SCIENTIFIC BACKGROUNDER

INFLAMMATORY MARKERS IN EXHALED BREATH CONDENSATE

HYDROGEN PEROXIDE AS A MARKER FOR ASTHMA AND COPD

Summary

This Scientific Backgrounder summarizes some of the current published knowledge regarding Exhaled Breath Condensate analysis for identification of inflammatory status, mainly asthma and Chronic Obstructive Pulmonary Disease (COPD).
It discusses the clinical potential for EBC analysis and some health economic aspects.
The main focus is on Hydrogen Peroxide (H_2O_2) as an indicator.

Exhalation Technology

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1 INTRODUCTION

Biomarkers in exhaled breath can be analysed to identify inflammatory conditions in the lungs.

Exhaled breath condensate (EBC) contains aerosolized airway lining fluid and volatile compounds that can provide non-invasive indications of ongoing biochemical and inflammatory activities in the lung.

Exhaled breath analysis focuses on two areas: measurement of Fractional exhaled Nitric Oxide (FE_{NO}), as direct, online measurement of the gas sample, and the off-line detection of biomarkers in exhaled breath condensate (EBC).

FE_{NO} has been known as an inflammation marker since the early 90's, and is now clinically accepted and standardized for clinical use.[1]

FE_{NO} has been demonstrated to be an accurate and reliable biomarker to diagnose and monitor asthma. For other conditions, such as Chronic Obstructive Pulmonary Disease, COPD, the clinical use is less certain, due to the contradictory lower FE_{NO} levels. [2, 3]

There is a growing interest in other biomarkers, some of which are easily quantified by off-line measurement of collected Exhaled Breath Condensate (EBC) of the exhaled gas fraction, for pulmonary diseases, such as COPD, bronchiectasis, Cystic Fibrosis and others. Additional biomarkers for on-line measurement include carbon dioxide (CO_2) and Exhaled Breath Temperature.

This Scientific Backgrounder summarizes some of the current published knowledge regarding EBC analysis for identification of inflammatory status, mainly asthma and Chronic Obstructive Pulmonary Disease (COPD). It discusses the clinical potential for EBC analysis and some health economic aspects. The main focus is on Hydrogen Peroxide (H_2O_2) as an indicator for COPD.

2 BIOMARKERS IN EXHALED BREATH CONDENSATE

Exhaled Breath Concentrate (EBC) contains aerosolised particles from the airway lining fluid (ALF). These aerosolised particles are generated from the entire respiratory tract (from mouth to alveolus) into air which is saturated with water vapour. As the expired air is cooled, this water vapour condenses around the aerosolised particles forming EBC, and the various mediators can then be quantified from the condensate.

EBC contains non-volatile compounds, including cytokines, lipids, surfactant, ions, oxidation products, and adenosine, histamine, acetylcholine, and serotonin. In addition, EBC traps potentially volatile water-soluble compounds, including ammonia, hydrogen peroxide, isoprostanes, leukotrienes, nitrogen oxides and ethanol, and other volatile organic compounds. EBC has readily measurable pH. [4, 5]

EBC is being increasingly used in respiratory medicine to measure the different biomarkers of airway inflammation and oxidative stress. The increased interest in the analysis of EBC from the 1990's and forward, resulted in the American Thoracic Society (ATS) and European Respiratory Society (ERS) organising a Task Force in 2001 to develop guidelines on EBC collection, measuring biomarkers and reviewing the current literature on EBC. This Task Force published their guidelines in 2005 based on the current literature and expert opinions at that time. [5]

Based on the consensus of the Task Force participants, the expression “EBC” is the preferred term to describe the method. As molecules from the airways may be captured by other techniques, not only by cooling exhaled breath, EBC strictly relates to exhaled samples collected by cooling the exhaled breath. [6] They conclude that the technique of EBC has been developing since the ATS/ERS Task Force publication and will continue to grow over the coming years and foresee its use in clinical practice.

3 METHOD STANDARDIZATION

EBC is mainly used as a research tool, due to the lack of appropriate standardization and the absence of reference values. The large number of measurable biomarkers and the diversity of the used methodologies are some of the points that currently hamper its wide clinical application.

There is a diversity in analytical methods for detecting EBC: Spectrophotometry, Spectrofluorometry [7] and electrochemistry, why it is has been difficult to compare results between studies.

