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REVIEW

Exhaled breath condensate in patients with asthma: implications for application in clinical practice

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Summary

Exhaled breath condensate (EBC) analysis, a rather appealing and promising method, can be used to evaluate conveniently and non-invasively a wide range of molecules from the respiratory tract, and to understand better the pathways propagating airway inflammation. A large number of mediators of inflammation, including adenosine, ammonia, hydrogen peroxide, isoprostanes, leukotrienes, prostanoids, nitrogen oxides, peptides and cytokines, have been studied in EBC. Concentrations of such mediators have been shown to be related to the underlying asthma and its severity and to be modulated by therapeutic interventions. Despite the encouraging positive results to date, the introduction of EBC in everyday clinical practice requires the resolution of some methodological pitfalls, the standardization of EBC collection and finally the identification of a reliable biomarker that is reproducible has normal values and provides information regarding the underlying inflammatory process and the response to treatment. So far, none of the parameters studied in EBC fulfils the aforementioned requirements with one possible exception: pH. EBC pH is reproducible, has normal values, reflects a significant part of asthma pathophysiology and is measurable on-site with standardized methodology although some methodological aspects of measurement of pH in EBC (e.g. the effect of ambient CO_2, sample de-aeration, time for pH measurement) require further research. However, EBC pH has not been evaluated prospectively as a guide for treatment, in a manner similar to exhaled NO and sputum eosinophils. EBC represents a simple and totally non-invasive procedure that may contribute towards our understanding of asthma pathophysiology. Besides the evaluation of new biomarkers, the standardization of the already existing procedures is warranted for the introduction of EBC in clinical practice.

Introduction

Asthma is an inflammatory disorder of the airways associated with airflow obstruction and bronchial hyperresponsiveness that vary in severity across the spectrum of the disease. Although asthma is considered to be a chronic inflammatory disease, evaluation and therapy guidance are mainly based on clinical symptoms and lung function tests that remain the cornerstones of clinical practice.

The field of non-invasive techniques for assessing airway inflammation has developed rapidly since nitric oxide (NO) was recognized as an important mediator in exhaled air [1]. The fraction of exhaled NO (FeNO) is the most extensively studied exhaled biomarker and increased levels of FeNO have been documented widely in patients with asthma [2]. FeNO is related primarily to eosinophilic inflammation [3], and has been used prospectively as a guide for treatment of asthma [4]. Sputum induction is considered to be a semi-invasive technique that has helped clinical researchers to identify the inflammatory process of many airway diseases including asthma [5]. The identification of sputum eosinophilia is a valuable parameter that predicts steroid response and has been used to guide treatment [6]. The value of sputum induction is not restricted to sputum eosinophilia, as inflammatory mediators can be measured in the supernatants. However, it is sometimes difficult to accomplish and involves some discomfort for the patients, it requires dedicated personnel and special equipment and may not be used repeatedly in part due to its pro-inflammatory effect [3, 7–9]. Additionally, sputum induction is associated with bronchospasm in a considerable proportion of

asthmatic patients [9] and all the above render its introduction in everyday clinical practice difficult.

The collection of exhaled breath condensate (EBC) is achieved by freezing the exhaled air with the use of special condensing devices. Despite some methodological problems of this procedure, its non-invasive nature gives the opportunity for repeated measurements on the same person and provides valuable information for the assessment of airway inflammation [10–12]. The recent ERS/ATS recommendations have made suggestions towards the standardization of the procedure [13] and there is emerging evidence that parameters obtained from EBC reflect changes in the level of airway lining fluid [14]. EBC contains a large number of mediators, including adenosine, ammonia, hydrogen peroxide (H_2O_2), isoprostanes, leukotrienes, prostanoids, nitrogen oxides, peptides and cytokines. Concentrations of these mediators are influenced by lung diseases and are modulated by therapeutic interventions. Published data support the evidence that EBC pH is the only rapidly evaluated marker, which simultaneously reflects the underlying inflammatory process as assessed by differential cell counts from induced sputum [15]. Additionally, pH is lowered during asthma exacerbation and is sensitive to treatment strategies with steroids [16]. Interest in this technique grew rapidly and accumulating evidence suggests that, even though EBC is currently a research tool, it has the potential of becoming a validated method that may provide valuable information in the understanding of the pathways propagating airway inflammation.

The aim of the present review is to summarize the major clinical studies that have used EBC for the evaluation of airway inflammation and the response to treatment in patients with asthma. Furthermore, we will try to evaluate which biomarkers have been validated adequately in order to be used in everyday clinical practice.

