

The Future of Hydrogen Peroxide as a Biomarker for Airway Inflammation

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Abstract: Airway inflammation has traditionally been assessed using invasive or semi-invasive techniques, prompting growing interest in non-invasive biomarkers. Exhaled breath condensate (EBC) offers a repeatable and well-tolerated method to evaluate airway inflammation and oxidative stress, with hydrogen peroxide (H₂O₂) emerging as a biomarker with relevant translational potential for disease monitoring and treatment response. This narrative review aimed to evaluate the potential H₂O₂ in EBC as a non-invasive biomarker for disease monitoring and treatment response in inflammatory respiratory diseases. Key clinical studies investigating H₂O₂ in patients with asthma, COPD, bronchiectasis and acute lung conditions were analyzed. Exhaled H₂O₂ levels are elevated in several inflammatory respiratory diseases, reflecting airway oxidative stress and disease instability, with higher concentrations observed in untreated or exacerbated patients, which decline in response to therapy. These findings indicate that H₂O₂ appears to be a promising tool for assessing airway oxidative stress and inflammation. Further research is needed to validate its clinical relevance and support its integration into standard clinical management.

Keywords: exhaled breath, diagnosis, disease monitoring, respiratory diseases, clinical practice

Introduction

Airway inflammation has been evaluated in various studies through either invasive techniques (eg, bronchoalveolar lavage fluid and bronchial biopsies)^{1,2} or semi-invasive methods such as sputum induction.³ Difficulties in reaching and exploring the nature of airway inflammation, especially in the distal portion of the bronchial tree, has limited the characterization of the inflammatory changes in clinical practice and for research purposes. In recent years, increasing attention has been given to non-invasive methods for evaluating and monitoring airway inflammation. In this context, exhaled breath has emerged as a valuable, non-invasive tool for collecting biological samples and assessing oxidative stress in respiratory diseases.⁴

Exhaled breath consists of a gaseous phase containing volatile organic compounds such as nitric oxide (NO), carbon monoxide (CO), ethane, and pentane, and a water-vapor phase containing aerosol particles. These particles can be condensed via a cooling system, resulting in what is known as exhaled breath condensate (EBC).⁵

EBC collection is completely non-invasive and repeatable. It is typically performed during tidal breathing using a nose clip and saliva trap, under controlled temperature and collection time, with a condenser made of inert material and no resistance or filter between the subject and the device.⁶ It is a well-tolerated method and can be applied to both adult and pediatric populations, including patients with acute symptoms. Most important, since EBC collection does not involve bronchoprovocation, unlike sputum induction or bronchoscopy, it does not alter the degree of airway inflammation during the procedure.⁷

EBC contains various non-volatile substances, including markers of oxidative stress. Increased oxidative stress, resulting from an imbalance between the generation and scavenging of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), is a key mechanism in several inflammatory respiratory diseases, including asthma and COPD.⁸ For this reason, it has been suggested that the concentrations of these mediators in EBC may reflect the inflammatory status of the disease and may be modulated by therapeutic interventions.

ROS are oxygen-containing reactive molecules produced mainly by oxidant enzymes and are subsequently neutralized by scavenging systems, which include both enzymatic and non-enzymatic reactions. Under normal physiological conditions, ROS are generated in nearly every subcellular organelle within the cell⁹ including the plasma membrane, cytosol, endoplasmic reticulum, Golgi apparatus, mitochondria, nucleus and others.¹⁰

Some ROS are purposefully produced through enzymatic pathways, including lipoxygenases and cyclooxygenases, to carry out specific biological functions and regulate cellular homeostasis.¹¹ Upon inflammatory stimuli, leukocytes and respiratory epithelial cells release ROS and reactive nitrogen species (RNS), disrupting intracellular redox balance and leading to oxidative stress and an increased production of ROS.¹²

H₂O₂ is a key oxidative metabolite commonly found in cells, tissues, and bodily secretions. Due to its small size and polarity, it freely diffuses across biological membranes. H₂O₂ is primarily produced through the dismutation of superoxide anions generated by activated inflammatory cells such as neutrophils, macrophages, and particularly eosinophils. Notably, H₂O₂ is volatile and can transition into the gaseous phase, making it detectable in exhaled air.¹³ During inflammatory processes, increased oxidative stress triggers respiratory bursts, resulting in excessive production of superoxide anions (O₂⁻) and a subsequent rise in H₂O₂ levels.¹⁴

Beyond being a mere byproduct of oxidative metabolism, H₂O₂ acts as a signaling molecule, modulating cytokine production, leukocyte recruitment, and epithelial barrier function. Because of its volatility and ability to equilibrate with air, H₂O₂ can be measured non-invasively in EBC.

The current narrative review examines the major clinical studies in humans on the use of H₂O₂ in EBC as a non-invasive biomarker for assessing airway inflammation, highlighting its potential applications in disease diagnosis, monitoring and treatment response, and discussing factors influencing the variability of exhaled H₂O₂.

Methods

We conducted a comprehensive literature search using PubMed, Cochrane, Google Scholar, EMBASE, ISRCTN, MEDLINE, and Web of Science. Studies including patients of all ages were considered. We focused on observational studies, including longitudinal, cross-sectional, cohort, case-control, as well as randomized controlled trials, involving patients with respiratory diseases from whom exhaled H₂O₂ was collected. Only full-text articles published in English were included, while studies that did not involve breath sample collection or that assessed biomarkers other than exhaled H₂O₂ were excluded.