There are some recommendations on how the sample collection can be standardized, as described in the review by Dodig et al “Exhaled breath condensate – from an analytical point of view: Ref:[8]

Due to the volatile art of EBC, parameters that should be standardized, or kept constant.

Pre-analytical parameter	**Description**
Sampling device	the surface of the collector should have an inert material (e.g. glass, Teflon, silicone)
Sample collection time	should be a constant to enable calculation of volume and concentration
Sample volume	The respiratory rate should be kept constant, which gives a direct correlation to sample volume
Ambient temperature and humidity	The allowed temperature and RH interval for sample collection should be determined.
Contamination of sample	Prevention of contamination from saliva may be achieved by using a mouthpiece and a two-way non-rebreathing valve. Contamination from bacteria may be reduced by using a mouthwash of chlorhexidine solution prior exhalation.

A thorough survey has been performed by Konstantinidi et al (2015) [9] covering 30 years of research on exhaled breath condensate in a disease-based approach. The search covered EBC biomarkers and a range of clinical lung conditions, e.g. COPD, asthma, cystic fibrosis and others.

The number of published articles is rapidly increasing, although the majority represent small-scale studies. They conclude that EBC analysis is an intriguing achievement, as it is a non-invasive, simple and painless method, but that larger cohort-studies are needed to establish reproducibility and reference values.

European Respiratory Society Task Force has in 2017 published a technical standard for exhaled biomarkers in lung disease. [10] Recommendations for standardisation of sampling, analysing and reporting of data and suggestions for research to cover gaps in the evidence is summarised.

Recommendations for condensing equipment:

- Material of the entire collection system including sample vials should be inert or must be standardised for each EBC component of interest.
- EBC collection devices work at different cooling temperatures ranging from zero to below −20°C.
- Increasing the condensing surface has been shown to increase EBC volume and the number of biomarkers detected.
- Different components in EBC are differentially sensitive to cold temperatures, and the concentration of some constituents depends on the condensing temperature.
- Efficacy is improved by the use of a closed condenser design with breath recirculation, especially in young children or by fractionated sampling to separate EBC originating from proximal and more distal airways.

Recommendations for future research: For each EBC component, the optimal condensing material and method should be defined. Comparative methodological studies on collection system and their efficacy are needed.

Recommendations for EBC collection, tidal breathing:

- Volume of exhaled breath, the volume of condensate collected from the exhaled volume and the time of collection have to be reported in order to assess efficacy of EBC collection.
- subjects should refrain from exercise for at least 1 hour preceding EBC collection
- Slow breathing cycles, *i.e.* quiet tidal breathing, are recommended to avoid dead-space ventilation, which contributes to EBC dilution and influence from ambient air.
- Use of nose clip recommended for mouth breathing
- Breath temperature can significantly change between winter and summer, which will influence the temperature gradient between exhaled breath and the collecting system
- It is highly recommended that a suitable filter be adapted on the inspiratory valve to avoid influence from ambient air.
- Measurements of at least pH and H_2O_2 have to be performed in real time or immediately after collection without freezing or storing EBC.

Recommendations for future research: Define exhaled volume for EBC collection and report time of collection and volume of EBC obtained or define time for EBC collection and report the other two variables in parallel.

ERS Task Force concludes that it is unlikely that “one standardisation” will fulfil the requirements of the different substances measurable as potential biomarkers in EBC. Likely, different steps of standardisation need to be adjusted for EBC components of interest. The relevance of future EBC publications depends not only on a scientific pro/con description of specific biomarker findings in a certain group of subjects, but also on the inclusion of a systematic and meticulous description of the methods and techniques used to collect, preserve and analyse EBC. Enabling technologies facilitates the use of this human sample that is known not only for its potential, but also for the difficulty in handling it.

4 CURRENT CLINICAL PRACTICE FOR AIRWAY DISEASES

4.1 Asthma

For a long time, lung function testing - spirometry, has been the dominating clinical test method for diagnosing and monitoring asthma, together with patient history and airway hyper-responsiveness testing.