Biomarkers in exhaled breath condensate in asthma

The major results of the most important clinical studies are summarized in Table 1.

Markers of oxidative stress

1. H_2O_2: Activated inflammatory cells such as eosinophils, macrophages and neutrophils respond with a respiratory burst, which results in the production of reactive oxygen species, the most commonly studied to date being H_2O_2 [17]. Most commonly, H_2O_2 is measured spectrophotometrically [18, 19] or spectrofluorimetrically [20–22], but various techniques have been used, including the recent development of an automated amperometric biosensor (Ecocheck, Viasys, Germany) [23].

Table 1. Major findings from studies relating to EBC in asthma

Parameters	H_2O_2	8-isoprostane	NO_x	Prostanoids	Leukotrienes	pH
Stable asthma	↑ [24–29]	↑ [15, 39]	↑ [26]	PGE_2 ↔ [62–64, 67, 68]	Cys-LTs↑ [41, 63, 74–76] LTE_4↑ [63, 67, 68, 72] LTB_4↑ [53, 63, 67, 72, 82, 106]	↓ [15, 50]
Exacerbation	↑ [27]	↑ [15, 39]			Cys-LTs↑ [42]	↓ [16]
Effect of smoking	↑ [26]		↓ [26]	↑ [62]	LTB_4↑ [83]	↓ [93]
Relation to lung function	FEV_1%, PEFR% and PC_{20} [24, 25, 27, 28]	Small airways function [43]			(−)PC_{20} (LTE_4) [78]	(−) FEV_1 % [15]
Relation to other inflammatory indices	(+) eos [28] No_x [26]	(+) FeNO [39]	(+) H_2O_2 [26] FeNO [50]		(+) RBM [79]	(+) eos [15] H_2O_2 [15] No_x [15, 16]
Effect of treatment	ICS↓ [26–29]	ICS↓ or ↔ [39–42] PPI↓ [44]	ICS↓ [26]		Allergen avoidance↓ [55] nasal steroids ↓[75] montelukast↓ [68, 79, 80] ICS↓ [67, 82]	↓ ICS [15,16]
Atopy	↔ [28]		NO_2/NO_3↑ [50]		Cys-LTs↑ [75] LTB4 ↔ [82]	↓ [94]

↑, increase; ↓, decrease; ↔, no change; (+), positive relation; (−), negative relation; eos, sputum eosinophils; RBM, reticular basement membrane; H_2O_2, hydrogen peroxide; FEV_1, forced expiratory volume in 1 s; PEFR, peak expiratory flow rate; PGE_2, Prostaglandin E_2; ICS, inhaled corticosteroids.

Most of the published studies have reported elevated levels of H_2O_2 in steroid-naïve asthmatic subjects, sometimes being 26-fold higher than normal subjects, although in a minority of asthmatic patients EBC H_2O_2 concentration is below the limit of detection [24–29] and despite the fact that a large variability in H_2O_2 concentrations in EBC has been reported in healthy subjects. H_2O_2 levels are influenced by the underlying disease severity [28], the smoking habit [26] and the presence of symptoms in unstable patients [27], whereas atopy did not have any effect on H_2O_2 [28]. An inverse correlation between H_2O_2, forced expiratory volume in 1 s (FEV_1), peak expiratory flow rate (PEFR) and PC_{20} was reported [24, 25, 27, 28], confirming *in vitro* observations that H_2O_2 is related to bronchial hyperresponsiveness and bronchoconstriction [30]. There is also evidence that H_2O_2 may be related to the underlying eosinophilic inflammation [28] as well as to products of NO metabolism [26].

H_2O_2 levels in stable asthmatic patients treated with inhaled corticosteroids (ICS) are lower compared with steroid-naïve subjects and similar to normal subjects [26–28]. However, most of the studies evaluating treatment effects are cross-sectional, not allowing definite conclusions. In two randomized double-blind placebo-controlled clinical trials, inhaled beclomethasone [29] and the oral administration of the lipid extract of New Zealand green-lipped mussel [31] significantly decreased exhaled H_2O_2 levels, whereas montelukast had no effect on H_2O_2 [32]. Nasal triamcinolone acetonide reduced H_2O_2 concentrations in EBC in patients with allergic rhinitis (AR) with and without asthma [33].