Two clinical researchers (NS and CG) independently reviewed all retrieved articles based on the predefined inclusion and exclusion criteria, with any discrepancies resolved through discussion with a third reviewer (SP) to reach consensus. Information extracted from each study included author, title, study population, disease type, analytical techniques, clinical application of exhaled H₂O₂, and key findings. References were managed using a reference management software, and all extracted data were organized in an Excel database maintained securely on University servers.

Study Characteristics

The included studies varied in terms of patient populations, analytical assays, and data normalization strategies. The main findings are summarized in Table 1 and extensively discussed. Populations ranged from pediatric to adult patients and encompassed different respiratory diseases, including asthma, COPD, bronchiectasis, and interstitial lung disease (Figure 1 and Table 2). Elevated H₂O₂ levels in EBC were consistently observed across these conditions, correlating with airway hyperresponsiveness and eosinophilic inflammation, as indicated by associations with eosinophil counts in induced sputum.¹⁵

Analytical techniques for H₂O₂ quantification included the Amplex Red assay,⁴⁵ as well as luminescent,⁴⁶ colorimetric,⁴⁷ magnetic resonance-based⁴⁸ and electrochemical⁴⁹ approaches.

Variations in sample collection, normalization strategies, and reporting units contributed to heterogeneity among studies. These differences were carefully recorded and considered in synthesizing the results, allowing for a nuanced interpretation of the clinical significance of exhaled H₂O₂.

Table 1 Summary of the Main Studies and Principal Results

First Author [ref]	Study Population	Disease	Analytical Techniques	Clinical Application of Exhaled H ₂ O ₂	Main Results
Dohlman ¹⁶	Asthma = n 22 Healthy controls = n 11	Asthma	Fluorimetric assay	Diagnosis Monitoring disease status	Pediatric patients with asthma showed notably higher levels of exhaled H ₂ O ₂ than healthy individuals, with the highest concentrations observed in those experiencing acute respiratory symptoms.
Al Obaidi ¹⁷	Cough-variant asthma= n 27 Classical asthma= n 43, Chronic cough = n 32 Healthy subjects = n 27	Asthma	Colorimetric assay	Diagnosis	Exhaled H ₂ O ₂ levels were significantly elevated in patients with cough-variant asthma compared to both chronic cough nonasthmatic patients and healthy controls. However, no significant difference was observed between cough variant asthma and classical asthma patients.
Murata ¹⁸	Asthma = n 29 COPD = n 33 Healthy controls = n 33	Asthma, COPD	Colorimetric assay	Diagnosis Monitoring disease status	H ₂ O ₂ levels were elevated in both asthma and COPD patients compared to healthy controls. H ₂ O ₂ levels in exhaled breath condensate may reflect the health status of individuals with COPD.
Antczak ¹⁹	Asthma = n 21 Healthy controls = n 10	Asthma	Spectrofluorimetric assay	Diagnosis	Asthmatic patients exhibited higher levels of exhaled H ₂ O ₂ compared to healthy subjects.
Antczak ²⁰	Asthma = n 17 Healthy controls = n 10	Asthma	Spectrofluorimetric assay	Diagnosis Treatment response	Patients with asthma showed elevated levels of exhaled H ₂ O ₂ compared to healthy subjects. Treatment with inhaled glucocorticoids over a four-week period reduced the concentration of H ₂ O ₂ in the expired air condensate of these patients.
Horváth ²¹	Unstable steroid-treated asthma= n 14 Stable steroid-treated asthma = n 30 Stable steroid-naïve asthma = n 72 Healthy controls = n 35	Asthma	Colorimetric assay	Diagnosis Monitoring disease status Treatment response	H ₂ O ₂ levels were elevated in steroid-naïve asthma patients compared to healthy subjects and were decreased in stable asthmatics receiving steroid treatment. However, unstable steroid-treated asthmatics showed increased H ₂ O ₂ levels. Exhaled H ₂ O ₂ also correlate with sputum eosinophil counts and airway hyperresponsiveness.
Jöbsis ²²	Steroid-treated asthma =n 41 Steroid-untreated asthma = n 25 Healthy controls = n 21	Asthma	Fluorimetric assay	Diagnosis Treatment response	Exhaled- H ₂ O ₂ concentration in EBC of asthmatic patients was significantly higher than in healthy controls, primarily due to elevated levels in stable asthmatic children who were not receiving anti-inflammatory treatment.
Svensson ²³	Asthma= n 19 Healthy controls = n 19	Asthma	Fluorimetric assay	Treatment response	A statistically significant difference in exhaled H ₂ O ₂ concentration was found between asthma patients and control subjects without asthma.

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Table 1 (Continued).

