Author/s:
Aldakheel, FM;Thomas, PS;Bourke, JE;Matheson, MC;Dharmage, SC;Lowe, AJ

Title:
Relationships between adult asthma and oxidative stress markers and pH in exhaled breath condensate: A systematic review

Date:
2016-06-01

Citation:
Aldakheel, F. M., Thomas, P. S., Bourke, J. E., Matheson, M. C., Dharmage, S. C. & Lowe, A. J. (2016). Relationships between adult asthma and oxidative stress markers and pH in exhaled breath condensate: A systematic review. Allergy European Journal of Allergy and Clinical Immunology, 71 (6), pp.741-757. https://doi.org/10.1111/all.12865.

Persistent Link:
https://hdl.handle.net/11343/291047

Received Date : 03-Jul-2015

Revised Date : 14-Feb-2016

Accepted Date : 15-Feb-2016

Article type : Review



**Title page**

**Title:** Relationships between adult asthma and oxidative stress markers and pH in exhaled breath condensate: *a systematic review*

**Authors:**

Fahad M. Aldakheel, MSc[1,2]; Paul S. Thomas, MD, FRACP, FRCP[3]; Jane E. Bourke, PhD[4]; Melanie C. Matheson, PhD[1]; Shyamali C. Dharmage, MBBS, MD, PhD[1,5]; and Adrian J. Lowe*, PhD[1,5]

**Affiliations:**

[1]Allergy and Lung Health Unit, the University of Melbourne, Melbourne, Australia.

[2]Department of Clinical Laboratory Sciences, King Saud University, Riyadh, Saudi Arabia.

[3]Department of Respiratory Medicine & Prince of Wales Hospital Clinical School, University of New South Wales, Sydney, Australia.

[4]Department of Pharmacology, Monash University, Melbourne, Australia.

[5]Murdoch Children's Research Institute, Melbourne, Australia.

**Corresponding author full contact details:**

Dr Adrian Lowe BBSC, MPH, PhD

The University of Melbourne, Level 3, 207 Bouverie Street, Carlton VIC 3053 Australia

Fax: +61 3 9349 5815

Email: lowea@unimelb.edu.au



**Authors contributions:**

**FA** designed the systematic review with input from **AL, PT, SD, JB** and **MM**, and wrote the first draft of the paper. **AL, PT, SD, JB** and **MM** critical revised and edited the manuscript and approved the final version.

**Declaration of all source of funding:**

Fahad Aldakheel is sponsored by King Saud University, which is represented by the Cultural Mission of Royal Embassy of Saudi Arabia in Canberra. Melanie Matheson, Shyamali C Dharmage, and Adrian J Lowe are supported by the National Health and Medical Research Council (NHMRC).

**Conflict of interest:**

The authors declare they have no conflict of interest in relation to this review.

**Word count for abstract: 196, text: 5,362.**

**Short title:** Adult asthma and EBC oxidative stress markers


## ABSTRACT

Oxidative stress has a recognised role in the pathophysiology of asthma. Recently, interest has increased in the assessment of pH and airway oxidative stress markers. Collection of exhaled breath condensate (EBC) and quantification of biomarkers in breath samples can potentially indicate lung disease activity and help in the study of airway inflammation, and asthma severity. Levels of oxidative stress markers in the EBC have been systematically evaluated in children with asthma; however, there is no such systematic review conducted for adult asthma. A systematic review of oxidative stress markers measured in EBC of adult asthma was conducted and studies were identified by searching MEDLINE and SCOPUS databases. Sixteen papers met the inclusion criteria. Concentrations of exhaled hydrogen ions, nitric oxide products, hydrogen peroxide and 8-isoprostanes were generally elevated and related to lower lung function tests in adults with asthma compared to healthy subjects. Assessment of EBC markers may be a non-invasive approach to evaluate airway inflammation, exacerbations, and disease severity of asthma, and to monitor the effectiveness of anti-inflammatory treatment regimens. Longitudinal studies, using standardised analytical techniques for EBC collection, are required to establish reference values for interpretation of EBC markers in the context of asthma.



**Key words:** Asthma; Exhaled breath condensate; Oxidative stress; pH; Reactive oxygen species.


**Abbrevitions:**

| | | | |
|---|---|---|---|
| CA: | Classic asthma. | $H_2O_2$: | Hydrogen peroxide. |
| CVA: | Cough variant asthma. | ICS: | Inhaled corticosteroid. |
| EBC: | Exhaled breath condensate. | NT: | Nitrotyrosine. |
| EIA: | Enzyme immunoassay. | $NO_x$: | Nitric oxide products. |
| ELF: | Epithelial lining fluid. | ROS: | Reactive oxygen species. |
| $H^+$: | Hydrogen ions | 8-isoP: | 8-isoprostane. |

## INTRODUCTION

Asthma is the result of a complex array of pathophysiological responses, including the activation of immune cells such as T lymphocytes, macrophages, neutrophils, eosinophils and mast cells (1, 2). In clinical practice, asthma and other inflammatory airway diseases can be diagnosed according to clinical manifestations, investigations of airway function and response to treatment (3, 4). Recently, there is increasing interest in assessing inflammatory biomarkers in the respiratory tract to: a) aid in the differential diagnosis of various respiratory disorders and the sub-typing of asthma phenotypes b) inform prognosis and c) predict and monitor therapeutic responsiveness. For this reason, techniques for non-invasive sampling of the airways have been developed.

Non-invasive breath analytical methods are increasingly contributing to the understanding of respiratory diseases, monitoring airway inflammation and disease severity. Exhaled breath condensate (EBC) collection is one such method (5-8). Collection of EBC is non-invasive, inexpensive, quick and easy to perform and allows for measurements of biomarkers in many respiratory conditions, including asthma (6, 9-12). Exhaled breath is saturated with water vapour, which is condensated by breathing through a cooling or freezing system. This condensate also contains a mixture of volatile and non-volatile compounds, which are produced from the airway epithelial lining fluid (ELF) (6, 12, 13). While volatile compounds are present in EBC due to the partitioning between the gaseous and the aqueous phase of the exhaled breath, the mechanism of formation of non-volatile compounds collected in EBC has not yet been fully clarified. However, it is assumed that respiratory surface liquid becomes aerosolised as a result of ***1)*** turbulence in the airways, or ***2)*** during the sudden opening of closed respiratory bronchioles and alveoli in the absence of bulk airflow, and it will depend on the gas velocity and the surface tension of the extracellular lining fluid. The balance between velocity and surface tension at the alveolar level determines the generation of aerosols (14).The EBC comprises mostly water but also includes a wide range of exhaled inflammatory biomarkers, albeit at often at very low concentrations, including nitric oxide products ($NO_x$), hydrogen peroxide ($H_2O_2$), hydrogen ions ($H^+$), eicosanoids (e.g. 8-isoprostane (8-isoP), leukotrienes, prostanoids), aldehydes, peptides, adenosine, ammonia, cytokines (e.g. interleukins (ILs), tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ)) and macromolecules such as mucin and DNA (5, 10, 15, 16). However, the variable dilution of the EBC constituents has impeded its application as a representation of airway ELF. Without a reliable measure of a dilution factor, the precise concentration of biomarkers in the ELF

cannot be accurately determined (17). Hence, dilution reference indicators are required to serve as a comparison for calculation of accurate concentrations of biomarker in respiratory fluid, and as a normalising indicator for inter-subject variability in droplet formation. It must be noted that the issue of dilution indicators is relevant only for non-volatile EBC biomarkers and not the volatile components, which are affected by different factors (18). Furthermore, although a potential limitation of this method is that the precise origin of EBC mediators from within the respiratory tract is unknown (6, 16, 19), evaluation of these biological mediators may reflect changes in the airway ELF associated with lung diseases including asthma (8, 13).

In asthma, the development of airway inflammation secondary to the actions of inflammatory mediators can lead to an imbalance between reactive oxygen species (ROS) and anti-oxidants, resulting in oxidative stress (11). Increased expression and secretion of ROS by activated inflammatory cells, including neutrophils, macrophages and eosinophils, can lead to further generation of inflammatory mediators, damaging the epithelial cells and increasing bronchial hyperreactivity (20-22). Many biomarkers of respiratory inflammation and oxidative stress have been detected within EBC samples in various inflammatory lung diseases (5, 11, 23). There are significant increases in the levels of most oxidative stress markers, including $NO_x$ (23) nitrotyrosine (NT) (6), $H_2O_2$ (24) and 8-isoP (25) reflecting increased inflammation of the airways. While $H^+$ ions are not strictly part of oxidative stress, increased acidity is also associated with inflammation and airway damage (6, 26).

We have previously systematically summarised the literature indicating that the levels of oxidative stress markers are increased in the EBC in children with asthma, with a particular link to severe asthma and exacerbations (8). In this manuscript, we systematically reviewed the evidence to determine if pH and oxidative stress markers in EBC are elevated in adults with asthma. We have also described the relationships between these markers and the severity of disease, lung function and other markers of airway inflammation.

## METHODS

This systematic review was carried out using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (27), involving a 27-item checklist "Table S1 in the online supporting information".

### *Search strategy*

Potential studies were systematically identified using MEDLINE (1950 to 25th of August 2014) and SCOPUS (1960 to 29th of August 2014). To identify studies examining the levels of oxidative stress markers in the EBC of patients with asthma, a combination of three groups of keywords were used as shown in "Figure S1" in the online supporting information for this paper. The first group of terms related to asthma, the second to EBC, and the third to oxidative stress markers. MeSH headings in MEDLINE and advanced search options in SCOPUS were applied by considering the inclusion criteria. The title and abstract for each study were reviewed for eligible inclusion criteria by one reviewer (FA). Complete assessment of the full text of the studies selected was conducted to ensure they met the inclusion criteria. Reference lists of the studies included and review papers were also searched.