During the 1990's, the biomarker Nitric Oxide had been investigated and developed as an alternative, non-invasive test to conventional lung function testing. Measurement of Fractional Exhaled Nitric Oxide (FE_{NO}) has been clinically tested in a range of studies and is now accepted as a clinical method for diagnosing asthma and monitoring effect of ICS treatment in asthmatics. [1, 11]

In line with increasing pressure on healthcare budgets, new therapeutic applications not only need to show clinical efficacy but also cost-effectiveness. The FE_{NO} method has also demonstrated positive health economic outcomes, [12] and is now included in several insurance policies in Europe and in the US.

Asthma can be both eosinophilic and neutrophilic inflammation, but FE_{NO} is a marker only for the eosinophilic part.[13].

4.2 COPD

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease state characterised by airflow limitation that is not fully reversible. COPD is characterised by damage to small airways due to an inflammatory process as well as an imbalance between oxidants and antioxidants, also known as oxidative stress. [14] Several cytokines and cell adhesion molecules enhancing a mainly neutrophilic inflammation have been associated with COPD.

COPD is a progressive disease, evolving in three pathogenetic stages, as proposed by Tuder (2018) [15] which include immediate responses to COPD-causing triggers (e.g., cigarette smoke and/or environmental pollution), a progressive stage in which there is activation of an endogenous process leading to alveolar destruction and remodelling, and finally a consolidated stage with an aging molecular and phenotypic signature.

Spirometry method Forced Expiratory Flow (FEV1) is the current main clinical method to diagnose and monitor COPD. [16] In the 2019 update of the Global Strategy for Diagnosis, Management and prevention of COPD (Global initiative for chronic obstructive lung disease, GOLD)[17], spirometry is still the main method recommended for diagnosis, often used together with a questionnaire assessment.

Although spirometry has been a standardized procedure for more than 30 years, there are still evidence for that it is being underused in primary care, for patients with breathing problems. [18, 19]

FE_{NO} has been shown as a very reliable marker for asthma, but less good for COPD due to the low FE_{NO} values contrary for asthma. [11] [20] Different patterns of FE_{NO} response in COPD has been identified (Perez Bogerd 2019), but would require more studies to demonstrate clinical significance. [21]

A thorough meta-analysis on the relation between FE_{NO} values and COPD have been performed by Lu et al 2018. They concluded that patient with COPD have mild elevated levels of FE_{NO}, and that ex-smokers had higher FE_{NO} values than current smokers, which confirms previous studies that smokers have reduced FE_{NO}.[22]

Other biomarkers, such as hydrogen peroxide (H_2O_2) and Exhaled Breath Temperature[23], has shown to be a more suitable marker for COPD.[24]

American Thoracic Society and European Respiratory Society has published a joint paper on current research questions for COPD [25]. It is not a clinical practice guideline, but recommendations for research areas.

They recommend, amongst other suggestions, the following research areas:

- Studies that determine which outcomes matter most to patients with COPD and, therefore, are truly patient-centred outcomes in this population.
- Studies that correlate physiological and anatomical outcomes with patient-centred outcomes, to identify high-quality surrogate outcomes that may be used in future research.
- Preferential use of patient-centred outcomes to inform judgments related to patient care until surrogate outcomes have been identified that strongly correlate with patient-centred outcomes
- Studies that relate potential biomarkers of disease activity (e.g. rate of lung function decline, increased exacerbation frequency, inflammation, lung tissue destruction, and repair responses induced by inhalational injury) to patient-centred outcomes to validate the biomarkers as clinically useful measures of disease activity.

4.3 Asthma - COPD overlap

The challenge of differentiation diagnosis, and identifying overlap, between asthma and COPD has been summarized in a review performed by Gibson et al [26]

Asthma-COPD overlap has drawn attention to the significant heterogeneity that exists within obstructive airway diseases. There is a need of novel approaches that identify and manage the components of this heterogeneity, such as multidimensional assessment and treatment.