First Author [ref]	Study Population	Disease	Analytical Techniques	Clinical Application of Exhaled H ₂ O ₂	Main Results
Ueno ²⁴	Severe asthma = n 21 Non severe asthma = n 34 Healthy controls = n 58 Smokers = n 7 Common cold = n 9	Asthma	Fluorimetric assay	Monitoring disease status Treatment response	H ₂ O ₂ levels were significantly higher in the asthma group compared to the control group, particularly in the mild persistent group. Additionally, FEV ₁ and PEF showed significant negative correlations with H ₂ O ₂ levels.
Sandrinì ²⁵	Asthma = n 20	Asthma	Colorimetric assay	Treatment response	No significant changes in H ₂ O ₂ concentration were observed following treatment with either montelukast or placebo.
Nowak ²⁶	Stable COPD= n 44 Healthy controls = n 17	COPD	Spectrofluorimetric assay	Diagnosis	Subjects with stable COPD show increased H ₂ O ₂ production in their airways. Current cigarette smoking does not differentiate COPD patients in terms of H ₂ O ₂ exhalation.
Kostikas ²⁷	Severe COPD= n 10 Mild COPD= n 10 Moderate COPD= n 10 Healthy controls = n 10	COPD	Spectrofluorimetric assay	Diagnosis Monitoring disease status	H ₂ O ₂ levels are elevated in the EBC of patients with COPD and appear to correlate with disease severity.
Dekhuijzen ²⁸	Stable COPD= n 12 Exacerbated COPD = n19 Healthy controls = n 10	COPD	Spectrofluorimetric assay	Diagnosis Monitoring disease status	Concentrations of H ₂ O ₂ were significantly elevated in patients with stable COPD compared to the control group, and were even higher in patients with acutely exacerbated COPD.
Antczak ²⁹	Exacerbated COPD= n 16 Healthy controls =n 13	COPD	Spectrofluorimetric assay	Diagnosis Treatment response	Exhaled H ₂ O ₂ levels were higher during infection-related exacerbation compared to the stable phase. Additionally, H ₂ O ₂ levels are elevated in the airways of stable COPD patients compared to healthy subjects.
Nagaraja ³⁰	Various respiratory diseases= n 77 * Healthy subjects with risk factors = n 23	Asthma COPD Pneumonia ILD Bronchiectasis	Electrochemical assay	Diagnosis Monitoring disease status Treatment response	H ₂ O ₂ levels in exhaled breath condensate may be valuable for detecting oxidative lung damage and early airway inflammation in healthy individuals with risk factors, as well as for monitoring the inflammatory response to treatment.
Inonu ³¹	COPD= n 25 Smokers = n 26 Non- smokers = n 29	COPD	Colorimetric assay	Diagnosis	H ₂ O ₂ concentrations were significantly elevated in both COPD patients and smokers compared to non-smokers
Ferreira ³²	Stable COPD = n 20	COPD	Spectrofluorimetric assay	Treatment response	H ₂ O ₂ levels did not show significant changes following treatment with inhaled beclomethasone or placebo.

Van Beurden ³³	Moderate COPD = n 41	COPD	Fluorimetric assay	Treatment response	Treatment with inhaled corticosteroids (ICS) appeared to reduce exhaled H ₂ O ₂ levels in stable COPD patients. Long-term oral administration of NAC reduces H ₂ O ₂ production in the airways of COPD patients, demonstrating the antioxidant effect of the drug. Oral NAC at 600 mg twice daily for two months quickly lowers H ₂ O ₂ levels in stable COPD patients. Nebulized NAC causes a temporary increase in exhaled H ₂ O ₂ levels. H ₂ O ₂ levels are elevated in the exhaled breath condensate of patients with bronchiectasis and correlate with disease severity. Patients treated with inhaled corticosteroids showed H ₂ O ₂ levels similar to those of steroid-naïve patients. Patients with bronchiectasis in a stable condition exhibited elevated levels of exhaled H ₂ O ₂ . These levels were not reduced by inhaled corticosteroids or long-term oral antibiotic treatment but were significantly influenced by chronic colonization with <i>Pseudomonas aeruginosa</i> . H ₂ O ₂ levels in exhaled breath condensate are elevated during a common cold compared to healthy controls. Patients with ARDS exhibited higher levels of exhaled H ₂ O ₂ compared to those without ARDS. Patients with acute respiratory failure showed higher H ₂ O ₂ concentrations than control patients, with the highest levels observed in the ARDS group. Following clinical improvement, H ₂ O ₂ concentrations decreased to levels comparable to those of the control group.
Kasielski ³⁴	Moderate COPD = n 44 Healthy controls = n 17	COPD	Spectrofluorimetric assay	Treatment response	
De Benedetto ³⁵	Moderate COPD = n 55	COPD	Spectrofluorimetric assay	Treatment response	
Rysz ³⁶	COPD = n 19	COPD	Fluorimetric assay	Treatment response	
Loukides ³⁷	Patients with bronchiectasis= n 37 Healthy controls = n 25	Bronchiectasis	Spectrofluorimetric assay	Diagnosis Monitoring disease status Treatment response	
Loukides ³⁸	Patients with bronchiectasis, n 30 Healthy controls = n 15	Bronchiectasis	Spectrofluorimetric assay	Diagnosis Monitoring disease status Treatment response	
Jöbsis ³⁹	Patients with common cold= n 20 Healthy controls = n 10	Respiratory infection	Fluorimetric assay	Diagnosis	
Baldwin ⁴⁰	Patients with ARDS = n 16 Patients without ARDS = n 27	ARDS	Fluorimetric assay	Diagnosis	
K i e t z m a n n ⁴¹	Patients with ARDS = n 7 Patients with polytrauma without chest trauma= n 4 Patients with polytrauma with chest trauma= n 8 Patients with pneumonia= n 4 Patients with pulmonary edema= n 3 Healthy controls = n 10	ARDS and others	Chemiluminescence assay	Diagnosis	
Fireman ¹⁵	Patients with OLD = n 20 Patients with PC = n 20 Patients with ILD = n 25 Healthy controls = n 10	OLD PC ILD COPD	Colorimetric assay	Diagnosis	

(Continued)

Table I (Continued).