***Inclusion criteria***

Studies were included if they met the following criteria; (1) studies of human subjects, (2) published in English, (3) peer reviewed (4) quantitatively examined at least one oxidative stress marker in EBC, and (5) measured associations between this EBC marker and asthma or wheezing in adults (≥18 years).

***Exclusion criteria***

Studies were excluded if (1) no original data were reported (e.g. review papers), (2) the level of EBC oxidative stress marker(s) were measured in diseases other than asthma, wheeze or exercise-induced bronchoconstriction (e.g. chronic obstructive pulmonary disease (COPD), pneumonia, cystic fibrosis or lung cancer) or (3) there was no healthy control group (i.e. without a history of pulmonary disorder).

***Data extraction***

Descriptive details for each eligible study were extracted in tabular format. Data extracted included: the first author and year of publication, study design, country of study, number and age of participants, method of diagnosing asthma, process of collecting EBC, the technique used for analysing EBC markers and any reported associations between the EBC markers and asthma status.

**RESULTS**

***Search results***

The systematic search resulted in a total of 1,356 studies from MEDLINE and 1,670 studies from SCOPUS. These two database search results were merged, resulting in the identification of a total of 1,167 duplicate records. After reviewing the titles and abstracts of the 1,859 unique records potentially eligible for inclusion, 202 studies were selected for full text screening. Of these186 studies were subsequently excluded after applying the exclusion criteria; yielding 16 relevant studies (*see* **Figure 1**).

***Characteristics of included studies***

In the sixteen studies included, results were reported from 1,338 participants, comprising 556 healthy controls and 832 patients with asthma. Among those included studies, level of hydrogen ions (pH), in the EBC was measured in six studies (28-33). Nitrite and nitrate in EBC were measured in only one study (29), and three studies assessed the levels of total nitric oxide ($NO_x$) products (29, 34, 35). The level of nitrotyrosine was reported in one study (36). Concentrations of hydrogen peroxide ($H_2O_2$) were addressed in eight studies (29, 32, 34, 37-41). EBC 8-isoprostanes (8-IsoP) concentrations were reported in four studies (30, 31, 42, 43). The majority of studies were conducted in European countries (n=10 (28, 34-42), or Japan (n=3 (29-31)). The remaining studies were from the USA (n=2 (33, 43)) and Iraq (n=1 (32)).

Control groups in these studies were consistently defined as those who lacked respiratory conditions and inflammatory airway diseases. Only six of the included studies reported that control groups were non-atopic (28, 31, 34, 39, 41, 42). The definitions of asthma in the included studies were based on clinical manifestations, responsiveness to bronchodilator drugs, measurements of pulmonary function, and physician diagnosis (n=4 (28, 37, 38, 40)). Asthma was defined in a range of ways in the remaining twelve studies (*see* **Table 1**). Seven out of sixteen studies assessed atopic status in adult patients with asthma, using skin prick testing, and the proportion of those patients with atopic asthma ranged from 33% to 100% (34, 35, 37-39, 41, 43).

The proportion of patients with asthma receiving inhaled corticosteroid (ICS) treatment also varied across the included studies, ranging from 15% to 80% (28, 29, 34-37, 39, 42). A number of different devices were used to collect EBC, with a custom-made condensing method used in seven studies (29, 32, 33, 37, 38, 41, 42), the EcoScreen in five studies (28, 30, 31, 36, 40), the R-Tube in two studies (35, 43) and the Heat-Exchanger Unit (RHES) in two studies (34, 39).

**Possible factors that influence the levels of EBC oxidative stress markers**

The breathing pattern, type of collection device, the condensing temperature and the materials used in the collection chamber can influence the collection of EBC (12). One study reported that EBC samples collected with the R-Tube were more acidic than those collected with EcoScreen in the same healthy individuals (28), suggesting that the results varied with the collection unit. Treatment with argon gas may also influence the measurement of EBC pH (28, 29, 33) as it removes residual carbon dioxide and stabilise pH (44).

***Findings of the studies***

The findings are divided into following sections: associations between each EBC maker and (1) asthma prevalence (2), asthma severity, and (3) airway inflammatory markers such as lung function parameters and fractionated exhaled nitric oxide (FeNO).

**Associations between EBC markers and prevalence of asthma**

***Acidity (pH)***

Airway acidification has been shown to be associated with the pathophysiology of asthma, leading to increased mucus viscosity and bronchial epithelial damage, contributing to bronchoconstriction (28, 32). EBC pH was assessed in six studies (28-33). In four of these studies, pH values in EBC from individuals with asthma were lower when compared to the healthy groups (28, 30-32). This was irrespective of whether the EBC was untreated (30-32), or treated with argon gas (28, 29, 33). On the other hand, one study failed to find any significant difference in the level of EBC pH between healthy individuals and patients with stable asthma (33). EBC pH was significantly lower in both patients with classic asthma (CA - patients with a history of cough, wheezing and dyspnea) and those with cough variant asthma (CVA – patients with a history of cough but not of wheezing or dyspnea) when compared to healthy controls (32). In one study, pH levels in untreated EBC samples obtained from subjects with asthma (mean age=56 years) were higher when compared to healthy controls (mean age=22 years) (29). This difference was no longer evident after deaeration of the EBC samples (29) (**Table2**).

***Nitric oxide (NO) products***

Nitric oxide (NO) plays an important biological role in the inflammatory process. NO levels are elevated in airway inflammation in response to pro-inflammatory molecules and

oxidant capacity, and the free radicals of NO react with oxygen to generate nitric oxide products as nitrite ($NO_2^-$) and nitrate ($NO_3^-$) (34, 35). Of the three studies that used the fluorometric-modified Griess reaction to measure the stable end-products of NO metabolism ($NO_x$), $NO_x$ levels were significantly higher in EBC from those with asthma compared with the healthy control groups in two studies (29, 34). In contrast, in a large epidemiological study (n=523), no significant differences in the levels of EBC $NO_2^-/NO_3^-$ ($NO_x$) between adults with and without asthma were found (35).

Nitrotyrosine (NT) is another stable end-product formed by the reaction of peroxynitrite ($ONOO^-$) and *p*-tyrosine proteins (22, 36). In a single study, the level of NT assessed by enzyme immunoassay (EIA) was detectable in EBC of healthy controls, and increased significantly in patients with mild asthma (36) (**Table 3**).

***Hydrogen peroxide ($H_2O_2$)***

Active inflammatory cells, particularly eosinophils, respond to stress with a respiratory burst, leading to an increased production of ROS. $H_2O_2$ is one form of ROS that results from enzyme-catalysed dismutation of superoxide anions (38, 41). Since $H_2O_2$ is released by the activation of inflammatory cells, including neutrophils and eosinophils, EBC levels of $H_2O_2$ might reflect the influx of inflammatory cells into the lungs as well as disease severity (37, 38). The levels of $H_2O_2$ in EBC were measured in eight studies, using spectrofluorometry (29, 32, 34, 37-41). Concentrations of exhaled $H_2O_2$ in individuals with asthma were significantly higher than those of healthy controls (29, 34, 37-41). EBC $H_2O_2$ levels in patients with CA or CVA were also significantly higher than healthy controls (32). Two studies reported higher values of EBC $H_2O_2$ in individuals with atopic asthma compared to healthy controls (34, 39) (**Table 4**).

***8-isoprostane (8-isoP)***

Isoprostanes are prostaglandin (PG)-like molecules formed by free radical-induced peroxidation of arachidonic fatty acids. 8-isoprostane, which is a member of the $F_2$-isoprostane family, has been demonstrated to be elevated in inflamed airways (30, 43). In four studies, 8-isoprostane concentrations in EBC of adults with asthma were measured using specific EIA (42) or radioimmunoassay (RIA) (30, 31, 43). It should be noted that RIA for this marker has been validated by high-performance liquid chromatography (HPLC) (45). In three of these studies, significantly higher concentrations of EBC 8-isoP were observed in

individuals with asthma compared to the healthy subjects (30, 31, 42). A smaller study (n=14) reported that EBC levels of 8-isoprostane in adults with asthma was not significantly elevated compared to healthy individuals, possibly due to limited power (43) (**Table 5**).

**Associations between EBC markers and asthma severity**

The relationship between EBC markers of oxidative stress and severity of asthma was examined in three studies (29, 33, 42). Among these, two studies examined the relationship between EBC pH and asthma severity (29, 33). It was found that patients with mild persistent asthma had significantly lower EBC pH than those with mild intermittent, moderate persistent and severe persistent asthma (29). In contrast, lower pH values were more often detected in those with acute asthma or less controlled asthma (33). Individuals with severe persistent asthma had significantly higher levels of EBC $NO_2^-$ than those patients with mild intermittent asthma. However, among patients with either mild or moderate persistent asthma; the levels of $NO_2^-$didnot show any difference compared to those with mild intermittent asthma (29). Unlike $NO_2^-$, levels of EBC $H_2O_2$ were significantly higher in participants with mild persistent asthma than those individuals with mild intermittent, moderate persistent and severe persistent asthma (29). A single study demonstrated that the EBC 8-isoprostanelevels were significantly higher in subjects with severe asthma when compared to those with mild to moderate asthma (42).

**Associations between EBC markers and lung function and fractional exhaled nitric oxide (FeNO)**

A number of studies examined the relationships between EBC markers and either lung function (peak expiratory flow (PEF), forced expiratory volume in one second ($FEV_1$)), or FeNO as a marker of airway inflammation. No associations were found between pH value in breath condensate and either lung function (FVC and $FEV_1$) (28, 29, 31) or FeNO measurement (28). In one study, significant inverse associations between the levels of exhaled$NO_2^-$, $NO_3^-$, and various aspects of lung function, including PEF and $FEV_1$, were demonstrated in adults with asthma and similarly increased levels of EBC $NO_x$ were associated with reduced levels of PEF (29) and $FEV_1$ (29). The $NO_x$ level in EBC was not associated with the FeNO (35), nor was the level of EBC NT correlated with $FEV_1$ in participants with mild asthma (36).