2. Isoprostanes: Isoprostanes are prostaglandin-like compounds formed by the free-radical lipid peroxidation of arachidonic acid and represent *in vivo* markers of oxidative stress. Isoprostanes also possess biological activity and could be mediators of the cellular effects of oxidant stress. A number of studies have shown these compounds to be extremely accurate markers of oxidative stress and have illuminated the role of oxidant injury in association with the production of nitrogen species [34–36]. The most studied isoprostane is 8-epi-PGF_{2a}, also known as 8-isoprostane [35–37].

The levels of 8-isoprostane are doubled in mild asthma and further increased in moderate and severe asthma [38, 39]. Montuschi et al. [39] showed a positive correlation of 8-isoprostane with FeNO in patients with mild asthma, but not in severe ones. Contradictory results have been reported in relation to the effect of ICS. Some studies showed no effect [39, 40], while others support a positive effect under specific conditions (exacerbation and aspirin sensitivity) but with 8-isoprostane levels still remaining higher than normal after treatment [41, 42]. Recent data showed a good correlation between 8-isoprostane and small airways function, indicating that 8-isoprostane reflects small airway inflammation, thus suggesting that it may be used complementary to spirometry in the monitoring of patients with asthma [43]. Measurement of 8-isoprostane concentration in EBC of asthma patients may be useful to evaluate the influence of Gastroesophageal reflux disease on asthma as well as to determine the timing of PPI therapy [44]. Prospective controlled trials are warranted for the evaluation of ICS treatment on 8-isoprostane levels, especially targeting small airway inflammation.

3. Aldehydes and Thiobarbituric Acid-Reactive Products (TBARs): Malondialdehyde is generated mainly by arachidonic acid and docosahexenoic acid, while other classes of aldehydes are produced during lipid peroxidation. Malondialdehyde levels in EBC were increased during asthma exacerbations whereas glutathione levels were decreased and negatively related to malondialdehyde levels [45]. In recovery, after steroid treatment, malondialdehyde levels decreased whereas glutathione levels increased [45]. In a study evaluating aldehydes in EBC and induced sputum in asthmatics, no significant correlations between the two were observed, suggesting that the two techniques must be evaluated independently [46].

TBARs are lipid peroxidation products that are produced after oxidant injury in the airways. TBARs in EBC are higher in asthmatic patients compared with controls [24], while a positive correlation between H_2O_2 and TBARs was observed, supporting a close relation between H_2O_2 and lipid peroxidation products.

In summary, EBC is a useful biological sample for the assessment of oxidative stress. H_2O_2 levels in steroid-treated patients are similar to those of normal subjects, while 8-isoprostane is not steroid responsive. This issue remains controversial and might be explained by the eosinophil-related mechanism of H_2O_2 production and the less eosinophilic pathway of lipid peroxidation. A new commercial device (Ecocheck) may offer a fast and easily performed alternative assay for the measurements of H_2O_2 levels 'in the field', given the fact that its detection limit is comparable with the already established methods [23]. Once EBC H_2O_2 may reflect the underlying eosinophilic inflammation in patients with asthma [27, 28], its routine measurement in daily clinical practice may be of great value, especially in the specific phenotype of eosinophilic asthma.

Nitric oxide-related products

NO plays important roles in the regulation of the smooth muscle tone of pulmonary blood vessels and bronchi. An increase in NO may be due to activation of inducible NO synthase (iNOS) expressed by epithelial cells in response to pro-inflammatory cytokines and oxidants [47]. NO is a free radical due to its unpaired electron and it may react with oxygen to yield nitrogen oxides (NO_x) or with a superoxide anion to yield peroxynitrite, a highly reactive

substance that may lead to the development of NO-derived products [47, 48]. Thus, NO release in the respiratory system can be measured indirectly by quantifying nitrite/nitrate, nitrotyrosine or nitrosothiols in EBC.

1. Nitrite/nitrate (NO_2^-/NO_3^-) [NO_x]: NO is enzymatically produced by NOS, and then oxidized to nitrite and nitrate by several mechanisms including macrophage activation. They can be produced by the reaction of NO with oxygen and by the decomposition of peroxynitrite. These, along with other oxides of nitrogen, are called NO_x and have been used to study NO metabolism [49].