First Author [ref]	Study Population	Disease	Analytical Techniques	Clinical Application of Exhaled H ₂ O ₂	Main Results
Mikuls ⁴²	Patients with rheumatoid arthritis = n 22 Healthy controls = n 23	Rheumatoid arthritis	Fluorimetric assay	Diagnosis	Patients with rheumatoid arthritis exhibited elevated levels of exhaled H ₂ O ₂ compared to controls, indicating that H ₂ O ₂ in exhaled breath condensate may serve as a useful biomarker.
Worlitzsch ⁴³	Patients with cystic fibrosis = n 63 Healthy controls = n 51	Cystic fibrosis	Fluorimetric assay	Diagnosis	H ₂ O ₂ levels were comparable between patients with CF and healthy individuals.
Ho ⁴⁴	Patients with cystic fibrosis = n 16 Healthy controls = n 14	Cystic fibrosis	Fluorimetric assay	Diagnosis	H ₂ O ₂ levels in exhaled breath condensate are not elevated in patients with CF, indicating that it is not a reliable marker of airway inflammation in this population.

Notes: *Included asthma, COPD, pneumonia, ILD, bronchiectasis.
Abbreviations: OLD, obstructive lung disease; PC, persistent cough; ILD, interstitial lung disease; COPD, chronic obstructive pulmonary disease; CF, cystic fibrosis; ICS, inhaled corticosteroids; EBC, exhaled breath condensate.

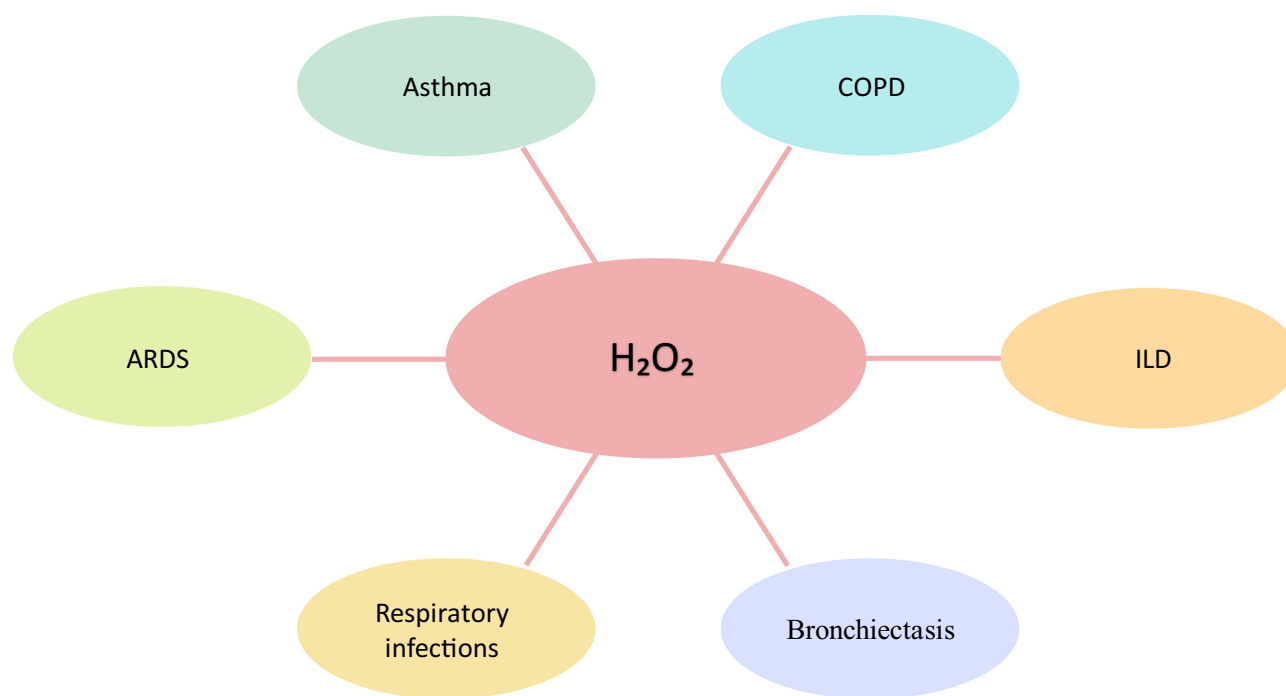


Figure 1 The schematic involvement of exhaled H_2O_2 in respiratory diseases.

The Significance and Clinical Implication of Exhaled H_2O_2 in Respiratory Disease

Diagnostic Potential of Exhaled H_2O_2 in Asthma

In the context of asthma, oxidative stress exerts multiple harmful effects, including lung tissue injury, bronchial hyperresponsiveness, epithelial cell damage and apoptosis, enhanced pro-inflammatory responses, inactivation of anti-oxidant enzymes, airflow obstruction, and increased mucus production. These changes significantly contribute to both disease severity and chronic progression.