Negative associations between concentrations of EBC $H_2O_2$ and PEF in patients with asthma were identified in two studies (29, 38). Likewise the level of EBC $H_2O_2$ was negatively correlated with $FEV_1$ (29, 38, 39, 41). In one study, a negative correlation between exhaled $H_2O_2$ and $FEV_1$ was only seen on day 29 after treatment with inhaled HFA-beclomethasone dipropionate glucocorticosteroids (400 μg/day) (37). Positive correlations were observed between levels of EBC 8-isoprostane and FeNO (42), but not with $FEV_1$ (31, 42).

**The effect of therapy on different EBC biomarkers**

Using ICS treatment appears to influence levels of oxidative stress markers in EBC from adults with asthma. Specifically, the pH of EBC for individuals who were hospitalised for asthma and treated with ICS increased by the time of discharge (28). Conversely, another study showed that levels of EBC pH was increased significantly in individuals with acute asthma who had received systemic glucocorticoid therapy, but not ICS, for longer than 48 hours (33). Furthermore, levels of EBC $NO_x$ were significantly lower in adult asthmatics treated with ICS (34). Exhaled NT was much higher in participants with mild asthma who were not treated with ICS compared to controls, and was significantly lower in those patients with severe asthma receiving both oral steroids and ICS when compared to those patients with moderate asthma receiving ICS only (36). This suggests that the use of combined ICS and systemic steroid treatment in patients with asthma might reduce the EBC levels of NT. There is growing evidence that the levels of EBC $H_2O_2$ in patients with asthma respond to ICS therapy (34, 37, 39). This evidence is also supported by Antzack *et al*, who found that daily administration of inhaled HFA-beclomethasone resulted in a rapid and sustained reduction in $H_2O_2$ in patients with asthma who had withheld use of preventer medication for 4 weeks (37). Inpatients with moderate asthma, treatment for two months with a proton pump inhibitor (PPI) was associated with increased EBC pH and reduced 8-isoprostane levels, but only in those with evidence of gastroesophageal reflux disease (30).

**DISCUSSION**

This systematic review found that pH was lower and markers of oxidative stress were generally elevated in the EBC of adults with asthma when compared to healthy controls. There was also some evidence that these differences were related to more severe asthma and worse lung function, and were altered with steroid treatment. Measurement of these mediators

in EBC might be a non-invasive method of monitoring disease severity, airway inflammation, exacerbations and responses to treatment in patients with asthma (28).

Potential factors that are expected to alter the pH value would include concomitant medication, respiratory tract infections, smoking and possibly age and gender (12, 15, 46). Other environmental conditions, including temperature and humidity might also impact on the volume collected of EBC and its biomarkers (6, 10, 28, 46). Acidic pH was more likely to be observed in the low room temperature (46), and this should be considered when collecting EBC and comparing results between populations. Interestingly, while two studies noted whether control participants were smokers (28, 29), no study indicated the smoking status of participants with asthma, which may have led to a potential bias. Degassing the EBC sample with argon (28, 29, 33) is thought to remove $CO_2$, which stabilises the pH (12, 15, 44).

As previously noted in paediatric asthma (8), pH values were lower in EBC obtained from asthma patients than in healthy individuals. Airway pH is reduced in the presence of endogenous or exogenous acids, which cause bronchial epithelial damage and subsequently increase the viscosity of airway mucus of asthma (33, 47). Lower EBC pH values were more often detected in those patients with severe asthma or less controlled asthma (28, 29, 33), indicating that there is an ongoing active inflammation. A lower EBC pH also reflects acute asthma exacerbations, which are then ameliorated with anti-inflammatory treatment such as ICS (47). Thus, a lower EBC pH might be a useful indicator for poorly controlled asthma in adults.

Increased generation of endogenous NO has a cytotoxic effect on lung cell and tissue by manipulating the permeability of cell membrane, or by priming lung macrophages, to produce ROS and RNS leading to DNA damage and tissue injury (48). $NO_x$ products, from the nitrate/nitrite–NO pathway, are stable end-products of NO metabolism, and have an important role in the pathogenesis of airway diseases such as asthma (34, 35). The levels of $NO_2^-$, $NO_3^-$ (29) and $NO_x$ (29, 34) are elevated in the EBC of individuals with asthma compared to healthy subjects, and their concentrations are associated with the asthma severity. However, one study failed to identify a significant difference in EBC $NO_x$ (35) in those with asthma. The finding that $NO_2^-$ in the EBC of adults with asthma is higher than in normal adults is consistent with the observations in childhood asthma (8) making measurement of NO products in EBC potentially useful for the assessment of airway inflammation.

Activation of inflammatory cells in asthma leads to an amplification of the inflammatory process as a consequence of increase production of the biological mediators, reactive oxygen species, superoxide radicals ($O_2^-$) and $H_2O_2$ (49). EBC $H_2O_2$, which is one of the reactive oxygen species, is released by inflammatory cells and can bind with membrane lipids and protein components of bronchial ELF (50, 51). There is consistent evidence of elevated levels of $H_2O_2$ in EBC from individuals with asthma, particularly in those who have current symptoms (8, 40, 52). Furthermore, the level of EBC $H_2O_2$ is negatively associated with $FEV_1$% (29, 37-39).

Elevated EBC $H_2O_2$ in patients with asthma could be the result of the activation of inflammatory leukocytes including eosinophils and neutrophils (20, 21, 53). The EBC $H_2O_2$ level was increased in patients with a history of cough, wheezing and dyspnea, or classic asthma, and was also increased in those individuals with cough variant asthma when compared to healthy individuals (32). This finding suggests that there is increased eosinophilic or leukocytic airway inflammation in both conditions (47, 54) supported by the presence of high number of eosinophils in bronchial biopsy and bronchoalveolar lavage samples of patients with CVA (55). Thus $H_2O_2$ in EBC might be a useful indicator to aid the assessment of not only CA but also CVA (54). Moreover, when compared to healthy controls, the mean levels of EBC $H_2O_2$ are increased further in those with asthma after challenge with N-formyl-methionyl-leucyl-phenylalanine (fMLP, delta [$Ca_2^+$]i), an indicator for activated neutrophils, suggesting that the $H_2O_2$ is released by the activation of neutrophils (53).

8-isoprostane, the most common form of $F_2$-isoprostane, is a non-enzymatic peroxidation product of polyunsaturated fatty acids (30, 43, 56). Elevated levels of EBC 8-isoprostane are believed to play a central role in asthma pathophysiology by activating prostanoid receptors to regulate airway and immune functions in asthma, leading to damage of the airway epithelium and pulmonary smooth muscle (57). Patients with asthma alone (30, 31) or atopic asthma (43) had higher concentrations of EBC 8-isoprostane compared to healthy adults. The concentration of EBC 8-isoprostane appears to be progressively elevated with asthma severity (8, 42). Therefore, the assessment of 8-isoprostane levels in EBC of patients with asthma would potentially reflect the inflammatory pattern involving oxidative stress.

Inhaled corticosteroids are commonly used as the primary treatment for asthmatic inflammation. The use of ICS in patients with asthma improves symptoms, inhibits oxidative

stress in the airways and reduces hyperresponsiveness (58). In cross-sectional studies, patients who were treated with ICS had significantly lower values of $NO_x$ and NT in the EBC compared to steroid-naïve asthmatic individuals (34, 36). These findings corroborate the expectation that corticosteroid treatment may suppress the inhibition of oxidative stress and the pro-inflammatory response in the inflamed airways (59, 60). EBC $H_2O_2$ levels derived from those with current symptoms of asthma are higher than those without symptoms. This may be explained by the fact that patients with asthma are receiving anti-inflammatory treatment such as corticosteroids therapy to inhibit asthma symptoms, which decreases their levels of hydrogen peroxide (40). However, not all EBC oxidative stress markers seem to be affected by ICS treatment in patients with asthma. Although concentrations of EBC 8-isoprostane were increased significantly in adults with asthma compared to healthy controls, the differences between those with and without ICS treatment were not significant, indicating that the level of 8-isoprostane is relatively resistant to ICS therapy (42).

EBC samples can be obtained with minimal risk and inconvenience from both adults and children, which is in contrast to other airway sampling techniques such as bronchial biopsies, bronchoalveolar lavage fluid and induced sputum. Similar to our previous review focused on paediatric asthma (8), concentrations of hydrogen ions, nitric oxide products, hydrogen peroxide and 8-isoprostanes were generally elevated and correlated with lower lung function tests in adults with asthma compared to healthy subjects. In regards to the levels of pH in non-degassed EBC, there were conflicting results between adult and paediatric reviews. One study reported that pH level of non-degassed EBC was higher in adult patients with asthma than in controls (29), whereas the non-degassed EBC were lower in children with asthma than healthy controls (61). It has been recommended that EBC samples must be treated with argon gas to remove carbon dioxide ($CO_2$) in order to improve the reproducibility of the pH level and stability of samples (44). In addition, levels of nitrotyrosine were significantly higher in adults with asthma compared to healthy controls (36), while there was no difference in children (62).

The overall findings of the studies included in this review indicate that EBC collection is a relatively robust technique, despite the differences in methods between studies including asthma definitions, condenser types, and the specificity of the analytical techniques. Asthma was defined in various ways, and the use of standard definition between studies may assist the comparability of results from different populations. There is also growing evidence that the concentration range of EBC biomarkers can be influenced by various factors, including EBC

collecting devices, humidity and temperature of ambient air, salivary contamination, and the type of analytical assays (6, 9-12).