Total nitrite/nitrate levels were found to be increased in patients with asthma and to be reduced by the use of ICS and smoking habits [26]. Atopy increased total nitrite/nitrate levels but to a lesser degree compared with concomitant asthma [50]. EBC nitrite/nitrate were significantly associated with FeNO [50] and oxidative stress as assessed by the concentration of H_2O_2 [26]. Additionally, nitrate (NO_3^-), the stable oxidative end product of NO metabolism, is increased in patients with asthma and, surprisingly, in normal smokers [51]. It still remains controversial whether the low FeNO values in smoking asthmatics are related to reduced endogenous NO production by feedback inhibition or represent a modulation of NO biochemistry, which – through oxidation processes – leads to a transiently higher concentration of NO-derived products [52].

2. Nitrotyrosine: The reaction of NO and superoxide anions O_2^- in the airway results in the formation of peroxynitrite, a highly reactive oxidant species. Nitrotyrosine is produced mainly by the reaction of peroxynitrite with tyrosine residues of proteins [48].

Nitrotyrosine EBC levels are increased in steroid-naïve adults [53] and children [54] with asthma, but are lower in patients with moderate to severe asthma receiving oral steroids [53]. This means that systemic steroids may inhibit the inflammatory response in the airways, therefore reducing local oxidative stress [52]. Regarding the association between nitrotyrosine and FeNO, conflicting results have been reported [53, 54], and yet significant correlations seem to exist in steroid-naïve patients [53]. In a single-blind placebo-controlled study, the administration of flunisolide significantly decreased EBC nitrotyrosine levels compared with placebo [55].

3. S-Nitrosothiols (RSNOs): They are produced by the interaction of peroxynitrite with thiol-containing macromolecules, such as cysteine and glutathione, which act as antioxidants and limit the nitrosative stress potential of NO and NO-related products [56, 57].

RSNOs levels in EBC are increased in bronchial asthma, with higher values detected in patients with severe asthma compared with mild ones [58]. A plausible explanation might be that nitrosative stress is greater in severe compared with mild asthma and consequently, there is a stronger adaptive response via the oxidation of NO.

In summary, products of NO metabolism, as nitrite/nitrate, nitrotyrosine and nitrosothiols, can be detected in EBC and may provide useful information for the plausible relation between NO metabolism and the pathophysiology of asthma, although the pathophysiological implications of the modulation of NO-derived products are mostly unknown. The most important information derived from studies so far is the effort to explain the disparity between EBC NO_x observations and the decline of FeNO after smoking and the possibility that smoking increases degradation of NO to NO_x. Further studies are needed for the evaluation of EBC NO-related products in smoking asthmatics and their response to treatment.

Arachidonic acid metabolites (eicosanoids)

The eicosanoids represent a largely heterogeneous family of C_{20}-unsaturated fatty acids derivatives. Prostanoids, leukotrienes and epoxides are produced through the actions of three types of oxygenases: cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 [59].

1. Prostanoids: They are synthesized via the COX pathway and include two families of substances: prostaglandins and thromboxanes [60, 61]. Prostaglandins E_2 (PGE_2, which presents both inflammatory and anti-inflammatory effects), D_2 (PGD_2) [41, 62–64] and PGF_{2a} [63], as well as thromboxane B_2 (TXB_2) [65] have been determined in EBC samples.

No difference in PGE_2 levels was found between asthmatics and normal controls, irrespective of steroid use [62, 66, 67]. However, smoking asthmatics had significantly higher PGE_2 values compared with asthmatic non-smokers and control subjects, possibly suggesting a protective bronchodilative mechanism against the increased inflammatory response in smoking asthmatics [62]. In the same study, no significant correlation was observed between PGE_2 levels and bronchial hyperresponsiveness, indicating no protective action against bronchial hyperreactivity [62]. A trial of ICS in children with asthma did not reduce PGE_2 in EBC, despite a significant reduction in LTE_4 and exhaled NO [67], and neither did the administration of montelukast in another trial [68]. Furthermore, exhaled PGE_2 levels were not reduced in patients with aspirin-induced asthma (AIA), suggesting that a deficiency of PGE_2 is not an important mechanism mediating AIA [41]. This was further supported by the absence of alteration in EBC PGE_2 concentrations after oral challenge with aspirin [69]. Finally, in one study, TXB_2 was increased in the EBC of asthmatic subjects compared with controls [65], whereas in another study in asthmatic children in whom TXB2 was detected, there was no difference in TXB2 concentrations in EBC between asthmatic children and healthy children [67].