Table 2 Disease Group Summaries and Role of Exhaled- H_2O_2

Disease	H_2O_2 Levels in EBC	Diagnostic Role	Monitoring Role
Asthma	↑ Especially in untreated/symptomatic cases	Helps distinguish asthmatic vs healthy subjects	Reflects response to ICS (↓ with treatment); varies with control level
COPD	↑ Even higher during exacerbations	Distinguishes COPD from healthy controls; ↑ with disease severity	Tracks disease stage and exacerbation; some effect with ICS or antioxidants
Bronchiectasis	↑ Especially with infections	Identifies oxidative stress and lung involvement	↓ after therapy; correlates with FEV_1 and symptom severity
Acute Respiratory Infections	↑ Markedly elevated	Indicates acute oxidative stress and inflammation	↓ with resolution of infection
ARDS	↑↑ Highest levels observed	May serve as marker of acute lung injury severity	Potential use in tracking inflammation
ILD	Variable	Mixed evidence; unclear role	↓ after treatment observed in some cases
Cystic Fibrosis	↔ Not elevated	Not effective as a diagnostic biomarker	No treatment-related changes detected

Notes: ↑ = Elevated. ↑↑ = Strongly elevated. ↔ = No significant change.

Abbreviations: ICS, inhaled corticosteroids; ILD, interstitial lung disease; COPD, chronic obstructive pulmonary disease; RA, rheumatoid arthritis; FEV_1 , forced expiratory volume in 1 second.

Several studies have investigated the potential role of H_2O_2 as a non-invasive biomarker for distinguishing asthmatic patients from healthy individuals. One of the earliest contributions¹⁶ assessed exhaled H_2O_2 levels in a cohort of 33 children. The study demonstrated that pediatric asthma patients exhibited significantly higher concentrations of exhaled H_2O_2 compared to healthy controls, with even greater levels observed in asthmatic individuals presenting with acute respiratory symptoms, involving both upper and lower airway infections. Although pulmonary function test results showed a marked deterioration from baseline in some patients, the correlation between impaired lung function and elevated H_2O_2 concentrations in exhaled breath condensate was not statistically significant. Other studies^{17–19} have since confirmed the finding that exhaled H_2O_2 concentrations are significantly higher in both adult and pediatric asthmatic patients compared to healthy individuals, particularly among those who are untreated. Moreover, all of the cited studies reported no significant correlation between exhaled H_2O_2 levels and FEV_1 , suggesting that H_2O_2 concentration may not directly reflect disease severity. In contrast, Antczak et al²⁰ had previously demonstrated a strong inverse correlation between exhaled H_2O_2 concentration and $\text{FEV}_1\%$ predicted, which may be explained by the role of reactive oxygen species in promoting bronchial hyperresponsiveness and subsequent bronchoconstriction.

Notably, exhaled H_2O_2 has also been investigated as a potential biomarker to distinguish between different asthma phenotypes. In a cross-sectional study,²¹ patients with cough variant asthma exhibited higher levels of H_2O_2 compared to those with non-asthmatic chronic cough and healthy controls. However, no significant differences in exhaled H_2O_2 were observed between patients with cough variant asthma and those with classical asthma.

Monitoring Asthma Status and Treatment Response

Many studies have utilized exhaled H_2O_2 to monitor asthma status and evaluate the effects of disease-modifying treatments, such as corticosteroids and other anti-inflammatory agents, on airway oxidative stress in asthmatic patients. Jobsis et al²² demonstrated that exhaled H_2O_2 levels were significantly elevated in asthmatic children not receiving anti-inflammatory treatment compared to healthy controls. In contrast, although asthmatic children treated with corticosteroids also exhibited higher H_2O_2 levels than healthy controls, this difference was not statistically significant. These findings have been supported by several other studies, which confirmed that treatment with inhaled corticosteroids (ICS) significantly reduces exhaled H_2O_2 concentrations. ICS-treated asthmatic patients consistently exhibit lower levels of exhaled H_2O_2 compared to steroid-naïve individuals.^{21,23,24} Notably, Antczak et al²⁰ reported a significant decrease in H_2O_2 concentrations in EBC after four weeks of ICS therapy, as compared to a placebo group, suggesting a rapid anti-inflammatory response to ICS treatment associated with a reduction in oxidative stress markers.

Horváth et al²¹ analyzed subgroups of asthmatic patients based on disease control status and found that exhaled H_2O_2 levels were reduced in steroid-treated patients with stable asthma. In contrast, patients with unstable asthma exhibited persistently elevated H_2O_2 levels despite corticosteroid therapy, suggesting that oxidative stress may remain uncontrolled in certain cases of poorly managed asthma. This contrasts with the findings of Ueno et al²⁴ who reported that H_2O_2 levels were lower in patients with severe asthma compared to those with mild persistent asthma. Moreover, the effect of montelukast on exhaled H_2O_2 has also been studied.²⁵ No significant changes in the concentration of H_2O_2 were detected with either montelukast or placebo treatment, suggesting that montelukast may not significantly influence airway oxidative stress as measured by exhaled H_2O_2 .

Diagnostic Potential of Exhaled H_2O_2 in COPD

A crucial aspect of COPD pathogenesis is the imbalance between proteolytic enzymes, such as elastase, and their inhibitors, notably α_1 -antitrypsin. The interplay between exogenous factors, such as cigarette smoke, environmental pollution, prior infections, and occupational exposures, and endogenous factors, including airway hyperreactivity, antioxidant capacity, genetic predisposition, age, and sex, explains the heterogeneity observed in COPD development.⁵⁰ Similarly, an imbalance between the production of reactive oxygen and nitrogen species (ROS/RNS) and the capacity of antioxidant defenses results in oxidative and nitrosative stress.⁵¹ This stress causes damage to cellular components, thereby contributing to both the severity and chronic progression of the disease.