The nature of control population can potentially influence the study findings. While smokers would not typically be considered as suitable healthy controls (63), only two studies reported the smoking status in the control groups (28, 29). In these studies, smokers in the control group had higher $NO_2^-$ (29) and significantly lower pH values (28) in EBC compared with non-smoking control subjects. Thus, the results from the remaining studies that may have included smokers in the non-asthmatic control groups may have underestimated the magnitude of difference in these markers. EBC $H_2O_2$ in a smoking group was five-fold higher than non-smoking group, suggesting that smoking leads to an influx of inflammatory cells such as alveolar macrophages and neutrophils into the lower airways (64). Similarly, smoking in participants in the asthma groups may similarly alter the EBC markers, and this should be documented in future studies.

One of the major problems related to the analysis of EBC is the levels of certain mediators in the mixture and the lack of a standard method to correct for dilution via the water content within breath. It has been reported that the variations in the concentration of biomarkers in EBC may be attributed to the diversity in the dilution and/or nature of condensate collected by different collection devices, sampling conditions, storage, and sensitivity of the analytical techniques used. It is also unknown whether differences in EBC biomarker levels are due to corresponding differences in ELF or varying sizes of breath droplets arising from an identical source (17, 65). Although measurements of electrolytes, urea, or the conductance of lyophilisation samples have been suggested as possible dilution reference indicators in EBC (18, 66), their role in EBC analysis is controversial. EBC data can be expressed as a change in ratio of one compound to another (18). Therefore, the use of dilution reference indicators has been proposed to calculate the true concentrations of inflammatory mediators and to correct for inter-individual variations in droplet formation but, such dilution methods are yet to be clearly defined or accepted (67).

Further research is needed to standardise the methods including EBC collection procedures, analytical techniques and establishing universal reference values before introducing this procedure into the clinical practice (6, 10). Moreover, additional investigation is required to determine if these oxidative stress markers in asthma can be influenced by

potential confounding factors such as age, gender, smoking, use of asthma medication and atopic status (47).

Most studies that assessed the associations of the EBC oxidative stress markers in inflammatory airways of asthma diseases have relied on a cross-sectional design. Additional longitudinal studies are needed in order to improve the utility of EBC collection and measurement of biomarkers to *(a)* predict responsiveness to specific pharmacological therapies such as corticosteroids in patients with asthma (8, 59, 68) *(b)* help determine the prognosis of disease, *(c)* evaluate stability these markers over time, *(d)* if the changes in these biomarkers are related to changes in asthma severity or *(e)* can predict remission of disease (68, 69).

As with all systematic reviews, it is possible that publication bias may have influenced the results of this study. If this is the case, the strength of the associations between these biomarkers and asthma and lung function may be exaggerated. It was not possible to assess the evidence for publication bias using funnel plots and other methods, as there were insufficient number of studies that reported associations in a uniform manner.

Furthermore, using high-resolution proton nuclear magnetic resonance (NMR) spectroscopy or mass spectroscopy of EBC for the metabolomic analysis of EBC is a reliable analytical approach both in adults and children (70), and might be used for studying oxidative stress (71). These detection techniques enable characterisation of the EBC metabolites, by producing a 'fingerprint' of the individual samples. This approach seems promising since it may help to distinguish between the heterogeneous spectrum of asthma and help to predict a drug's clinical efficacy (70).

In conclusion, this systematic review demonstrates that oxidative stress markers in the expired breath condensate are consistently elevated in adults with asthma. These findings suggest measuring EBC has the potential to be a useful tool to assess the activation of oxidative stress-related soluble markers in asthma. The studies included in this review varied in terms of the definition of asthma and outcomes, type of EBC condenser, analytical techniques and reference ranges for each marker, indicating that there is a need for further assessments and validation studies to resolve a number of practical issues. To allow EBC analysis to become a useful clinical tool, future studies should evaluate the ability of EBC biomarkers to predict future asthma exacerbations and tailor asthma treatment.

## REFERENCES


1. Bousquet J, Clark T, Hurd S, Khaltaev N, Lenfant C, O'byrne P*, et al.* Gina guidelines on asthma and beyond*. *Allergy* 2007;**62**:102-112.

2. Vignola AM, Chanez P, Campbell AM, Souques F, Lebel B, Enander I*, et al.* Airway inflammation in mild intermittent and in persistent asthma. *Am J Respir Crit Care Med* 1998;**157**:403-409.

3. Dragonieri S, Schot R, Mertens BJ, Le Cessie S, Gauw SA, Spanevello A*, et al.* An electronic nose in the discrimination of patients with asthma and controls. *J Allergy Clin Immunol* 2007;**120**:856-862.

4. Pekkanen J, Pearce N. Defining asthma in epidemiological studies. *Eur Respir J* 1999;**14**:951-957.

5. Lee W, Thomas PS. Oxidative stress in copd and its measurement through exhaled breath condensate. *Clinical and translational science* 2009;**2**:150-155.

6. Chow S, Campbell C, Sandrini A, Thomas PS, Johnson AR, Yates DH. Exhaled breath condensate biomarkers in asbestos-related lung disorders. *Respir Med* 2009;**103**:1091-1097.

7. Baraldi E, Carraro S. Exhaled no and breath condensate. *Paediatric Respiratory Reviews* 2006;**7, Supplement 1**:S20-S22.

8. Thomas P, Lowe A, Samarasinghe P, Lodge C, Huang Y, Abramson M*, et al.* Exhaled breath condensate in pediatric asthma: Promising new advance or pouring cold water on a lot of hot air? A systematic review. *Pediatr Pulmonol* 2013.

9. Griese M, Latzin P, Beck J. A noninvasive method to collect nasally exhaled air condensate in humans of all ages. *Eur J Clin Invest* 2001;**31**:915-920.

10. Montuschi P. Analysis of exhaled breath condensate in respiratory medicine: Methodological aspects and potential clinical applications. *Ther Adv Respir Dis* 2007;**1**:5-23.

11. Chan HP, Tran V, Lewis C, Thomas PS. Elevated levels of oxidative stress markers in exhaled breath condensate. *J Thorac Oncol* 2009;**4**:172-178.

12. Liu J, Conrad DH, Chow S, Tran VH, Yates DH, Thomas PS. Collection devices influence the constituents of exhaled breath condensate. *Eur Respir J* 2007;**30**:807-808.

13. Shahid SK, Kharitonov SA, Wilson NM, Bush A, Barnes PJ. Increased interleukin-4 and decreased interferon-γ in exhaled breath condensate of children with asthma. *Am J Respir Crit Care Med* 2002;**165**:1290-1293.

14. Hunt J. Exhaled breath condensate: An evolving tool for noninvasive evaluation of lung disease. *J Allergy Clin Immunol* 2002;**110**:28-34.

15. Horvath I, Hunt J, Barnes P. Exhaled breath condensate: Methodological recommendations and unresolved questions. *Eur Respir J* 2005;**26**:523-548.

16. Loukides S, Kontogianni K, Hillas G, Horvath I. Exhaled breath condensate in asthma: From bench to bedside. *Curr Med Chem* 2011;**18**:1432-1443.

17. Effros RM, Hoagland KW, Bosbous M, Castillo D, Foss B, Dunning M*, et al.* Dilution of respiratory solutes in exhaled condensates. *Am J Respir Crit Care Med* 2002;**165**:663-669.

18. Effros RM. Exhaled breath condensate: Delusion or dilution? *CHEST Journal* 2010;**138**:471-472.

19. Chow S, Thomas PS, Malouf M, Yates DH. Exhaled breath condensate (ebc) biomarkers in pulmonary fibrosis. *Journal of Breath Research* 2012;**6**:016004.

20. Sahiner UM, Birben E, Erzurum S, Sackesen C, Kalayci O. Oxidative stress in asthma. *The World Allergy Organization journal* 2011;**4**:151.

21. Celik M, Tuncer A, Soyer OU, Saçkesen C, Tanju Besler H, Kalayci O. Oxidative stress in the airways of children with asthma and allergic rhinitis. *Pediatr Allergy Immunol* 2012;**23**:556-561.

22. Ricciardolo FL, Di Stefano A, Sabatini F, Folkerts G. Reactive nitrogen species in the respiratory tract. *Eur J Pharmacol* 2006;**533**:240-252.

23. Ratnawatti, Morton J, Henry RL, Thomas PS. Exhaled breath condensate nitrite/nitrate and ph in relation to pediatric asthma control and exhaled nitric oxide. *Pediatr Pulmonol* 2006;**41**:929-936.

24. Diamant Z, Boot J, Mantzouranis E, Flohr R, Sterk P, Gerth van Wijk R. Biomarkers in asthma and allergic rhinitis. *Pulm Pharmacol Ther* 2010;**23**:468-481.

25. Baraldi E, Ghiro L, Piovan V, Carraro S, Ciabattoni G, Barnes PJ*, et al.* Increased exhaled 8-isoprostane in childhood asthma. *CHEST Journal* 2003;**124**:25-31.

26. Kazani S, Israel E. Utility of exhaled breath condensates across respiratory diseases. *Am J Respir Crit Care Med* 2012;**185**:791-792.

27. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: The prisma statement. *Ann Intern Med* 2009;**151**:264-269.

28. Antus B, Barta I, Kullmann T, Lazar Z, Valyon M, Horvath I*, et al.* Assessment of exhaled breath condensate ph in exacerbations of asthma and chronic obstructive pulmonary disease: A longitudinal study. *Am J Respir Crit Care Med* 2010;**182**:1492-1497.