5 H_2O_2 AS BIOMARKER FOR ASTHMA AND COPD – EXPECTED VALUES

Perhaps the most studied EBC mediator in both asthma and COPD is hydrogen peroxide H_2O_2. Hydrogen peroxide is normally present in exhaled breath, probably derived from the pulmonary circulation. [27] Additionally, activation of airway epithelial and endothelial cells, neutrophils, alveolar macrophages and eosinophils leads to production of superoxide radicals and hence H_2O_2 production in airway inflammation. [28, 29]

H_2O_2 is less reactive and soluble than other reactive oxygen species, with its neutral charge and low molecular weight allowing it to cross membranes to exit into the extracellular spaces. H_2O_2 is volatile and readily equilibrates with air, thus its presence can be easily detected in EBC as a marker of pulmonary inflammation and oxidative stress. [6]

H_2O_2 levels have been demonstrated to be higher in EBC of COPD patients compared to healthy controls. [30, 31] It has also been demonstrated to rise further during exacerbations [32] and to correlate with severity of COPD as measured using FEV_1. [33]

Current smoking increases hydrogen peroxide levels in EBC in healthy subjects [34] but does not appear to have this effect in stable COPD patients. [30]

A study by Murata et al [35] measured the H_2O_2 levels and pH values using derivatives of reactive oxygen metabolites exhalation test kit (Diacron) and a pH analyser, respectively, in EBC obtained using an ECoScreen device.

They examined the relationships among oxidative stress and the asthma control test (ACT) or COPD assessment test (CAT) scores, pulmonary function, fractional exhaled nitric oxide (FE_{NO}), and the extent of low attenuation areas on HRCT. The H_2O_2 levels were elevated and pH was lower in both asthma and COPD:

Studied group	H_2O_2	pH
Asthmatic patients N=29	8.75 ± 0.88 µM, p < 0.01	7.14 ± 0.07, p < 0.05
COPD patients N=33	7.44 ± 0.89 µM, p < 0.01	pH; 6.87 ± 0.10, p < 0.01
Healthy controls (non-smokers) N=33	3.42 ± 0.66 µM	7.35 ± 0.04

Neither the H_2O_2 levels nor pH correlated with the ACT scores and FE_{NO} in asthma patients. Neither the H_2O_2 levels nor pH significantly correlated with the pulmonary function in asthma and COPD.

The CAT scores significantly correlated with the H_2O_2 levels in patients with COPD ($r = 0.52$, $p < 0.01$).

De Benedettino et al [31] has demonstrated the usefulness of exhaled H_2O_2 in COPD patients, using a technique which allows longer storage of the expired air condensate before the H_2O_2 assay.

The technique was applied in 13 healthy non-smoking subjects (six male, age range 22-40 yrs) and in seven patients (five male, age range 58-81 yrs) with mild or moderate COPD.

There was high repeatability on measurements, with a coefficient of variation equal to zero. The mean +/- SD H_2O_2 level in exhaled air from normal subjects was 0.12 +/- 0.09 µM, whereas it was significantly increased in COPD patients: 0.50 +/- 0.11 µM; $p = 0.0001$ compared to healthy subjects. In three healthy control subjects, a normal H_2O_2 level in expired air increased to 0.70-0.80 µM during an acute upper respiratory tract infection.

Peters et al[36] has made a study to evaluate the effect of ambient H_2O_2 when measuring EBC, by comparing Exhaled breath condensate (EBC) collected from 12 COPD patients and nine healthy control subjects either with an inhalation filter or without. An ECoScreen device was used for measuring EBC. They reported that H_2O_2 concentration in ambient air is significantly higher than H_2O_2 concentration in EBC samples. Inhaled H_2O_2 may be one factor in the heterogeneity and limited reproducibility of study results, and the use of an inhalation filter reduces variability of H_2O_2 values in exhaled air and shows an endogenous production.

6 ANALYTICAL METHODOLOGY

The analytical technology for measuring H_2O_2 is performed In Vitro. An EBC sample is collected in a special collector which preserves the sample and can then be analysed.

Commercially produced EBC collection devices are becoming available that may assist this process, such as the R tube® (Respiratory Research), ECoScreen® device (Viasys), Turbo Deccs (Medivac) and the new **Inflammacheck by Exhalation Technology.**

Inflammacheck uses an electrochemical sensor where the enzyme peroxidase is used in the reaction. This makes the sensor highly selective for H_2O_2.

7 CLINICAL APPLICATIONS FOR H_2O_2 in EBC

7.1 In Asthma

There are several publications on the usability of measuring H_2O_2 as a marker for asthma.