Various studies have demonstrated the ability of exhaled H_2O_2 in EBC to distinguish patients with COPD from healthy subjects. In particular, it has been shown that patients with COPD typically exhibit higher levels of exhaled H_2O_2

compared to healthy controls,^{26,27} and its production further increases during exacerbations^{28–30} according to the increase of oxidative stress. In addition to differentiating COPD patients from healthy subjects, exhaled H_2O_2 has been linked with clinical parameters. Significant correlations have been observed between exhaled H_2O_2 levels and lung function as measured by FEV_1 , neutrophil count in induced sputum, and dyspnea severity (MRC scale) were observed. Notably, these associations were present only in patients with moderate to severe disease, highlighting the potential role of H_2O_2 as a biomarker of disease progression and severity.²⁷ Moreover, Inonu et al³¹ showed that H_2O_2 levels in EBC were similar between smokers and COPD patients, and both were higher than those in non-smokers, suggesting that the oxidant burden in the lungs of smokers is comparable to that in COPD patients.

Monitoring COPD Status and Treatment Response

The potential role of H_2O_2 in monitoring COPD status and treatment response has been explored in several pilot studies. Specifically, H_2O_2 levels in EBC have been investigated in patients with stable versus unstable COPD, revealing that H_2O_2 concentrations are higher in unstable COPD patients compared to those with stable disease, although the latter still exhibit elevated levels compared to healthy subjects.²⁷ Similarly, patients with moderate and severe COPD show increased H_2O_2 levels relative to those with mild disease, indicating a correlation between H_2O_2 concentration and disease severity.²⁸ Murata et al¹⁸ also identified a significant correlation between COPD Assessment Test (CAT) scores and H_2O_2 levels in COPD patients, further linking exhaled H_2O_2 concentrations to patient-reported health status.

The effects of COPD treatments on H_2O_2 levels in EBC are variable. One study reported that ICS therapy did not significantly affect H_2O_2 concentrations.³² Conversely, Van Beurden et al³³ demonstrated that ICS treatment reduced H_2O_2 levels in EBC, with no return to baseline values after a second washout period, suggesting a possible carry-over effect. Moreover, the impact of antibiotic therapy during infectious exacerbations²⁹ and long-term oral N-acetylcysteine treatment^{34,35} on exhaled H_2O_2 levels has also been evaluated. These studies demonstrated a reduction in H_2O_2 concentrations in EBC, although levels in stable COPD patients remained higher than those in healthy controls. Conversely, nebulized N-acetylcysteine administration in stable COPD patients resulted in a transient increase in exhaled H_2O_2 levels, indicating a mild pro-oxidant effect.³⁶

Other Respiratory Diseases

The ability of H_2O_2 to assess airway inflammation and monitor treatment response has been demonstrated in a variety of respiratory diseases beyond asthma and COPD. Studies have found higher concentrations of H_2O_2 in the EBC of patients with bronchiectasis compared to healthy subjects, indicating enhanced oxidative stress in these patients. Moreover, a significant negative correlation has been observed between H_2O_2 levels and lung function, as assessed by FEV_1 , suggesting a strong inverse relationship between oxidative stress and disease severity.^{30,37} Later studies confirmed these preliminary findings, further reporting that higher H_2O_2 levels are observed in patients with *Pseudomonas aeruginosa* colonization, in those receiving long-term oral antibiotic therapy, and in patients with more severe symptoms.³⁸ Moreover, elevated levels of exhaled H_2O_2 have been detected in acute respiratory infections³⁹ and in various types of acute lung diseases, particularly in patients with ARDS.⁴⁰

Patients with acute lung infiltrates, whether focal or diffuse, showed significantly higher H_2O_2 concentrations compared to healthy controls, with the highest median levels observed in the ARDS group.⁴¹

Whereas data regarding interstitial lung disease (ILD) remain controversial, a pilot study¹⁵ reported no significant difference in H_2O_2 concentrations in EBC between ILD patients and healthy controls. Conversely, another pilot study by Mikuls et al⁴² found increased levels of exhaled H_2O_2 in patients with rheumatoid arthritis-associated ILD compared to healthy controls, suggesting that H_2O_2 could be a potentially useful biomarker.

Contrary to findings in other respiratory diseases, studies conducted on cystic fibrosis have consistently shown that no elevated levels of H_2O_2 are detected in EBC, suggesting that H_2O_2 may not serve as a reliable marker of inflammation in this specific condition.^{43,44}

The potential of H_2O_2 as a biomarker for monitoring treatment response in various respiratory diseases has been investigated. In particular, Nagaraja et al³⁰ evaluated the inflammatory response to treatment by comparing exhaled H_2O_2 levels before and after therapeutic intervention. Their findings demonstrated a significant decrease in H_2O_2 levels

following treatment in patients with asthma, COPD, bronchiectasis, pneumonia and interstitial lung disease, supporting the potential of exhaled H₂O₂ as a non-invasive biomarker for assessing inflammatory response to treatment in respiratory conditions.