29. Ueno T, Kataoka M, Hirano A, Iio K, Tanimoto Y, Kanehiro A*, et al.* Inflammatory markers in exhaled breath condensate from patients with asthma. *Respirology (Carlton, Vic)* 2008;**13**:654-663.

30. Shimizu Y, Dobashi K, Zhao JJ, Kawata T, Ono A, Yanagitani N*, et al.* Proton pump inhibitor improves breath marker in moderate asthma with gastroesophageal reflux disease. *Respiration* 2007;**74**:558-564.

31. Zhao JJ, Shimizu Y, Dobashi K, Kawata T, Ono A, Yanagitani N*, et al.* The relationship between oxidative stress and acid stress in adult patients with mild asthma. *J Investig Allergol Clin Immunol* 2008;**18**:41-45.

32. Al Obaidi AHA. Expired breath condensate hydrogen peroxide concentration and ph for screening cough variant asthma among chronic cough. *Annals of thoracic medicine* 2007;**2**:18.

33. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TAE*, et al.* Endogenous airway acidification implications for asthma pathophysiology. *Am J Respir Crit Care Med* 2000;**161**:694-699.

34. Ganas K, Loukides S, Papatheodorou G, Panagou P, Kalogeropoulos N. Total nitrite/nitrate in expired breath condensate of patients with asthma. *Respir Med* 2001;**95**:649-654.

35. Nadif R, Rava M, Decoster B, Huyvaert H, Le Moual N, Bousquet J*, et al.* Exhaled nitric oxide, nitrite/nitrate levels, allergy, rhinitis and asthma in the egea study. *Eur Respir J* 2014:erj02024-02013.

36. Hanazawa T, Kharitonov SA, Barnes PJ. Increased nitrotyrosine in exhaled breath condensate of patients with asthma. *Am J Respir Crit Care Med* 2000;**162**:1273-1276.

37. Antczak A, Kurmanowska Z, Kasielski M, Nowak D. Inhaled glucocorticosteroids decrease hydrogen peroxide level in expired air condensate in asthmatic patients. *Respir Med* 2000;**94**:416-421.

38. Antczak A, Nowak D, Shariati B, Krol M, Piasecka G, Kurmanowska Z. Increased hydrogen peroxide and thiobarbituric acid-reactive products in expired breath condensate of asthmatic patients. *Eur Respir J* 1997;**10**:1235-1241.

39. Loukides S, Bouros D, Papatheodorou G, Panagou P, Siafakas NM. The relationships among hydrogen peroxide in expired breath condensate, airway inflammation, and asthma severity. *Chest* 2002;**121**:338-346.

40. Svensson S, Olin A-C, Lärstad M, Ljungkvist G, Torén K. Determination of hydrogen peroxide in exhaled breath condensate by flow injection analysis with fluorescence detection. *Journal of Chromatography B* 2004;**809**:199-203.

41. Emelyanov A, Fedoseev G, Abulimity A, Rudinski K, Fedoulov A, Karabanov A*, et al.* Elevated concentrations of exhaled hydrogen peroxide in asthmatic patients. *CHEST Journal* 2001;**120**:1136-1139.

42. Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, Barnes PJ. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med* 1999;**160**:216-220.

43. Sood A, Qualls C, Seagrave J, McDonald J, Shohreh R, Chiavaroli A*, et al.* Effect of allergen inhalation on airway oxidant stress, using exhaled breath condensate 8-isoprostane, in mild asthma. *J Asthma* 2013;**50**:449-456.

44. Lin J, Bonnichsen M, Thomas P. Standardization of exhaled breath condensate: Effects of different de-aeration protocols on ph and $h_2o_2$ concentrations. 2011.

45. Montuschi P, Barnes PJ, Ciabattoni G. Measurement of 8-isoprostane in exhaled breath condensate. Methods Mol Biol. 2010;**594**:73-84.

46. Dodig S, Čepelak I. Exhaled breath condensate–from an analytical point of view. *Biochemia medica* 2013;**23**:281-295.

47. Grob N, Aytekin M, Dweik R. Biomarkers in exhaled breath condensate: A review of collection, processing and analysis. *Journal of Breath Research* 2008;**2**:037004.

48. Freeman B. Free radical chemistry of nitric oxide: Looking at the dark side. *CHEST Journal* 1994;**105**:79S-84S.

49. Kharitonov SA, Barnes PJ. Biomarkers of some pulmonary diseases in exhaled breath. *Biomarkers* 2002;**7**:1-32.

50. Buszewski B, Kęsy M, Ligor T, Amann A. Human exhaled air analytics: Biomarkers of diseases. *Biomed Chromatogr* 2007;**21**:553-566.

51. Miekisch W, Schubert JK, Noeldge-Schomburg GF. Diagnostic potential of breath analysis—focus on volatile organic compounds. *Clin Chim Acta* 2004;**347**:25-39.

52. Huang M, Adcock PIM, Barnes PPJ. Hydrogen peroxide in exhaled breath condensate in patients with asthma. *Chest* 2011;**140**.

53. Antczak A, Nowak D, Bialasiewicz P, Kasielski M. Hydrogen peroxide in expired air condensate correlates positively with early steps of peripheral neutrophil activation in asthmatic patients. *Arch Immunol Ther Exp (Warsz)* 1999;**47**:119-126.

54. Bousquet J, Chanez P, Lacoste J, White R, Vic P, Godard P*, et al.* Asthma: A disease remodeling the airways. *Allergy* 1992;**47**:3-11.

55. Niimi A, Amitani R, Suzuki K, Tanaka E, Murayama T, Kuze F. Eosinophilic inflammation in cough variant asthma. *Eur Respir J* 1998;**11**:1064-1069.

56. Carpagnano GE, Kharitonov SA, Resta O, Foschino-Barbaro MP, Gramiccioni E, Barnes PJ. 8-isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep apnea after night and is reduced by continuous positive airway pressure therapy. *Chest* 2003;**124**:1386-1392.

57. Voynow JA, Kummarapurugu A. Isoprostanes and asthma. *Biochimica et Biophysica Acta (BBA)-General Subjects* 2011;**1810**:1091-1095.

58. Kroegel C. Global initiative for asthma (gina) guidelines: 15 years of application. 2009.

59. Kato G, Takahashi K, Izuhara K, Komiya K, Kimura S, Hayashi S. Markers that can reflect asthmatic activity before and after reduction of inhaled corticosteroids: A pilot study. *Biomarker insights* 2013;**8**:97-105.

60. Djukanović R, Roche W, Wilson J, Beasley C, Twentyman O, Howarth P*, et al.* Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990;**142**:434-457.

61. Rosias PPR, Dompeling E, Dentener MA, Pennings HJ, Hendriks HJE, Van Iersel M*, et al.* Childhood asthma: Exhaled markers of airway inflammation, asthma control score, and lung function tests. *Pediatr Pulmonol* 2004;**38**:107-114.

62. Celio S, Troxler H, Durka SS, Chládek J, Wildhaber JH, Sennhauser FH*, et al.* Free 3-nitrotyrosine in exhaled breath condensates of children fails as a marker for oxidative stress in stable cystic fibrosis and asthma. *Nitric Oxide* 2006;**15**:226-232.

63. Liu J, Sandrini A, Thurston MC, Yates DH, Thomas PS. Nitric oxide and exhaled breath nitrite/nitrates in chronic obstructive pulmonary disease patients. *Respiration* 2007;**74**:617-623.

64. Nowak D, Antczak A, Krol M, Pietras T, Shariati B, Bialasiewicz P*, et al.* Increased content of hydrogen peroxide in the expired breath of cigarette smokers. *Eur Respir J* 1996;**9**:652-657.

65. Effros RM, Peterson B, Casaburi R, Su J, Dunning M, Torday J*, et al.* Epithelial lining fluid solute concentrations in chronic obstructive lung disease patients and normal subjects. *J Appl Physiol* 2005;**99**:1286-1292.

66. Konstantinidi EM, Lappas AS, Tzortzi AS, Behrakis PK. Exhaled breath condensate: Technical and diagnostic aspects. *The Scientific World Journal* 2015;**2015**.

67. Ahmadzai H, Huang S, Hettiarachchi R, Lin J-L, Thomas PS, Zhang Q. Exhaled breath condensate: A comprehensive update. *Clin Chem Lab Med* 2013;**51**:1343-1361.

68. Robroeks C, Van De Kant K, Jöbsis Q, Hendriks H, Van Gent R, Wouters E*, et al.* Exhaled nitric oxide and biomarkers in exhaled breath condensate indicate the presence, severity and control of childhood asthma. *Clin Exp Allergy* 2007;**37**:1303-1311.

69. Rosias PP, Robroeks CM, Niemarkt HJ, Kester AD, Vernooy JH, Suykerbuyk J*, et al.* Breath condenser coatings affect measurement of biomarkers in exhaled breath condensate. *Eur Respir J* 2006;**28**:1036-1041.

70. Motta A, Paris D, Melck D, de Laurentiis G, Maniscalco M, Sofia M*, et al.* Nuclear magnetic resonance-based metabolomics of exhaled breath condensate: Methodological aspects. *Eur Respir J* 2012;**39**:498-500.

71. Montuschi P, Paris D, Montella S, Melck D, Mirra V, Santini G*, et al.* Nuclear magnetic resonance–based metabolomics discriminates primary ciliary dyskinesia from cystic fibrosis. *Am J Respir Crit Care Med* 2014;**190**:229-233.