A thorough meta-analysis on H_2O_2 in asthmatics have been made by Teng et al. [37] Eight clinical studies on asthmatic adults and children were summarized. It was concluded that EBC H_2O_2 concentrations were significantly higher in patients with asthma who were non-smokers compared with healthy subjects. Higher values of EBC H_2O_2 were observed at each level of asthma, classified either by severity or control level, and the values were negatively correlated with FEV_1. In addition, EBC H_2O_2 concentrations were lower in patients with asthma treated with corticosteroids than in patients with asthma not treated with corticosteroids.

A study in children with asthma has been performed by Trischler et al. [38] A method of fractionated sampling was used, which provides the ability to collect condensate from different parts of the lung. The primary hypothesis was that airway H_2O_2 concentrations are significantly higher than alveolar concentrations. Also, to correlate these results with data of exhaled nitric oxide (FE_{NO}), lung function measurements and the asthma control test (ACT).

It was concluded that H_2O_2 concentrations in exhaled breath condensate were significantly higher in the airway fraction than in the alveolar fraction in asthmatic children and young adolescents. Only the H_2O_2 concentrations of the alveolar fraction correlated with asthma control in children 12 years and older suggesting that alveolar H_2O_2 plays a role in asthma control.

7.2 In COPD

There are well-designed clinical trials performed where measuring the H_2O_2 levels have been used for monitoring the effect of treatment. A double-blinded placebo controlled study performed by Kasileski et al [39] showed a significant reduction in H_2O_2 after long term (> 6 months) treatment with N-acetylcysteine.

In another clinical study by van Beurden et al [40] the aim was to investigate the effect of Inhaled Corticosteroids (ICS) on oxidative stress, and different depositions of ICS in the lungs. Measurement of H_2O_2 was used as primary outcome in this study. The study concluded that H_2O_2 significantly reduced from baseline after 2 weeks treatment.

H_2O_2 concentration	**Baseline (week 0)**	**1st treatment period end (week 4)**	**2nd treatment period start (week 8)**	**2nd treatment period end (week 12)**
Sequence 1 N=	0.32 µmol/l (0.03 µmol/l)	0.21 µmol/l (p = 0.04)	0.19 µmol/l (0.03 µmol/l	0.19 µmol/l
Sequence 2 N=	0.42 µmol/l (0.08 µmol/l)	0.22 µmol/l (p = 0.01)	0.17 µmol/l (0.02 µmol/l)	0.22 µmol/l
Combined Reduction, significance		P < 0.01		None

The overall change in H_2O_2 concentration was not significant for either treatment in the second treatment period. There was no difference between patients who used ICS prior to the study (n = 24)

and patients who did not (n = 11): 0.35 and 0.41 µmol/l, respectively. The higher the baseline H_2O_2 concentration, the greater the change after treatment ($p < 0.05$). There was no difference between smokers and ex-smokers (H_2O_2 concentration 0.40 and 0.35 µmol/l, respectively).

H_2O_2 in stable and instable COPD was studied by Dekhuijzen et al. [32] H_2O_2 from EBC was measured from patients with stable COPD, patients with exacerbated COPD and compared with a healthy control group.

Group	FEV1 % predicted	H_2O_2	P-value
Stable COPD N = 12	51%	0.205 +/- 0.054 microM	Compared
Unstable COPD (acutely exacerbated) N =19	36%	0.600 +/- 0.075	P < 0.001
Healthy controls N = 10	108%	0.029 +/- 0.012 microM	P < 0.05

These findings demonstrate that patients with stable COPD exhibit increased oxidant production in the airways and that oxidant production increases further during exacerbations. [32]

In an Indian study by Nagaraja et al, 100 patients were included, a control group with risk factors (smoking etc) and COPD diagnosed patients. [41]

The ECoScreen device was used. Of the 100 subjects studied, 23 were healthy individuals with risk factors (smoking, exposure to air pollution, and urbanization).

Study group	H_2O_2 before treatment	H_2O_2 after treatment
Total N = 100		
Healthy with risk factors N = 23	200-2220 nmol/l (smokers) 340-760 nmol/l (non-smokers	NA
COPD patients, acute exacerbations	540-3040 nmol/l	240-480 nmol/l
Asthma patients	400-1140 nmol/l	100-320 nmol/l
Bronchiectasis patients	300-340 nmol/l	200-280 nmol/l
Interstitial Lung Disease	220-720 nmol/l	210-510 nmol/l

Gerritzen et al have studied the effect of treatment with prednisolone, in COPD patients. H_2O_2, along with IL-8 and a serum marker sICAM, were used as biomarkers.

