



Update on exhaled breath condensate analyses in occupational disease

Piero Maestrelli, Paola Mason, and Gabriella Guarnieri

Purpose of review

The present work represents an update of the review published in this journal by Corradi *et al.*, regarding the use of exhaled breath condensate (EBC) to investigate occupational lung diseases.

Recent findings

The relevant literature was searched in the Medline database, assessed through PubMed using key terms such as 'breath AND condensate AND occupational'. Eleven pertinent publications were retrieved between January 2018 and October 2019. One article only was related to occupational allergy, and the conclusion is that EBC hydrogen peroxide is not an useful marker in laboratory animal allergy. The biomarkers of exposure most often assessed with EBC are metals. However, it is controversial whether this approach has any advantage over the conventional environmental monitoring. The biomarkers of effect studied by the majority of investigations were those related to oxidative stress. They appear consistently elevated upon occupational exposures to various agents, including welding fumes, crystalline silica, nanomaterials and chemicals.

Summary

Although EBC represent a suitable tool to sample airway lining fluid in a noninvasive manner, it remains a niche approach to the investigation of occupational diseases. The confounding influence of EBC dilution should be better addressed in the expression of the results.

Keywords

airway inflammation, metals, noninvasive, occupational medicine, oxidative stress

INTRODUCTION

Exhaled breath condensate (EBC) is the result of cooling exhaled breath through contact with a cold surface or condenser. Samples are collected as a fluid or frozen material and analyzed immediately or later for volatile and nonvolatile macromolecules [1]. Much of the volume of EBC is water vapor evaporated from the epithelial lining fluid of the respiratory tract. A small fraction is represented by aerosol droplets generated by a process of respiratory fluid film or bubble bursting by reopening of respiratory bronchioles during inhalation and the subsequent emission of fragmented droplet aerosol during the next exhalation [1]. These microdroplets carry an array of nonvolatile molecules (proteins, lipids, electrolytes, acidic and alkaline small molecules, metals, etc.). In addition, EBC contains a large number of slightly volatile substances (carbon dioxide, nitric oxide, hydrocarbons, etc.). Therefore, EBC provides a dilute sampling of the epithelial lining fluid.

Occupational respiratory diseases are caused by materials inhaled in the workplace and are

characterized by a reaction to these stimuli, including oxidative stress and inflammation. In addition, the respiratory system is the major way by which inhaled substances can enter into the body.

A review on the use of EBC to investigate occupational lung diseases was published by Corradi *et al.* [2] in this journal and the authors concluded that the analysis of EBC is one of the most promising methods currently available for the study of pulmonary biomarkers of exposure, effect and susceptibility in occupational settings. The advantages of this tool to sample the airways are that it is noninvasive, can be applied in field studies at the workplace and is suitable for longitudinal assessment.

Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova, Padova, Italy

Correspondence to Piero Maestrelli, MD, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova, Padova, Italy. Tel: +39 049 8212564; e-mail: piero.maestrelli@unipd.it

Curr Opin Allergy Clin Immunol 2019, 19:000–000

DOI:10.1097/ACI.0000000000000607

KEY POINTS

- EBC represent a suitable tool to sample airway lining fluid in a noninvasive manner and to study pulmonary biomarkers of exposure, effect and susceptibility in occupational settings.
- The biomarkers of exposure most often assessed with EBC are metals. However, it is controversial whether this approach has any advantage over the conventional environmental monitoring.
- The biomarkers of effect studied by the majority of investigations were those related to oxidative stress. They appear consistently elevated upon occupational exposures to various agents and therefore rather unspecific.
- It is recommended to express the results of EBC content of volatile components as quantity per minute of ventilation and EBC content of nonvolatile substances per 100l of exhaled breath to minimize the confounding influence of dilution.

STANDARDIZATION OF EXHALED BREATH CONDENSATE

EBC is relatively easy to collect with homemade systems or with a variety of commercial equipment. However, the efficacy of condensation depends on a variety of variables, including cooling temperature, breath volume over time, condensing surface area and the gradient of temperature between exhaled breath and sampling system. A first attempt to provide methodological recommendation was done in 2005 by a task force of European Respiratory Society (ERS) and American Thoracic Society [3]. An update of recommendation for standardization of EBC sample collection and evaluation of different analytic approaches was recently published by an ERS Task Force as an ERS technical standard [1]. The general

conclusion was that different steps of standardization are needed to be adjusted according to the EBC component of interest. However, two factors that drive the reproducibility issue should be considered anyway: first, the absence of a valid dilution factor method; and second, many biomarkers have been detected near the lower limit of the assay.

There was a consensus that efficacy of the collection should be assessed by reporting the volume of exhaled breath, the volume of the condensate and the time of collection. The recommended methods to express the results to minimize the confounding influence of dilution are to recalculate the EBC content of volatile components as quantity per minute of ventilation and the content of nonvolatile substances per 100l of exhaled breath.