2. Leukotrienes: Leukotrienes are synthesized in the leucocytes from arachidonic acid through the action of

5-lipoxygenase and are classified into two classes: LTB_4 and cysteinyl leukotrienes (Cys-LTs, i.e. LTC_4, LTD_4 and LTE_4) [61, 70, 71]. Leukotrienes have been investigated thoroughly in the setting of bronchial asthma and AR in adults and children.

Exhaled Cys-LTs are increased in both adults [63, 72] and children [72, 73] with asthma, especially in patients with unstable asthma [74]. Increased CysLTs during asthma exacerbations in children returned to normal after treatment with oral corticosteroids [42]. CysLTs are also increased in AR [75], exercise-induced bronchoconstriction (EIB) [76] and AIA [41]. EBC cys-LTs are increased after AMP challenge, but not after metacholine challenge, indicating that the AMP acts indirectly but more selectively by releasing cys-LTs from primed mast cells [77]. LTE4 concentrations in EBC are elevated in patients with asthma [63, 67, 68, 72, 78] and are negatively associated with AHR to methacholine [79]. In a recent study, an association between EBC cys-LTs and reticular basement membrane thickness was shown in a subgroup of children who were not treated with montelukast, a cys-LTs receptor antagonist [79], suggesting that cys-LTs may play an important role in airway remodelling, which is partially reversible in those patients treated with montelukast. However, the cross-sectional methodology does not allow strong conclusions to be drawn. Nasal steroids [75], montelukast [68, 79, 80] and allergen avoidance [81] significantly affect exhaled cys-LTs concentrations.

The role of LTB_4 – a potent chemoattractant of neutrophils – in asthma is largely unknown. LTB_4 has been found to be increased in the EBC of asthmatic adults [53, 63] and children [67, 82] but not in atopic children without asthma [82]. In a cross-sectional study, the use of ICS was related to decreased EBC levels of LTB_4 in asthmatic children [82]. The above effect of ICS seemed to be dose-dependent. However, in another study, no effect of ICS was observed [81]. Montelukast also decreased LTB_4 levels in a time-dependent manner [80]. Smoking asthmatics had higher levels of LTB_4 compared with smokers without asthma [83] and, interestingly, they had values similar to COPD patients who shared a characteristic of asthma pathophysiology, the significant reversibility of airway obstruction after bronchodilation [83]. In the same study, a good correlation between EBC and sputum measurements was observed [83]. Despite the interesting data for LTB_4 in asthma, it is still considered to be a more significant mediator for COPD.

In summary, the detailed study of eicosanoids in EBC provides useful information for asthma pathophysiology. PGE_2 may play a protective role, through its bronchodilative properties, in smoking asthmatics where inflammation is further increased. Cys-LTs are participating in the inflammatory process of different aspects of the disease, like exercise-induced asthma, aspirin-sensitive asthma and airway remodelling. Finally, the implication of LTB_4 in asthmatic inflammation is not well established, although there is evidence that supports an important role for LTB_4 in more severe forms of asthma, in analogy to what was hypothesized in COPD [84], which is probably related to neutrophilia.

pH

Measurement of pH with pH electrodes after deaeration for the removal of CO_2 with a CO_2-free gas (e.g. argon or nitrogen) is a technically validated EBC measurement in the published literature [15, 16]. Measurement of EBC pH is a simple, robust, reproducible and relevant marker of disease [85]. In healthy subjects, EBC pH after deaeration has a mean pH of 7.7 with a range of normal considered by the investigators to be 7.4–8.8 [86]. A recent study, although, suggests that the standardization of EBC pH to a certain level of PCO_2 in EBC represents the most reproducible method for the measurement of EBC pH, both in normal subjects and in asthmatics [87]. There is a debate as to whether orally collected EBC pH assays reflect acidification of the lower airways, because the high ammonia content of the mouth may conceivably interfere with the assay [88]. This concern has not been proven and extensive data do not reveal an effect of oral ammonia on EBC pH assays [88–90]. Measurement of pH of deareated EBC is not affected by hyperventilation, temperature of collection, duration of collection or storage, or acute airway obstruction with metacholine [85], but is dependent on the collection devices used [91, 92].