Factors Influencing the Variability in Exhaled H₂O₂ Concentration

Several factors may influence H₂O₂ concentration in EBC; the key results are summarized in Table 3.

Diurnal Variability

Several studies have investigated the circadian rhythm of exhaled H₂O₂, demonstrating a distinct diurnal pattern characterized by fluctuations in H₂O₂ levels throughout the day. In particular, Nowak et al⁵² reported two peak concentrations occurring around 12:00 and 24:00 hours, with the lowest levels observed at approximately 08:00 and 20:00 hours. A plausible explanation for this variation lies in the circadian regulation of inflammatory cell activity.⁵³ Specifically, phagocytes and airway epithelial cells, both key sources of H₂O₂, undergo fluctuations in number and function throughout the day. These rhythmic changes may contribute directly to the temporal variations in exhaled H₂O₂ levels.

Moreover, circadian variations have been reported in several biological mechanisms relevant to oxidative stress and inflammation, including the expression of leukocyte receptors⁵⁴ circulating levels of adhesion molecules⁵⁵ and overall antioxidant capacity.⁵⁶ These interrelated factors likely underlie the observed circadian fluctuations in exhaled H₂O₂ concentrations, as documented both in healthy individuals and in patients with chronic inflammatory airway diseases, such as COPD.⁵⁷

Flow Rate and Breathing Pattern Variability

The influence of respiratory parameters, such as expiratory flow rate and breathing pattern, on the concentration of H₂O₂ in EBC has been evaluated. In a explorative study, Schleiss et al⁵⁸ demonstrated that faster expiratory flow rates resulted in altered H₂O₂ concentrations, suggesting that exhaled H₂O₂ is primarily generated within the bronchial airways, with minimal alveolar contribution. The same study also highlighted the role of length of expiration: when a fixed volume of air is collected, lower flow rates, leading to longer expiration times, were associated with higher H₂O₂ concentrations.⁵⁹ However, this finding remains controversial, as other studies have reported no significant correlation between H₂O₂ levels and expiratory flow rate.¹⁶ Additionally, breathing pattern has been shown to affect H₂O₂ concentrations; specifically, an increase in tidal volume was associated with a significant decrease in H₂O₂ levels across all subjects.⁶⁰

Table 3 Factors Influencing the Variability in Exhaled H₂O₂ Concentration

Factor	Description	Effect on H ₂ O ₂ Levels	References
Sampling time	Circadian rhythms regulate inflammatory cell activity and oxidative stress pathways.	Two peaks (~12:00, ~24:00), lowest levels at ~08:00 and ~20:00.	[52–57]
Expiratory flow rate	Faster flows reduce contact time with airway surfaces.	Altered concentrations; lower flow: higher H ₂ O ₂ .	[16,58,59]
Breathing pattern	Changes in tidal volume affect airway shear forces and gas exchange.	Increased tidal volume: decreased H ₂ O ₂ levels.	[60]
Age	Potential increase in ROS production with age due to phagocyte activity.	Conflicting data: some report positive correlation, others no association.	[52,61–64]
Gender	Hypothesized influence of sex hormones on ROS production.	No significant differences reported	[65]
Cigarette smoke	Induces ROS release from macrophages and neutrophils; transient oxidative stress after smoking.	Acute smoking: temporary increase; baseline levels in COPD patients may not differ in smokers vs non-smokers.	[26,66–69]

Abbreviation: ROS, reactive oxygen species.

Gender and Age Influence

The relationship between exhaled H_2O_2 and age has been investigated in several studies, producing conflicting results. Research conducted in pediatric population evaluated H_2O_2 concentrations in EBC and found no evidence of age dependence.^{61,62} In contrast, Nowak et al⁵² reported a positive correlation between H_2O_2 levels and age in never-smokers, attributing this to an age-related increase in ROS production by activated phagocytes, as supported by recent findings.⁶³ However, Jobsis et al⁶⁴ noted that their control group was not age-matched, suggesting that age-related differences in exhaled H_2O_2 might have influenced their results. Nonetheless, they argued that it is unlikely that healthy children produce more ROS in their airways than healthy young adults. They emphasized the need for future studies to establish normative reference values for exhaled H_2O_2 across a broad age range.

In addition to age, the potential influence of gender on exhaled H_2O_2 has been investigated, based on the hypothesis that sex hormones may modulate the production of this inflammatory mediator. However, no significant effect of gender on H_2O_2 concentrations has been documented.⁶⁵

Influence of Cigarette Smoke

Although cigarette smoke contains high levels of oxidants, the hydrogen peroxide detected in EBC does not appear to originate directly from the smoke itself.²⁶ Rather, cigarette smoke stimulates alveolar macrophages and polymorphonuclear leukocytes to release ROS, including H_2O_2 . This mechanism provides the rationale for investigating the effect of tobacco smoking on exhaled H_2O_2 levels in various populations.