When EBC components are used as biomarkers of exposure, the issue of contamination from outdoor or indoor ambient air is relevant. Therefore, it is highly recommended that suitable filters be adapted on the inspiratory valve. In addition, the device contamination by its components (i.e. metals) or by cleaning procedures should be carefully assessed.

LITERATURE SEARCH

The relevant literature was searched in the Medline database, assessed through PubMed in October 2019, using key terms such as 'breath AND condensate AND occupational'. The number of titles retrieved was 182, which ranged from 2001 to 2019. The distribution of the number of articles over the years is shown in Fig. 1. The publications increased rather rapidly after 2007, with a peak between 2010 and 2011 and a temporary drop between 2014 and 2015. According to the time span of the literature for this review, we considered the articles published between January 2018 and October 2019. There were 15 titles, but only 11

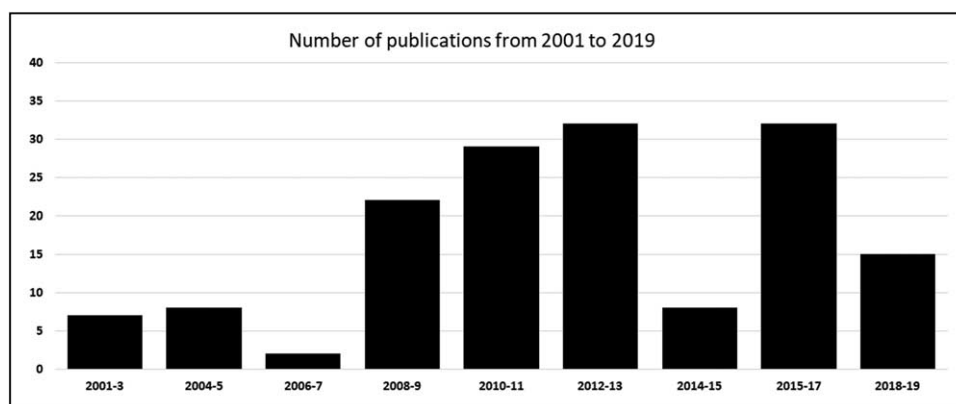


FIGURE 1. Distribution of the publications related to the use of exhaled breath condensate in occupational setting retrieved in PubMed since 2001.

publications were pertinent after reading the abstracts.

EXHALED BREATH CONDENSATE BIOMARKERS IN OCCUPATIONAL SETTINGS

Two articles regard protocols of studies still to be performed and do not provide original data. Hopf *et al.* [4] describe a project aimed to assess whether exposure to metalworking fluids (MWF) is associated with biomarkers of oxidative stress and inflammation as well as genotoxic effects. Workers from France and Switzerland exposed to MWF and suitable controls will be followed for three consecutive working days. EBC will be used to assess exposure biomarkers such as metals, metabolites of polycyclic aromatic hydrocarbons and nitrosamine, and biomarkers of effect such as malondialdehyde, 8-isoprostane, 8-hydroxyl-2'-deoxyguanosine, nitrates and nitrites.

Santonen *et al.* [5] report a European human biomonitoring initiative involving 400 workers exposed to hexavalent chromium from eight European countries. EBC will be used as a new method for chromium biomonitoring in association with chromium in red cells and the traditional urinary total chromium measurement.

There was only one article related to occupational allergy [6], compared with the four investigations published between 2008 and 2009 quoted in the previous review on ERS by Corradi *et al.* [2,7–10]. Tafuro *et al.* [6] performed an observational study with the aim to validate biomarkers of airway inflammation in laboratory animal workers. The fraction of exhaled nitric oxide (FeNO), EBC hydrogen peroxide and serum pneumoproteins (surfactant protein-A, surfactant protein-D and Club Cell 16) were compared in 63 laboratory animal workers and 64 controls. In a multivariate analysis, FeNO and surfactant protein-D levels were positively associated with exposure to laboratory animals. EBC hydrogen peroxide levels were not significantly different between laboratory animal workers and controls as a whole, but the sensitized laboratory animal workers had significantly higher levels than the sensitized controls. The conclusion of the author is that EBC hydrogen peroxide levels do not seem to be an useful marker in laboratory animal allergy.

