isoprostane was found to significantly increase during an acute asthma exacerbation and, after a course of oral steroids, its levels declined but remained higher than in controls (9). Taken together, these observations suggest that steroids are not entirely effective on this biomarker of oxidative stress.

**Leukotrienes.** Cysteinyl leukotrienes (Cys-LTs) have a well-known role in the pathophysiology of asthma, increasing vascular permeability, mucus production and airway responsiveness. Increased levels of EBC Cys-LTs were demonstrated in children with both stable and unstable asthma (10). The rise in leukotriene levels in asthmatic subjects has been confirmed using a reference analytical method (gas chromatography/mass spectrometry) (11).

EBC Cys-LT levels seem to be affected by treatment with leukotriene receptor antagonists, the higher their baseline value, the greater their reduction after the treatment (12-13). These findings suggest that examining the levels of Cys-LT in EBC could help us to identify the children most likely to respond to antileukotriene therapy.
EBC Cys-LTs seem to reflect specific aspects correlating with asthmatic airway inflammation too, such as bronchial hyper-reactivity and airway remodeling.

A correlation was demonstrated between EBC Cys-LT levels and the maximum drop in FEV1 after an exercise challenge, suggesting a role for these mediators in exercise induced bronchoconstriction (14). In addition, a significant correlation emerged between basal EBC Cys-LT levels and airway hyper-responsiveness, measured as PC15 (15).

A recent study demonstrated a correlation between Cys-LTs in EBC and the thickness of the reticular basement membrane (RBM), which is an indicator of airway remodeling (12).

In addition to Cys-LTs, also increased EBC concentrations of leukotriene B4 have been demonstrated in asthmatic children (16-17).

**Products of nitric oxide (NO) metabolism.** Increased nitrite/nitrate levels were demonstrated in children with asthma (18). It has been suggested that simultaneously measuring exhaled NO and EBC NO metabolites can provide a more accurate estimation of NO production, and of the amount of ongoing oxidation in the lung (19).

3-nitrotyrosine is a marker of oxidative stress deriving from the reaction between the powerful oxidant peroxynitrite and a tyrosine. This biomarker is significantly increased in the EBC of asthmatic children (20).

**Acidity.** Some studies measured the pH of EBC collected in asthmatic children (21). This is a robust and reproducible biomarker of airway acidity (22) and the normal range of values for EBC pH has recently been defined (23). EBC pH is significantly reduced in patients with acute asthma exacerbation (24-25), but also in patients with stable asthma (26-27), suggesting that an altered airway pH homeostasis may be involved in the pathogenesis of asthma.

**Metabolomics.** Finally, a recent study has shown that metabolomic analysis can be applied to EBC to enable the identification of metabolite patterns capable of distinguishing asthmatic from healthy children (28). Metabolomics is defined as the analysis and interpretation of global metabolic data, which express the multiparametric metabolic response of living systems to pathophysiological stimuli, using modern spectroscopic techniques and appropriate statistical approaches. Further applications of metabolomic analysis in asthmatic subjects may lead to a better understanding of the different phenotypes underlying the “asthma syndrome”, providing a better rationale for the classification and treatment of patients (29).

**Methodological issues**

EBC is easy to collect in children starting from the age of 4 year old (3). Its collection in younger children and infants represents a challenging issue. Griese et al. (30) developed a device based on the aspiration of air exhaled from the nose, which then condenses inside a cold trap. The authors
demonstrated that this method can be used in children as young as 4 week old. Unfortunately, the
device they proposed only enables the collection of nasally exhaled air, so processes involving the
upper airways may affect the composition of the condensate collected. Moeller et al. (31) developed
a condenser consisting of two syringes connected together and surrounded by two ice packs. The
infant breathes through a face mask connected to the syringes. Using this device the authors
demonstrated that collecting both nasal EBC (with the mask over the nose and the mouth closed)
and oral EBC (collected while closing the nose with the soft part of the mask) is feasible in sedated
infants (31). Vogelberg et al. recently adapted a commercially available condenser to collect
condensate in children from 4 month to 7 year old. They customized the device connecting a face
mask to the condenser though a two-ways non-rebreathing valve (32).
These methods still carry some drawbacks in that it is difficult to separate oral and nasal exhaled air
and to collect a sufficient amount of condensate. Studies aiming to develop devices for EBC
collection in infants and toddlers are nonetheless very important because they pave the way to the
non-invasive assessment of airway inflammation in the younger age groups.

Conclusions
EBC analysis enables the study of the lung in an entirely non-invasive way - a feature particularly
important when working with children. Although the EBC technique has yet to be thoroughly
standardized and many biomarkers are still awaiting validation, it is a promising technique. Several
studies highlighted its potential for clarifying the pathogenic mechanisms of asthma. Moreover, new
approaches recently applied to EBC (e.g. metabolomics) may have a significant role in the
ccharacterization of the different asthma phenotypes.
References


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