Fourteen patients with moderate to severe COPD exacerbation have been monitored during treatment with prednisolone.

During treatment H_2O_2 concentrations in breath condensate declined significantly ($p<0.001$) as well as IL-8 and sICAM in serum ($p=0.002$, respectively, $p<0.001$). There was no significant change in sE-selectin ($p=0.132$). No significant improvement was found in spirometry. [42]

A study by Neville et al [43], using the Inflammacheck Device, has been designed with the purpose to determine whether the device can measure EBC H_2O_2 consistently and whether it can be used to differentiate asthma and COPD from healthy controls. The result is correlated with disease stage, spirometry, FE_{NO}, and symptom control scores.

The study is a cross-sectional, feasibility, pilot study of EBC H_2O_2 levels and other markers of disease severity and symptom control in patients with asthma and COPD and volunteers with no history of lung disease.

Preliminary results have been presented, with 177 subjects included (67 asthma [32 GINA 2/3, 34 GINA 4/5], 60 COPD [30 GOLD stages 1/2, 30 GOLD 3/4] and 50 controls). Data from 153 of these were analysed.

Compared to healthy controls, mean EBC H_2O_2 levels were significantly higher in participants with COPD (2.55 µM vs 3.17 µM, p<0.001) and asthma (2.55 µM vs 3.11 µM, p=0.008). There was no significant difference between asthma and COPD, but levels were higher in severe COPD. Sensitivity analyses confirmed the differences were still significant comparing milder asthma and COPD with healthy controls. A 2.0 µM threshold of H_2O_2 provided 79% sensitivity (63% specificity) and an 81% positive predicted value for diagnosis of COPD at 53% prevalence. Patient factors were not related to H_2O_2 levels. All participants agreed the device was easy to use and the test easy to perform.

8 RESEARCH APPLICATIONS

8.1 Carbon Dioxide as inflammation marker

Carbon Dioxide (CO_2) has also been shown to be a biomarker for inflammatory lung conditions. It has been difficult to obtain consistent readings with traditional sensors.

Cambridge Respiratory Innovations Ltd. (CRiL), has received exciting early data from the first longitudinal study to show the development of an asthma exacerbation using Tidal Breathing CO_2 ($TBCO_2$) waveform analysis, with a LED sensor technology.

Exhaled Breath temperature (EBT) as inflammation marker

Several studies have been made on the correlation between Exhaled Breath temperature and smoking, and where EBT is a marker for the risk for smokers to develop COPD.

EBT values are within a narrow range, why the thermosensors for detecting EBT need to be precise and very sensitive.

Carpagnaro et al [44] analyzed the EBT in current smokers. They also monitored the effects both of cigarette smoking on EBT and of what happens after smoking cessation. The study population consisted of 25 smokers recruited from a smoking cessation programme, and 25 healthy, non-smokers ans control group. They concluded that EBT is significantly higher in smokers compared to controls. EBT increases after cigarette smoking and progressively decreases with the increase of time from when the last cigarette was smoked.

A study by Lázár et al[45] studied EBT (using X-halo, Delmedica Investments) in 19 control non-smoking subjects, 19 control smoking/ ex-smoking subjects, 20 patients with stable COPD and 17 patients with COPD at onset and also after recovery from an acute exacerbation.

Their results demonstrated that the peak EBT was different between the subject groups, with lower values in the patients with stable COPD (34.00/33.35–34.34/°C); median /interquartile range/) than in the smoking/ex-smoking control subjects (34.51/34.20– 34.68/°C).
The EBT was higher at the onset of AECOPD (34.58/34.12–34.99/°C) compared to in a stable condition, and positively correlated with the sputum leukocyte count and neutrophil percentage

The conclusion from this study was that the peak exhaled breath temperature, recorded during multiple tidal breaths, increases with an acute exacerbation of COPD, and may be related to accelerated airway inflammation.

A longitudinal study by Labor et al showed that EBT can act as a marker to predict future COPD in smokers. [46]

Measurements of EBT were made 2 times: at the pulmonology clinic at the recruitment visit before and after a smoked cigarette. Patients were reassessed for a COPD diagnosis after 2 years.