***Table 1:*** *Characteristics of the included studies*

| Study (Author/Year), Country | Study Type | Subjects / Sample Size (*N*) / Age (*Years*) | | | Diagnosis of Asthma | EBC Collecting Device | Biomarker(s) Assessed |
|---|---|---|---|---|---|---|---|
| **Antus (2010), Hungary (28)** | Longitudinal study | Non-atopic healthy controls | 36 | 59.9 ± 3.8 | Defined based on physician-diagnosed asthma. Patients with asthma were admitted to the hospital due to a serious exacerbation of the disease. | EcoScreen | pH |
| | | Asthma exacerbation | 20 (6 steroid-naïve) | 56.5 ± 4.1 | | | |
| | | ICS-treated | 14 | | | | |
| **Ueno (2008), Japan (29)** | Cross-sectional | Healthy controls | 58 | 20–40 | Defined based on the Asthma Prevention and Management Guidelines 2006, Japan. | Custom–made glass condensing device | pH, $NO_2^-$, $NO_3$, $NO_x$ & $H_2O_2$ |
| | | Asthma | 55 (11 steroid-naïve) | 21–78 | | | |
| | | Mild intermittent | 18 | 26–77 | | | |
| | | Mild persistent | 7 | 21–73 | | | |
| | | Moderate persistent | 9 | 25–63 | | | |
| | | Severe persistent | 21 | 31–78 | | | |
| | | ICS-treated | 44 | | | | |
| **Shimizu** | Longitudinal | Healthy controls | 26 | 46.7 ± 2.8 | Defined according to the | EcoScreen | pH & 8-isoP |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **(2007), Japan (30)** | study | Moderate asthma with GERD<br>Asthma with GERD QUEST score ≥4<br>Asthma with GERD QUEST score <4 | 36<br>20<br>16 | 45.2 ± 2.4 | Global Initiative for Asthma (GINA) Guidelines. | | |
| **Zhao (2008), Japan (31)** | Cross-sectional | Non-atopic healthy controls<br>Mild asthma | 20<br>44 | 35.2 ± 2.8<br>37.6 ± 1.5 | Defined as based on the National Institutes of Health–World Health Organization (NIH-WHO) guidelines. | EcoScreen | pH & 8-isoP |
| **Al Obaidi (2007), Iraq (32)** | Cross-sectional | Healthy controls<br>Cough variant asthma (CVA)<br>Classic asthma (CA) | 27<br>27<br>43 | | Cough Variant Asthma (CVA) was defined based on the Allergy and Asthmology Centre in Tikrit Teaching Hospital, and asthma was defined based on the National Heart, Lung and Blood Institute (NHLBI) criteria. | Custom–made glass condensing device | pH & $H_2O_2$ |
| **Hunt (2000),** | Longitudinal | Healthy Controls | 19 | 20.5 ± 2.3 | Ever asthma was defined | Custom–made | pH |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **The USA** | study | Stable asthma | 12 | 21.5 ± 2.0 | based on the history of | glass | |
| **(33)** | | Acute asthma | 22 (6 had received systemic glucocorticoid therapy for >48 hours) | 19.5 ± 2.1 | three or more episodes of $\beta_2$-agonist–reversible airway obstruction. | condensing device | |
| **Ganas (2001), Greece** | Cross-sectional | Non-atopic healthy controls | 10 | 21-32 | Defined based on the American Thoracic Society | Heat-Exchanger | $NO_x$& $H_2O_2$ |
| **(34)** | | Atopic asthma | 50 (30 steroid-naïve) | 19–30 | (ATS) criteria. | Unit (RHES) | |
| | | ICS-treated | 20 | 19-29 | | | |
| **Nadif (2014), France** | Cross-sectional | Controls | 268 (38.8% atopic) | 43.2 ± 15.9 | Ever asthma was defined based on the history of | R-Tube | $NO_x$ |
| **(35)** | | Asthma | 255 | 36.5 ± 16.8 | asthma attacks in the past a positive answer to either: ‘‘*Have you ever had attacks of breathlessness at rest with wheezing?*’’, or ‘‘*Have you ever had* | | |
| | | ICS-treated in the past 3 months | 38 | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | *asthma attacks?''*, or *if they were recruited as asthmatic cases at the first survey.* | | |
| **Hanazawa (2000), The UK (36)** | Cross-sectional | Healthy controls<br>Asthma<br>Mild asthma with steroid-naïve<br>Moderate asthma with only ICS-treated<br>Severe asthma with oral prednisolone and ICS-treated | 15<br>39<br>15<br>12<br>12 | 32.3 ± 4.5<br><br>30.1 ± 1.34<br>45.1 ± 5.6<br>47.2 ± 4. | Defined according to the American Thoracic Society (ATS) and the National Institutes of Health/World Health Organization (NIH/WHO) Guidelines. | EcoScreen | NT |
| **Antczak (2000), Poland (37)** | Double-blind, placebo-controlled study (Longitudinal study) | Healthy controls<br>Asthma<br>HFA-placebo<br>Inhaled HFA-beclomethasone | 10<br>17 (7 atopic)<br>6<br>11 | 34.3 ± 5.5<br><br>37.3 ± 8<br>41.4 ± 9 | Defined based on physician-diagnosed asthma according to the history of wheezing dyspnoea and previous documentation of bronchodilator-induced bronchial reversibility with the use of spirometry with | Custom–made glass condensing device | $H_2O_2$ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | flow-screen. | | |
| **Antczak (1997), Poland (38)** | Cross-sectional | Healthy controls<br>Asthma | 10<br>21 (7 atopic) | 32 ± 7<br>37 ± 9 | Defined based on physician-diagnosed asthma according to the history of wheezing dyspnoea and previous documentation of bronchodilator-induced bronchial reversibility with the use of spirometry with flow-screen. | Custom–made glass condensing device | $H_2O_2$ |
| **Loukides (2002), Greece (39)** | Cross-sectional | Non-atopic healthy controls<br>Stable asthma<br>Mild-intermittent asthma<br>Mild-persistent asthma<br>Moderate asthma<br>Steroid-naïve | 15<br>50 (32 atopic)<br>10<br>20<br>20<br>20 | 19-37<br>19-43<br>22-43<br>19-36<br>19-43<br>19-43 | Defined based on the National Heart, Lung and Blood Institute (NHLBI) criteria. | Heat-Exchanger Unit (RHES) | $H_2O_2$ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | ICS-treated | 20 | 19-36 | | | |
| **Svensson (2004), Sweden (40)** | Cross-sectional | Healthy controls<br>Asthma<br>Without current symptom<br>With current symptoms | 9<br>19<br>10<br>9 | | Defined based on physician-diagnosed asthma. | EcoScreen | $H_2O_2$ |
| **Emelyanov (2001), The UK (41)** | Cross-sectional | Non-atopic healthy controls<br>Atopic unstable asthma with steroid-naïve | 17<br>70 | 19–34<br>18–62 | Defined based on the American Thoracic Society (ATS) criteria. | Custom–made glass condensing device | $H_2O_2$ |
| **Montuschi (1999), The UK (42)** | Cross-sectional | Non-atopic healthy controls<br>Asthma<br>Mild asthma with steroid-naïve<br>Moderate asthma with ICS<br>Severe asthma with oral steroid | 10<br>44<br>12<br>17<br>15 | 34.1 ± 2.8<br><br>27.8 ± 1.34<br>47 ± 5.15<br>38.9 ± 4.2 | Defined based on the American Thoracic Society (ATS) criteria. | Custom–made glass condensing device | 8-isoP |
| **Sood (2013),** | Longitudinal | Healthy controls | 6 | 39.9 ± 9.7 | Defined based on the | R-Tube | 8-isoP |

| | | | | | |
|---|---|---|---|---|---|
| **The USA (43)** | study | Mild atopic asthma | 8 | 31.4 ± 8.5 | guidelines of the National Asthma Education and Prevention Program (NAEPP). |

**ABBREVIATIONS:** EBC = Exhaled breath condensate. ICS = Inhaled corticosteroids. $NO_2^-$ = Nitrite. $NO_3^-$ = Nitrate.$NO_x$ = Total nitric oxide.$H_2O_2$ = Hydrogen peroxide. 8-isoP = 8-isoprostnae. GERD = Gastroesophageal reflux disease. CVA = Cough variant asthma. CA = Classic asthma. $FEV_1$ = Forced expiratory volume in 1 second.

***Table 2:*** *Evaluation of pH in EBC*

| Study (Author/Year), Country | Analytical Technique | Subjects / pH Results | | | Conclusion |
|---|---|---|---|---|---|
| **Antus (2010), Hungary (28)** | pH-$CO_2$ Standardization method | **Mean ± SEM:** | | | EBC pH was significantly lower in patients with asthma exacerbation compared with non-atopic control subjects, and slightly increased after ICS therapy. |
| | | Non-atopic healthy controls | 6.41 ± 0.04 | | |
| | | Asthma exacerbation | 6.24 ± 0.03 | ***P*< 0.01** | |
| | | ICS-naïve on admission | 6.22 ± 0.04 | | |
| | | ICS-treated | 6.25 ± 0.22 | ***n.s.*** | |
| | | ICS-treated on discharge | 6.33 ± 0.03 | ***P*< 0.05** | |
| **Ueno (2008), Japan (29)** | pH meter* | **Mean ± SEM:** | | | EBC pH was significantly higher in patients with asthma compared with healthy controls before deaeration. Participants with persistent asthma (including mild, moderate and severe) had significantly lower pH than participants with mild intermittent asthma. |
| | | Healthy controls before EBC deaeration | 6.16 ± 0.07 | | |
| | | Asthmatics before EBC deaeration | 6.84 ± 0.06 | ***P*< 0.01** | |
| | | Healthy controls after EBC deaeration | 7.69 ± 0.01 | | |
| | | Asthma after EBC deaeration | 7.66 ± 0.06 | ***n.s.*** | |
| | | Mild intermittent (*reference group*) | 6.98 ± 0.01 | | |
| | | Mild persistent | 6.39 ± 0.16 | ***P*<0.01** | |
| | | Moderate persistent | 6.88 ± 0.16 | ***P*<0.05** | |