In healthy subjects, cigarette smoking has been shown to increase H_2O_2 concentrations in EBC, accompanied by enhanced neutrophil chemotactic activity.⁶⁶ However, Guatura et al⁶⁷ observed that although there was a significant rise in mean exhaled H_2O_2 levels 30 minutes after smoking a single cigarette, baseline H_2O_2 levels were similar between smokers and non-smokers. This suggests that in healthy individuals smoking induces a transient increase in airway oxidative stress, which may return to baseline within a few hours of abstinence. Additionally, in asthmatic patients, acute cigarette smoking causes a slight decrease in exhaled NO concentration, which is associated with an increase in exhaled H_2O_2 levels. This rise in H_2O_2 indicates an acute release of reactive oxygen species in the airways, potentially leading to enhanced formation of peroxynitrite, a reactive nitrogen species, that may contribute to the observed reduction in exhaled NO.⁶⁸

In patients with COPD, studies have not demonstrated significant differences in exhaled H_2O_2 levels between smokers and ex-smokers, suggesting a possible saturation effect or disease-related ceiling in oxidative biomarker expression.⁶⁹

Future Research Direction

Future research in this field should focus on several key areas to advance our understanding and clinical application of EBC biomarkers. Notably, recent evidence on exhaled H_2O_2 remains limited, highlighting a clear gap in current knowledge and emphasizing the need for updated and rigorous research.

First, it is important to establish reference values for the various inflammatory biomarkers in healthy individuals. This baseline is essential to interpret changes observed in disease states accurately. Second, studies assessing the reproducibility of these measurements are needed to ensure reliability and consistency across different settings and times. Additionally, large-scale longitudinal studies will be crucial to observe how these biomarkers fluctuate over time and correlate with disease progression or response to treatment. Another important direction is to explore how EBC markers relate to clinical symptoms, lung function tests, and other established methods for measuring airway inflammation, which will help clarify their clinical significance. Finally, a deeper understanding of the mechanisms behind the formation of EBC and its relationship to the airway lining fluid is necessary. This knowledge will improve the interpretation of EBC findings and their connection to underlying airway pathology. Overall, progress in this area depends on conducting rigorous studies focused on reproducibility, developing more sensitive and specific assays for detecting mediators, and establishing standardized normal values. This is especially important given that many laboratories currently report inconsistent results for the same biomarkers, highlighting the need for greater harmonization and methodological refinement.

Conclusions

This umbrella review outlines the promising potential of exhaled H_2O_2 as a biomarker in inflammatory lung diseases. Measurements of exhaled H_2O_2 in EBC provide valuable insights into the pathophysiology of inflammatory lung diseases. Thanks to its completely noninvasive nature, EBC is suitable for repeated measurements, offering information on airway inflammation both during stable phases of the disease and exacerbations. Furthermore, in pediatric populations, noninvasive techniques to assess airway inflammation are essential, given the limitations of relying mainly on symptoms and lung function tests to evaluate the patient's underlying pathobiological state.

Although EBC mainly consists of water vapor with other chemical compounds present in trace amounts, modern highly sensitive diagnostic tests enable effective analysis of these samples. Exhaled H_2O_2 in EBC has shown great potential in distinguishing patients with inflammatory airway diseases such as asthma or COPD from healthy subjects.

Given the complex pathophysiology of these conditions, H_2O_2 may be even more informative when used as part of a multimodal biomarker panel, complementing other physiological and biochemical measures.

However, the interpretation of H_2O_2 levels remains controversial, with heterogeneity across studies likely due to differences in methodology, patient populations, and analytical techniques.

Additionally, H_2O_2 levels correlate strongly with disease severity, disease control, and response to conventional treatments across various respiratory diseases. However, most studies analyzing the effects of therapies on exhaled H_2O_2 are observational; therefore, controlled trials are needed to evaluate its utility in specific disease management.

Nevertheless, the clinical use of exhaled H_2O_2 is limited by high variability in measurements influenced by factors such as diurnal variation, breathing pattern, and flow rate. Several methodological challenges also need to be addressed, including the standardization of sample collection, validation of analytical techniques, and harmonization of the diverse collection devices, which vary significantly in efficiency.⁷⁰

In conclusion, although measurement of exhaled H_2O_2 is not yet a routine clinical practice for guiding respiratory disease management, it remains a promising tool with expected broader adoption. Notably, a significant gap exists in recent evidence, highlighting the need for up-to-date research to validate the clinical relevance and reproducibility of exhaled H_2O_2 . Despite encouraging preliminary results, technical limitations and unresolved methodological issues need to be resolved. Further research is required to firmly establish the clinical role of H_2O_2 in EBC before recommending this technique for routine respiratory care.

With rapid technological advancements, more compact, user-friendly, and precise analyzers for EBC assessment are expected to become available soon, facilitating the diagnosis and monitoring of disease activity in patients with asthma and other inflammatory airway conditions.

Data Sharing Statement

Data will be made available on reasonable request from the corresponding author.

Acknowledgments

In rare case of ambiguity in the text, generative AI (ChatGPTversion 4) was employed to provide contextual clarification where the meaning was not explicitly clear. The AI tool was not used for literature classification, idea generation, writing or preparation.

Author Contributions

CG wrote the original draft, was responsible for the data curation and the methodology applied to the paper. AB and SP supervised the paper, validated and edited the original draft. NS was responsible for the conceptualization of the paper, supervised the paper, and edited the original draft. All authors approved the final version of the paper. All authors have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This paper was not funded.

Disclosure

C. Gagliani, N. Scichilone, A. Benfante have nothing to disclose. S. Principe received Unrestricted Grant from European Union's HORIZON Research and Innovation program and Unrestricted Grant from Innovative Medicines Initiative 2 Joint Undertaking (JU). The authors declare that they have no other conflicts of interest.

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