Results: A subsample of 122 subjects (57.4% male), mean (SD) age 52.4 (6.2) yrs with 37.6 (17.4) packyears of smoking was reassessed after 2 years. At enrolment 32.8% were asymptomatic, 54.1% were symptomatic and 13.1% were GOLD stage I (postbronchodilator FEV_1/FVC)

EBT change after a cigarette was significantly associated with QoL and marginally with a progression to COPD. Conclusion: Our preliminary data shows that EBT could serve as one of the early markers in predicting future COPD in smokers.

Carpagnaro et al has initiated a study to establish reference values of EBT for use in future research studies. [47] The study was performed in healthy Caucasian subjects that has never smoked.

An alternative to general reference values, is to assess EBT when patients are in a steady state of their disease and to use this 'personal best' to monitor them and guide their treatment. Individual devices outfitted with microprocessors and memory have been created, which can be used for personalized monitoring and disease management by telemedicine.[23]

8.2 Fractionated H_2O_2 measurements

There is interesting ongoing research on fractionated sampling of EBC from alveolar parts; vs upper airways. One study where fractionated H_2O_2 measurements in adults with COPD are published is by Möller et al. [48]

In this study, EBC was collected as fractionated samples from the airways and from the lung periphery in 10 non-smokers, eight asymptomatic smokers, and in eight chronic obstructive pulmonary disease (COPD) patients. H_2O_2 concentration and acidity (pH) were analyzed in the airway and the alveolar fraction.

In all subjects studied, H_2O_2 was 2.6 times higher in the airway versus the alveolar fraction. Airway H_2O_2 was twofold higher in smokers and fivefold higher in COPD patients compared to non-smokers. In all study groups, there was no significant difference in deaerated pH between the airway and the alveolar sample. They conclude that exhaled H_2O_2 is released at higher concentrations from the airways of all subjects studied, implying that the airways may be the dominant location of H_2O_2 production. Because many lung diseases cause inflammation at different sites of the lung, fractionated sampling of EBC can reduce variability and maintain an anatomical allocation of the exhaled biomarkers.

8.3 Differentiation of eosinophilic vs neutrophilic inflammation using H_2O_2

Comparison of H_2O_2 in EBC with eosinophilic count in induced sputum was studied by Fireman et al[49]. They showed a significant positive correlation between H_2O_2 and eosinophils and also neutrophils in induced sputum. They conclude that both eosinophil and neutrophil cells are the source of H_2O_2 production.

Wewel et al studied changes in H_2O_2 and FE_{NO} in lung cancer patients undergoing chemotherapy. The see a relationship between H_2O_2 levels and neutrophils, but not the same relation for FE_{NO}.[50]

Therefore, it seems that H_2O_2 is detecting inflammation in a broader spectrum than e.g. FE_{NO}, and could aid in differential diagnosis between asthma, where inflammation is eosinophilic, and COPD, where inflammation is mainly driven by neutrophils.

8.4 Cystic Fibrosis

Inflammatory markers are present in Cystic Fibrosis. Robroeks et al has studied a range of inflammatory markers, and found FE_{NO} and EBC could contribute in diagnosis and monitoring.[51, 52]. Jöbsis et al[53], concluded in a study that H_2O_2 is an indicator that cystic fibrosis patients (children) with an acute pulmonary exacerbation have abnormally high concentrations of hydrogen peroxide.

Future longitudinal studies should reveal whether non-invasive monitoring of airway inflammation in CF adds to better follow-up of patients.

8.5 Clinical use in environmental / occupational health

A recent study, published April 2014, has used EBC for monitoring the effect of exposure to nanoparticles from cigarette smoke. [54]

The aim was to evaluate the feasibility of using EBC for assessing the lung deposited dose of combustion nanoparticles and for determining the resulting oxidative stress by measuring H_2O_2 and malondialdehyde (MDA).

Although H_2O_2 and MDA concentrations in EBC increased during exposure, only H_2O_2 showed a transient normalization 1 hr after exposure and increased afterward. In contrast, MDA levels stayed elevated during the 2 hr post exposure.