| | | | | | |
|---|---|---|---|---|---|
| | | Severe persistent | 6.84 ± 0.08 | ***P*<0.05** | |
| **Shimizu (2007), Japan (30)** | pH meter | **Mean ± SEM:** | | | EBC pH value was lower in patients with asthma with GERD than in the healthy controls.<br>EBC pH value in the (QUEST score ≥4) subgroup of asthma patients was significantly increased after treatment.<br>However, EBC pH level in the (QUEST score <4) subgroup of asthma patients did not change significantly after treatment. |
| | | Healthy controls | 7.5 ± 0.2 | | |
| | | Moderate asthma with GERD | 7.3 ± 0.3 | ***P*<0.05** | |
| | | Asthma with GERD QUEST score ≥4 before treated with PPI lansoprazole | 7.2 ± 0.1 | | |
| | | Asthma with GERD QUEST score <4 before treated with PPI lansoprazole | 7.3 ± 0.6 | ***n.s.*** | |
| | | Asthma with GERD QUEST score ≥4 after treated with PPI lansoprazole | 7.3 ± 0.1 | | |
| | | Asthma with GERD QUEST score <4 after treated with PPI lansoprazole | 7.4 ± 0.4 | ***n.s.*** | |
| **Zhao (2008), Japan (31)** | pH-meter | **Median [IQR]:** | | | EBC pH value was significantly lower in patients with mild asthma than in non-atopic healthy controls. |
| | | Non-atopic healthy controls | 7.70 [7.62–7.74] | | |
| | | Mild asthma | 7.53 [7.41–7.68] | ***P*<0.05** | |
| **Al Obaidi (2007), Iraq (32)** | pH meter | **Mean ± SD (95% CI):** | | | EBC pH values were significantly lower in CVA and CA than that in healthy controls.<br>Value of pH was lower in CA as compared to that in CVA, but the difference did not |
| | | Healthy controls | 7.82 ± 0.65 (95% CI 7.61 to 7.99) | | |
| | | Cough variant asthma (CVA) | 6.26 ± 0.43 (95% | ***P*<0.001** | |

| | | | | | |
|---|---|---|---|---|---|
| | | | CI 6.09 to 6.43) | | reach significant value. |
| | | Classic asthma (CA) | 5.97 ± 1.03 (95% CI 5.65 to 6.29) | ***NR*** | |
| **Hunt (2000), The USA (33)** | pH meter* | **<u>Mean ± SEM:</u>** | | | EBC pH value was significantly lower in patients with stable asthma than in healthy controls. No significant difference between EBC pH in patients with stable asthma and healthy controls.<br>EBC pH level increased significantly in patients with acute asthma who had received systemic glucocorticoid therapy for >48 hours compared to those who treated for less than 48 hours. |
| | | Healthy controls | 7.65 ± 0.20 | | |
| | | Stable asthma | 5.80 ± 0.10 | ***P*= 0.95** | |
| | | Acute asthma | 5.23 ± 0.21 | ***P*<0.001** | |
| | | Acute asthma after receiving systemic glucocorticoid therapy for >48 hours | 7.4 ± 0.23 | ***P*<0.001** | |

*******EBC samples were treated with argon gas for deaeration after the collection.*

**<u>ABBREVIATIONS:</u>** EBC = Exhaled breath condensate. SEM = Standard error of mean. ICS = Inhaled corticosteroids. n.s. = Not significant. GERD = Gastroesophageal reflux disease. PPI = Proton pump inhibitor. IQR = Interquartile range. SD = Standard deviation. 95% CI = 95% Confidence interval. CVA = Cough variant asthma. CA = Classis asthma. NR = Not reported.

***Table 3:*** *Evaluation of nitric oxide products in EBC*

| Study (Author/Year), Country | Analytical Technique | EBC Biomarkers | Subjects / Nitric oxide products | | | Conclusion |
|---|---|---|---|---|---|---|
| **Ueno (2008), Japan (29)** | Fluorometric assay (The lower limit of detection, 0.01 μM) | **$NO_2^-$** | **Mean ± SEM (μmol/ml):** | | | EBC $NO_2^-$ level in asthma group was significantly higher than healthy controls. Participants with severe persistent asthma had significantly higher $NO_2^-$ level than participants with mild intermittent asthma. |
| | | | Healthy controls | 0.78 ± 0.18 | | |
| | | | Asthma patients | 3.13 ± 0.42 | ***P*<0.001** | |
| | | | Mild intermittent (*reference group*) | 6.98 ± 0.01 | | |
| | | | Mild persistent | 6.39 ± 0.16 | ***P*<0.01** | |
| | | | Moderate persistent | 6.88 ± 0.16 | ***P*<0.05** | |
| | | | Severe persistent | 6.84 ± 0.08 | ***P*<0.05** | |
| | | **$NO_3^-$** | **Mean ± SEM (μmol/ml):** | | | EBC $NO_3^-$ level in asthma group was significantly higher than healthy controls. Participants with mild persistent asthma had significantly higher $NO_3^-$ level than participants with mild intermittent asthma. |
| | | | Healthy controls | 5.91 ± 0.46 | | |
| | | | Asthma patients | 14.88 ± 0.73 | ***P*<0.001** | |
| | | | Mild intermittent(*reference group*) | 14.43 ± 0.75 | | |
| | | | Mild persistent | 18.22 ± 2.18 | ***P*<0.05** | |
| | | | Moderate persistent | 15.62 ± 1.67 | ***NR*** | |
| | | | Severe persistent | 13.85 ± 1.46 | ***NR*** | |
| | | **$NO_x$** | **Mean ± SEM (μmol/ml):** | | | EBC $NO_x$ levels in asthma group were significantly higher than healthy controls. No significant differences in the $NO_x$ |
| | | | Healthy controls | 6.59 ± 0.47 | | |
| | | | Asthma patients | 18.01 ± 0.91 | ***P*<0.001** | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Mild intermittent (*reference group*) | 16.65 ± 1.05 | | levels across the asthmatic severity. |
| | | | Mild persistent | 20.60 ± 3.01 | ***n.s.*** | |
| | | | Moderate persistent | 18.60 ± 2.13 | ***n.s.*** | |
| | | | Severe persistent | 18.07 ± 1.78 | ***n.s.*** | |
| **Ganas (2001), Greece (34)** | Fluorometric modified Greiss reaction | **$NO_x$** | **Mean (95% CI) µM:** | | | EBC $NO_x$ levels were significantly higher in patients with asthma compared to non-atopic healthy controls. |
| | | | Non-atopic healthy controls | 0.63, (95% CI 0.20 to 0.41) | | |
| | | | Atopic asthma | 1.08 (95% CI 0.86 to 1.33) | ***P*<0.001** | Patients treated with ICS exhibited significantly lower levels of $NO_x$ in compared to those who were steroid-naive. |
| | | | Asthma with steroid-naive | 1.33 (95% CI 1.00 to 1.65) | | |
| | | | Asthma with ICS-treated | 0.71 (95% CI 0.55 to 0.87) | ***P*<0.001** | |
| **Nadif (2014), France (35)** | Fluorometric modified Greiss reaction | **$NO_x$** | **Median [IQR] (µmol/mg):** | | | EBC $NO_x$ levels in patients with asthma were not significantly higher than non-asthmatic controls. EBC $NO_x$ levels were unrelated to ICS use. |
| | | | Controls | 2.33 [1.15–4.81] | | |
| | | | Asthma | 2.16 [1.13–5.11] | ***P*= 0.50** | |
| **Hanazawa (2000), The UK (36)** | EIA (The lower limit of detection, 3.9 ng/ml) | **NT** | **Mean ± SEM (ng/ml):** | | | NT levels were increased significantly in patients with mild asthma compared to those patients with moderate or severe asthma and healthy controls. |
| | | | Healthy controls | 6.3 ± 0.8 | | |
| | | | Mild asthma with steroid-naïve | 15.3 ± 2.0 | ***P*<0.01** | |
| | | | Moderate asthma with ICS steroid | 5.0 ± 0.6 | ***NR*** | |

| | | |
|---|---|---|
| Severe asthma with ICS and oral steroid | 3.3 ± 0.6 | ***P*<0.05** |

**ABBREVIATIONS:** EBC = Exhaled breath condensate. $NO_x$ = Total nitric oxide products. $NO_2^-$ = Nitrite. $NO_3^-$ = Nitrate. NT = Nitrotyrosine. SEM = Standard error of mean. NR = Not reported. n.s. = Not significant. CI = 95% Confidence interval. IQR = Interquartile range.ICS = Inhaled corticosteroids. EIA = Enzyme immunoassay.