A number of studies considered exposures to an array of inhalable workplace materials: metals, silica, mineral dust, chemicals, fumes and gases, nanocomposites. EBC was used to detect biomarkers of effect in all these studies. In addition, Riccelli *et al.* [11] investigated the chromium and nickel content in EBC as a biomarker of exposure to stainless steel

tungsten inert gas welding fumes. Their results showed that chromium EBC is a reliable biomarker of exposure even at very low environmental concentrations, whereas nickel EBC concentration remained below the lower limit of detection. The EBC biomarker of effect – hydrogen peroxide, malondialdehyde, 4-hydroxy-nonenal – did not show any close relationship with EBC biomarker of exposure, that is chromium, indicating that low concentrations of airborne metal have only slight direct effects on oxidative stress of the airway or that other components of welding fumes, not measured in the study, are probably involved in lung oxidative stress. The changes in biomarkers of effect with exposure exhibited different patterns, that is related to daily work-shift for hydrogen peroxide, while related to weekly exposure for malondialdehyde and 4-hydroxy-nonenal. Therefore, the choice of the time of sampling EBC in relation with work exposure is critical to detect a proper signal. It remains controversial whether biological monitoring of metal exposure using EBC should be suggested routinely. The issue is extensively discussed by the review of Ghio *et al.* [12]. They considered the measurements of EBC metals in the pathogenesis of diseases and in occupational or environmental exposures. The conclusion is that EBC is a research tool in the investigation of the participation of metals in various respiratory diseases, particularly those associated with smoking as chronic obstructive pulmonary disease. However, the quantification of EBC metal concentration is currently not reliable for clinical use in either supporting or determining any diagnosis. The studies of occupationally exposed workers confirm that EBC metals concentrations can document a significant exposure to specific metals, but it may be more efficient to measure them on filters collected for environmental monitoring. In fact, the failure to assess the variability in the dilution of airway particles in EBC and the capacity of the lung to actively metabolize and transport metals pose limitation to the EBC analysis.

A study by Corradi *et al.* [13] explored the ability of EBC markers of oxidative stress to distinguish lung functional impairment in a small group of workers exposed to low concentrations of crystalline silica. The study was negative. It was interesting the observation that the EBC 4-hydroxynonenal mean concentration was higher in the few ($n = 8$) workers with silica exposure above the threshold limit value of 0.025 mg/m^3 proposed by the American Conference of Governmental Industrial Hygienists.

Wu *et al.* [14] have used EBC to detect acute pulmonary responses to occupational wild land fire smoke. EBC samples were collected from firefighters before, after and next morning following prescribed

burn shift and regular work shifts in nonburning days. There was limited evidence that smoke exposure causes an acute pulmonary response since only the postshift 8-isoprostane concentrations were marginally higher than the pre shift concentration on the burn days.

Two companion articles from the same group in Czech Republic [15[■],16[■]] examined inflammatory markers and markers of oxidative stress in EBC collected from researchers exposed to nanomaterials generated during various tasks associated with nanocomposite synthesis, production and postprocessing. The authors were able to characterize the exposure to nanoparticles in term of mass concentrations and particle number. All markers of oxidation of nucleic acids and proteins were elevated in the preshift EBC samples compared with controls and significant post-shift elevations were documented in lipid oxidation markers. The most robust markers of lung inflammatory response were leukotriene B4 and tumor necrosis factor. Since mixed exposures to aerosols, originating from welding, smelting and nanocomposite processing were detected, the role of nanoparticles and the other substances implicated in the lung oxidative stress and inflammatory response remains to be determined.

A novel approach applied to the analysis of EBC was used by Maniscalco *et al.* [17[■]], who compared NMR-based metabolomics of EBC in group of workers exposed to levels of airborne inhalable dust, phenol, formaldehyde and volatile organic compounds below regulatory limits. In this pilot study, the supervised analysis – Orthogonal Projections to Latent Structure Discriminant Analysis – of EBC from 20 blue-collar workers identified a clear separation between EBC samples before (not exposed) and after a 30-min exposure to the factory environment without wearing protective mask (exposed). The relevant markers detected after exposure were fatty acids, alcohols and phenylalanine. Furthermore, this approach was able to demonstrate the effectiveness of wearing protective masks to prevent the absorption of environmental chemicals.

EBC is mentioned by Fishwick and Forman [18] among the individual tools that can be considered in health surveillance at work for prevention of occupational asthma as it is relatively easy to collect and can be analyzed both for specific constituents or acidity (pH). However, the authors conclude that any potential role in health surveillance is as yet unproven.

CONCLUSION

Although EBC represent a suitable tool to sample airway lining fluid in a noninvasive manner, it

remains a niche approach to the investigation of occupational diseases. Apparently, the use of EBC to determine relevant biomarkers in occupational allergy has nearly been abandoned during the last years. The biomarkers of exposure most often assessed with EBC are metals. However, it is controversial whether this approach has any advantage over the conventional environmental monitoring. Probably, EBC would be more suitable for kinetic studies of metal absorption by the lung, when EBC metal concentrations were compared with airborne and blood concentrations. The biomarkers of effect studied by the majority of investigations were those related to oxidative stress. They appear consistently elevated upon occupational exposures to various agents and therefore rather unspecific. These findings suggest that oxidative stress represent a general response to inhalation environmental toxics. It has to be underlined that none of the studies quoted in this review complied with the recommendation of ERS technical standard to express the results to minimize the confounding influence of EBC dilution.