The measurement of EBC-pH has been proposed to be a promising non-invasive tool in the assessment of patients with asthma. It was found to reflect acute exacerbations and to normalize with anti-inflammatory therapy [16]. Patients with stable persistent asthma, both adults and children, appear to have lower EBC pH values than healthy controls [15, 50]. The above difference was further affected by exacerbations [16], underlying disease severity [15] and smoking [93]. Airway acidification was also found in children with AR and atopic dermatitis [94]. However, in the epidemiologic setting, EBC pH did not differ between children with and without parentally reported symptoms suggestive of asthma [95]. In that study, there was no consistent association between EBC pH and lung function, airway hyperresponsiveness and airway inflammation, as expressed by FeNO [95]. There is some evidence that EBC pH is significantly correlated with the number of eosinophils obtained from induced sputum in moderate asthmatics, as well as with parameters expressing oxidative stress and NO metabolism in EBC [15, 16]. Glutaminase expression and activity in the human airway epithelium may be related to the regulation of airway pH [96]. Other interesting evidence showed that a NO synthase-dependent host response to viral infection mediated by S-nitrosothiols, rather than direct infec-

tion itself, plays a role in decreased airway surface pH during human rhinovirus infection [97]. Finally, the neutralization of airway pH caused a decrease in FeNO, especially in persistent asthma, providing a potential clinical and therapeutic implication for EBC pH [98].

In summary, EBC pH is associated with important components of asthma pathophysiology (eosinophilic inflammation, NO metabolism, host defence during exacerbations), it is easy to perform, measurable in the field, and reproducible, it has established normal values and may be measured with portable equipment. However, EBC pH has failed to be valuable in the epidemiological setting in children with and without parentally reported symptoms suggestive of asthma [95]. A study that will evaluate whether pH could be used for the guidance of asthma management, in a manner similar to FeNO [4] and sputum eosinophils [8], is urgently warranted.

Cytokines and other mediators

Various cytokines have been analysed in EBC. The main limiting factors regarding cytokine evaluation are the measurement techniques, because detection limits and intra-inter assay variability are affected by the method used [13]. The use of protein arrays as well as the multiplex analysis with cytometric bead arrays improve the validation of cytokines in EBC [99, 100]. An increased ratio of IL-4/IFN-γ was observed in EBC, consistent with the predominance of T helper type 2 (Th2)-type inflammation in the airways of children with asthma [101]. Many cytokines, chemokines and growth factors, like IL-4, IL-8, IL-17, TNF-α, RANTES, IFN-γ-inducible protein 10, TGF-β, macrophage-derived chemokine (MDC), eotaxin and macrophage inflammatory protein 1α and 1β, were significantly up-regulated in asthmatic airways [99–101]. Among the up-regulated molecules, RANTES expression was significantly correlated with parameters representing airway calibre, FEV_1 and respiratory resistance values [100]. In addition, the levels of both TNF-α and TGF-β were significantly correlated with methacholine threshold and peak expiratory flow variability [99].

Adenosine concentration in EBC is significantly increased in EBC of asthmatic patients during exercise but not in healthy control subjects [102]. Exercise-induced changes in adenosine concentration correlated significantly with the decline in FEV_1 values. EBC adenosine is also increased in AR, indicating that non-asthmatic patients with AR may have subclinical inflammation in their lower airways [103]. Metallic elements studied in EBC may be useful in distinguishing asthma from COPD since the latest was found to have more elevated levels [104].

Recently, a useful observation emerged from Carraro et al. [105], showing that investigation of metabolomics can be applied to EBC, characterizing the airway biochemical fingerprints. In this study, the presence of acetylated compounds suggests a new metabolic pathway that may have a role in asthma pathophysiology.

Conclusions

There is some evidence that certain markers in EBC may correlate with asthma severity, lung function impairment, airway remodelling and different aspects of the disease such as aspirin sensitization or EIB. Despite the encouraging positive results to date, the introduction of EBC in everyday clinical practice requires the resolution of some methodological pitfalls, the standardization of EBC collection and finally the identification of a reliable biomarker that is reproducible, has normal values and provides information about the underlying inflammatory process and the response to treatment. EBC pH is reproducible, normal values are available, it is measurable on-site with a standardized methodology, but has not been evaluated prospectively as a guide for treatment. Despite promising data, EBC still have some problems that are mainly attributable to the different assays used for determination, different devices used in collection and the large intra- and inter-assay variability specifically in those values that are close to the detection limit. It is crucial in order to progress in the field to perform studies relating to reproducibility, to develop more sensitive and specific assays for the mediators and finally to try to establish normal values because most of the laboratories do not present similar results for the same mediator. EBC represents a simple and totally non-invasive procedure that may contribute towards our understanding of asthma pathophysiology. Furthermore, besides the evaluation of new biomarkers, standardization of the already existing procedures is warranted for the introduction of EBC in clinical practice.

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