***Table 4:*** *Evaluation of Hydrogen Peroxide ($H_2O_2$) in EBC*

| Study (Author/Year), Country | Analytical Technique | Subjects / $H_2O_2$ Results | | | Conclusion |
|---|---|---|---|---|---|
| **Ueno (2008), Japan (29)** | Spectrofluorometry (The lower limit of detection was 0.05 μM) | **Mean ± SEM (μmol/ml):** | | | EBC $H_2O_2$ level in the asthma group was significantly higher than that controls. EBC $H_2O_2$ level in patients with mild, moderate and severe persistent asthma were significantly higher than that in mild intermittent. |
| | | Healthy controls | 0.59 ± 0.03 | | |
| | | Asthma patients | 1.89 ± 0.07 | ***P*<0.001** | |
| | | Mild intermittent (*reference group*) | 1.85 ± 0.10 | | |
| | | Mild persistent | 2.26 ± 0.25 | ***P*<0.05** | |
| | | Moderate persistent | 1.65 ± 0.13 | ***P*<0.01** | |
| | | Severe persistent | 1.89 ± 0.11 | ***P*<0.05** | |
| **Al Obaidi (2007), Iraq (32)** | Spectrofluorometry Colorimetric assay | **Mean ± SD (95% CI) μmol:** | | | EBC $H_2O_2$ levels in patients with CVA and CA were significantly higher than that for controls. No significant difference in EBC $H_2O_2$levels between CA and CVA. |
| | | Healthy controls | 0.29 ± 0.07 (95% CI 0.27 to 0.31) | | |
| | | Cough variant asthma(CVA) | 0.83 ± 0.34 (95% CI 0.69 to 0.96) | ***P*<0.001** | |
| | | Classic asthma (CA) | 0.89 ± 0.36 (95% CI 0.78 to 1.00 | ***P*<0.001** | |
| **Ganas (2001), Greece (34)** | Spectrofluorometry (The lower limit of detection was 0.10 μM) | **Mean (95% CI) μM:** | | | EBC $H_2O_2$ level was significantly higher in patients with asthma compared to non-atopic healthy controls. Patients treated with ICS exhibited |
| | | Non-atopic healthy controls | 0.30 (0.20–0.40) | | |
| | | Atopic asthma | 0.60 (0.48–0.70) | ***P*<0.005** | |
| | | Atopic asthma with ICS- | 0.72 (0.55–0.89) | | |

| | | | | | |
|---|---|---|---|---|---|
| | | naïve | | | significantly lower levels of EBC $H_2O_2$ compared to those who were steroid-naive. |
| | | Atopic asthma with ICS-treated | 0.41 (0.29–0.52) | ***P*<0.05** | |
| **Antczak (2000), Poland (37)** | Spectrofluorometry | **Median ± IQR (μM):** | | | The baseline EBC $H_2O_2$ level was higher in patients with asthma than that in healthy controls.<br>EBC $H_2O_2$ levels were decreased after treated with inhaled HFA-beclomethasone in active-treatment group and were significantly lower than that in placebo group. |
| | | Healthy controls (baseline) | 0.03 ± 0.02 | | |
| | | Asthma (baseline) | 0.54 ± 0.21 | ***P*<0.01** | |
| | | HFA-placebo - after one day | 0.66 ± 0.19 | | |
| | | HFA-beclomethasone - after one day | 0.57 ± 0.21 | ***NR*** | |
| | | HFA-placebo - after 29 days | 0.69 ± 0.26 | | |
| | | HFA-beclomethasone - after 29 days | 0.33 ± 0.10 | ***P*<0.05** | |
| **Antczak (1997), Poland (38)** | Spectrofluorometry (The lower limit of detection was 0.1 nM) | **Mean ± SD (nM):** | | | EBC $H_2O_2$ level in patients with asthma was significantly higher than in healthy controls. |
| | | Healthy controls | 0.01 ± 0.03 | | |
| | | Asthma | 0.26 ± 0.29 | ***P*<0.05** | |
| **Loukides (2002), Greece (39)** | Spectrofluorometry (The limit of detection was 0.1μM $H_2O_2$) | **Mean [ 95% CI] (μM):** | | | EBC $H_2O_2$ levels were significantly higher in all stable asthmatics compared to non-atopic healthy controls.<br>EBC $H_2O_2$ level in moderate asthma was significantly higher than those with mild-intermittent and mild-persistent asthma.<br>EBC $H_2O_2$ levels were significantly lower in asthma patients who treated with ICS |
| | | Non-atopic healthy controls | 0.20 [0.16–0.24] | | |
| | | Stable asthma | 0.67 [0.56–0.77] | ***P*<0.0001** | |
| | | Mild-intermittent asthma (*reference group*) | 0.27 [0.23–0.32] | | |
| | | Mild-persistent asthma | 0.59 [0.47–0.70] | ***P*<0.001** | |
| | | Moderate asthma | 0.94 [0.76–1.12] | ***P*<0.0001** | |
| | | Non-atopic asthma | 0.60 [0.40–0.80] | | |

| | | | | | |
|---|---|---|---|---|---|
| | | Atopic asthma | 0.7 [0.6 to 0.8] | ***P*= 0.80** | than those who were steroid-naïve. |
| | | Steroid-naïve | 0.87 [0.68–1.05] | | |
| | | ICS-treated | 0.66 [0.52–0.80] | ***P*<0.05** | |
| | | Mild-persistent asthma with steroid-naïve (*reference group*) | 0.65 [0.40–0.87] | | |
| | | Mild-persistent asthma with ICS | 0.52 [0.41–0.64] | ***P*<0.0001** | |
| | | Moderate asthma with steroid-naïve | 1.09 [0.83–1.34] | ***NR*** | |
| | | Moderate asthma with ICS | 0.74 [0.46–1.02] | ***P*<0.0001** | |
| **Svensson (2004), Sweden (40)** | Spectrofluorometry (The lower level of detection, 50 nM) | **Mean ± SEM (nM):** | | | EBC $H_2O_2$ levels were significantly higher in all stable asthmatics compared to controls. |
| | | Healthy controls | 260 ± 10 | | |
| | | Asthma | 780 ± 430 | ***P*= 0.03** | |
| | | Asthma without symptoms | 720 ± 320 | | EBC $H_2O_2$ level obtained from the asthmatic patients with symptoms were higher than those obtained from asthmatic patients without symptoms. |
| | | Asthma with symptoms | 850 ± 570 | ***NR*** | |
| **Emelyanov (2001), The UK (41)** | Spectrofluorometry Colorimetric assay | **Mean ± SD (µM):** | | | EBC $H_2O_2$ levels were significantly higher in all unstable asthmatics compared to non-atopic healthy controls. |
| | | Non-atopic healthy controls | 0.024 ± 0.016 | | |
| | | Atopic unstable asthma with steroid-naïve | 0.127 ± 0.083 | ***P*<0.001** | |

**ABBREVIATIONS:** EBC = Exhaled breath condensate. $H_2O_2$ = Hydrogen peroxide. SEM = Standard error of mean. SD = Standard deviation. 95%. CVA = Cough variant asthma. CA = Classis asthma. IQR = Interquartile range. ICS = Inhaled corticosteroids. NR = Not reported.

***Table 5:*** *Evaluation of 8-isoprostane (8-isoP) in EBC*

| Study (Author/Year), Country | Analytical Technique | Subjects / 8-isoP Results | | | Conclusion |
|---|---|---|---|---|---|
| **Shimizu (2007), Japan (30)** | EIA (The detection limit of the assay was 4 pg/ml) | **Mean ± SEM:** | | | EBC 8-isoP level was higher in moderate asthma patients with GERD than in the healthy control subjects. EBC 8-isoP levels in the (QUEST score ≥4) subgroup of asthma patients were significantly decreased after treatment. However, EBC 8-isoP level in the (QUEST score<4) subgroup of asthma patients did not change significantly after treatment. |
| | | Healthy controls | 6.6 ± 1.2 | | |
| | | Moderate asthma with GERD | 27.7 ± 2.3 | ***P*<0.05** | |
| | | Asthma with GERD QUEST score ≥4 before treated with PPI lansoprazole | 32.7 ± 3.4 | | |
| | | Asthma with GERD QUEST score <4 before treated with PPI lansoprazole | 24.6 ± 3.8 | ***P*= 0.09** | |
| | | Asthma with GERD QUEST score ≥4 after treated with PPI lansoprazole | 19.2 ± 3.4 | | |
| | | Asthma with GERD QUEST score <4 after | 21.0 ± 2.7 | ***n.s.*** | |

| | | | | | |
|---|---|---|---|---|---|
| | | treated with PPI lansoprazole | | | |
| **Zhao (2008), Japan (31)** | EIA (The detection limit of the assay was 4 pg/ml) | **Median [IQR] (pg/ml):** | | | EBC 8-isoP level was significantly higher in asthma patients than in non-atopic healthy controls. |
| | | Non-atopic healthy controls | 3.5 [2.6–7.9] | | |
| | | Mild asthma | 16.2 [11.7–19.1] | ***P*<0.05** | |
| **Montuschi (1999), The UK (42)** | EIA (The detection limit of the assay was 4 pg/ml) | **Mean ± SEM (pg/ml):** | | | EBC 8-isoP level was significantly higher in asthma patients than in non-atopic healthy controls. EBC 8-isoP levels were significantly higher in subjects with severe than with mild to moderate asthma. |
| | | Non-atopic healthy controls | 15.8 ± 1.6 | | |
| | | Mild asthma with steroid-naive | 33.7 ± 2.8 | ***P*<0.001** | |
| | | Moderate asthma with ICS | 38.3 ± 3.7 | ***P*<0.001** | |
| | | Severe asthma with oral steroid | 49.1 ± 5.0 | ***P*<0.001** | |
| **Sood (2013), The USA (43)** | ELISA/RIA | **Mean ± SD (pg/ml)** | | | EBC 8-isoP level in subjects with asthma was not significantly higher than that for the controls. EBC 8-isoP level in subjects with asthma did not change after inhalational challenge. |
| | | Healthy controls | 1.54 ± 1.39 | | |
| | | Mild atopic asthma | 2.50 ± 0.99 | ***P*= 0.22** | |

**ABBREVIATIONS:** EBC = Exhaled breath condensate. 8-isoP = 8-isoprostanes. EIA = Enzyme immunoassay. ELISA = Enzyme-linked immunosorbent assay. RIA = Radioimmunoassay. SEM = Standard error of mean. PPI = Proton pump inhibitor. IQR = Interquartile range. ICS = Inhaled corticosteroids.SD = Standard deviation.

**Figure Legends**

***Figure 1: Flow chart illustrating the study profile.*** **RCT:** Randomised control trial. **EBC:** Exhaled breath condensate. **FeNO:** Fractional exhaled nitric oxide.