Acknowledgements

None.

Financial support and sponsorship

The work was supported by grant BIRD167128/16 from the Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Horváth I, Barnes PJ, Loukides S, *et al.* A European Respiratory Society technical standard: exhaled biomarkers in lung disease. *Eur Respir J* 2017; 49:1–26.
2. Corradi M, Gergelova P, Mutti A. Use of exhaled breath condensate to investigate occupational lung diseases. *Curr Opin Allergy Clin Immunol* 2010; 10:93–98.
3. Horváth I, Hunt J, Barnes PJ, *et al.*, ATS/ERS Task Force on Exhaled Breath Condensate. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 2005; 26:523–548.
4. Hopf NB, Bourgard E, Demange V, *et al.* Early effect markers and exposure determinants of metalworking fluids among metal industry workers: protocol for a field study. *JMIR Res Protoc* 2019; 8:e13744.
5. Santonen T, Alimonti A, Bocca B, *et al.* Setting up a collaborative European human biological monitoring study on occupational exposure to hexavalent chromium. *Environ Res* 2019; 177:108583.
6. Tafuro F, Selis L, Goldoni M, *et al.* Biomarkers of respiratory allergy in laboratory animal care workers: an observational study. *Int Arch Occup Environ Health* 2018; 91:735–744.
7. Klusackova P, Lebedova J, Kacer P, *et al.* Leukotrienes and 8-isoprostane in exhaled breath condensate in bronchoprovocation tests with occupational allergens. *Prostaglandins Leukot Essent Fatty Acids* 2008; 78:281–292.

8. Ferrazzoni S, Scarpa MS, Guarnieri G, *et al.* Exhaled nitric oxide and breath condensate pH in asthmatic reactions induced by isocyanates. *Chest* 2009; 136:155–162.
 9. Do R, Bartlett KH, Dimich-Ward H, *et al.* Biomarkers of airway acidity and oxidative stress in exhaled breath condensate from grain workers. *Am J Respir Crit Care Med* 2008; 178:1048–1054.
 10. Corradi M, Sanchez Mdel M, Acampa O, *et al.* Noninvasive assessment of lung pathobiology in hairdressers. *Med Lav* 2008; 99:49–57.
 11. Riccelli MG, Goldoni M, Andreoli R, *et al.* Biomarkers of exposure to stainless steel tungsten inert gas welding fumes and the effect of exposure on exhaled breath condensate. *Toxicol Lett* 2018; 292: 108–114.
- It is a well conducted study on exhaled breath condensate (EBC) content of metals upon exposure to low concentrations of chromium and nickel.
12. Ghio AJ, Madden MC, Esther CR. Transition and posttransition metals in ■ exhaled breath condensate. *J Breath Res* 2018; 12:027112.
- Excellent review on the role of measurement of EBC metals in respiratory diseases and in environmental exposure.
13. Corradi M, Folesani G, Robuschi B, *et al.* Noninvasive techniques to assess restrictive lung disease in workers exposed to free crystalline silica. *Med Lav* 2019; 110:83–92.
 14. Wu CM, Adetona A, Song CC, *et al.* Measuring acute pulmonary responses to ■ occupational wildland fire smoke exposure using exhaled breath condensate. *Arch Environ Occup Health* 2019; 1–5. [Epub ahead of print]
- The work proposes an interesting design to investigate the short-term effect of exposure to fire smoke in firefighters.
15. Pelclova D, Zdimal V, Schwarz J, *et al.* Markers of oxidative stress in the ■ exhaled breath condensate of workers handling nanocomposites. *Nanomaterials (Basel)* 2018; 8:1–19.
- Interesting study on lung oxidative stress associated with exposure to nanomaterials using EBC.
16. Pelclova D, Zdimal V, Komarc M, *et al.* Deep airway inflammation and respiratory ■ disorders in nanocomposite workers. *Nanomaterials (Basel)* 2018; 8:1–13.
- As for the companion article [15■], it is an interesting study on lung inflammation, assessed using EBC, associated with exposure to nanomaterials.
17. Maniscalco M, Paris D, Melck D, *et al.* Biomonitoring of workers using nuclear ■ magnetic resonance-based metabolomics of exhaled breath condensate: a pilot study. *Toxicol Lett* 2018; 298:4–12.
- This is an original approach in the use of EBC as a suitable matrix to investigate airway metabolomics.
18. Fishwick D, Forman S. Health surveillance for occupational asthma. *Curr Opin Allergy Clin Immunol* 2018; 18:80–86.