Using EBC as a marker for asbestosis has been studied by Chow et al. [55]

Eighty-six male subjects were studied (48 ex-smokers and 38 never smokers). None of the subjects were using inhaled corticosteroids. EBC H_2O_2 was also increased in patients with asbestosis compared to normal controls (13.68 (8.63e21.68) vs. 5.89(3.99e8.69) mM, $p<0.05$. A trend towards increasing H_2O_2 levels with increasing severity of lung disease was observed. H_2O_2 levels were also significantly correlated with exhaled NOx (rZ0.61, $p<0.0001$) and protein (rZ0.74, $p<0.0001$).

8.6 Veterinary applications

The analysis of biomarkers in exhaled breath (EB) and exhaled breath condensate (EBC) allow non-invasive and repeatable assessment of respiratory health and disease in mammals. A review of published studies has been performed by Chow et al. [56] They conclude that compared to human medicine, research data from EB and EBC analysis in veterinary medicine are limited and more patient variables influencing concentrations of EB/EBC analytes may be present. In addition, variations in methodologies between studies may influence results.

H_2O_2 in EBC has been measured in horses by Duz et al [57], they did not observe any significant changes between horses with Lower Airway Inflammation and controls.

Two later studies by de Preez et al (2017 and 2019), concludes that H_2O_2 and pH is possible to measure from EBC in horses with good inter- and intraday consistency for both pH and H_2O_2, although ambient humidity and temperature influence the measurements .[58] They conclude that H_2O_2 and pH are suitable markers for lower airway inflammation in horses[59].

A veterinary study done in cats, showed a significant correlation between increased H_2O_2 in EBC and Lower Airway inflammation, compared to control group.[60]

9 HEALTH ECONOMY ASPECTS

Chronic Obstructive Pulmonary Disease (COPD) is a major cause of chronic morbidity and mortality throughout the world. Many people suffer from this disease for years and die prematurely from it or its complications. COPD is the fourth leading cause of death in the world, and further increases in its prevalence and mortality can be predicted in the coming decades. [14]

An economic analysis of patient response to a survey in the U.K. [61] showed that COPD places a high burden on the healthcare system and society with annual direct costs estimated at £819.42 per patient, and indirect cost at £819.66 per patient resulting in total per patient costs of £1639.08. The cost impact of the disease was particularly marked in secondary care, as a result of inpatient hospitalizations, amounting to 54% of direct costs. These results suggest that reducing patient requirement for hospital care could alleviate the burden of COPD on the U.K. healthcare system. This will require considerable improvements to the way the disease is managed by healthcare professionals in primary care, with earlier diagnosis and the use of interventions aimed at preventing exacerbations and delaying the progression of disease.

Effective diagnostic tools would play a role in health economics. Measurement of H_2O_2 in EBC may contribute to an earlier and more accurate diagnosis of COPD, and for optimization and monitoring of treatment. Similar studies are performed in France, Italy, Canada and other countries. The summary results of these studies are discussed in a review article from 2002. [62]

Berg et al [12] reported an economic evaluation of the use of FE_{NO} measurements in the diagnosis and management of asthma. This study was designed to compare the cost-effectiveness of a FE_{NO} - driven asthma management strategy with standard diagnostics and treatment guidelines, from the perspective of a German payer. A similar approach would be possible for H_2O_2 in EBC.

10 CONCLUSION

Exhaled breath condensate (EBC) is a non-invasive method of sampling the airways that can be repeated easily and is acceptable to patients.

Biomarkers in EBC, such as Hydrogen Peroxide, can be used for diagnosis and monitoring effect of medical treatment.

Recommendations for standardization of EBC measurements, and recommended areas for further research, have been published by American Thoracic Society and European Respiratory Society.

Once the method of measuring H_2O_2in EBC becomes standardized, clinical applications may include diagnosis of COPD, identification of preclinical disease, phenotyping of COPD patients, evaluation of response to therapies and defining the prognosis of individual patients.

There are health economic advantages for simple diagnostic methods for COPD, as patients may get diagnosed in an earlier stage, and get proper treatment to control the disease and avoiding hospitalization.

There are a range of clinical options for further research, where the area of most interest would be differentiated diagnosis between eosinophilic and neutrophilic inflammation conditions. Diagnosis and monitoring of Cystic Fibrosis and other pulmonary conditions are also of high importance.

There are also options for a usage within environmental monitoring and for veterinary applications.

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