Exhaled Breath Condensate: Pilot Study of the Method and Initial Experience in Healthy Subjects

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ABSTRACT

Analysis of Exhaled breath condensate (EBC) is a re-discovered approach to monitoring the course of the disease and reduce invasive methods of patient investigation. However, the major disadvantage and shortcoming of the EBC is lack of reliable and reproducible standardization of the method. Despite many articles published on EBC, until now there is no clear consensus on whether the analysis of EBC can provide a clue to diagnosis of the diseases. The purpose of this paper is to investigate our own method, to search for possible standardization and to obtain our own initial experience. Thirty healthy volunteers provided the EBC, in which we monitored the density, pH, protein, chloride and urea concentration. Our results show that EBC pH is influenced by smoking, and urea concentrations are affected by the gender of subjects. Age of subjects does not play a role. The smallest coefficient of variation between individual volunteers is for density determination. Current limitations of EBC measurements are the low concentration of many biomarkers. Standardization needs to be specific for each individual biomarker, with focusing on optimal condensate collection. EBC analysis has a potential become diagnostic test, not only for lung diseases.

KEYWORDS

exhaled breath condensate; standardization; healthy subjects

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Received: 13 December 2017
Accepted: 27 April 2018
Published online: 2 July 2018

Acta Medica (Hradec Králové) 2018; 61(1): 8–16
https://doi.org/10.14712/18059694.2018.17

INTRODUCTION

Exhaled breath condensate (EBC) is a biological fluid that consists mainly of water, but it also contains small droplets of airway lining fluid (36). Condensate carries molecules <65 kDa (25). EBC contains both, volatile and non-volatile compounds (35). Adenosine, ammonia, hydrogen peroxide, isoprostanes, leukotrienes, nitrogen oxides, peptides, cytokines belong to the compounds detected in EBC (42). In the clinical setting, non-volatile components, such as cytokines, are used for diagnostics and for monitoring of disease progression (87, 61, 27).

First reports on the EBC collection were published in early 1980s.The report on the EBC collection from the 1990s shows that the condensate was obtained by passing expired gas through tubing submerged in an ice-water bath (7). The first EBC studies documented, that the exhaled breath condensate test is simple, non-invasive and easy to perform. Homemade and commercially manufactured condensers are available today. Various homemade devices have been described, for example a Teflon or a Polypropylen tube, dipped in a bucket filled with ice, a double glass layer container or a device, where exhaled air condensation takes place between the two layers (70, 83, 102). Commercially manufactured condensers are also available, for example EcoScreen, Turbodeccs, ANACON or RTube (90, 26, 85, 14).

Nowadays, there is an incrased interest in non-invasive diagnostics and investigation. Collection of EBC fulfils requirements of a non-invasive, repeatable test and thus applicable in the pediatric population (20, 88, 100) and in adult patients, especially in those, who have to control parameters daily. EBC has the potential to become a routine method used for diagnostics, mainly for lung diseases such as bronchial asthma (98, 33, 92, 16, 74), cystic fibrosis (101), idiopathic pulmonary fibrosis (82), bronchiectasia (64), tuberculosis (69), lung carcinoma (2, 46), acute lung injury and acute respiratory distress syndrome (19), chronic obstructive pulmonary disease (59), scleroderma with pulmonary involvement (38, 67), sleep apnea syndrome (18, 89), silicosis (77, 60) and other occupational lung diseases (24, 76, 75, 80, 92). Recently, EBC has been used in monitoring gastrointestinal diseases, such as gastroesophageal reflux disease (91, 81, 39, 58), inflammatory bowel disease (56, 52, 44), coeliac disease (5, 43). Other studies were carried out to monitor systemic sclerosis (28, 93), liver diseases (6), abdominal surgery (66, 84, 65), obese population (13), impacts of oxidative stress (78, 94, 63) or for toxicity screening (34, 79, 57).

If the breath is captured and analyzed correctly, it can be used to provide information of the current health status with a potential to predict future outcomes progression of the disease (50). Nowadays, the PubMed (at https://www.ncbi.nlm.nih.gov/pubmed) registers more than 1,300 scientific papers on EBC. However, several methodological issues, including standardization of EBC technique and validation of analytical methods, need to be addressed before this approach can be considered and taken into real practice. EBC composition may be influenced the time of exhalation, condenser temperature, use of nose clips, temperature and duration of condensate storage, saliva contamination, smoking, eating, drinking of coffee may influence composition of EBC. The American Thoracic Society and European Respiratory Society developed guidelines for EBC collection and measurement of exhaled biomarkers, to suggest recommendations on the possible use and limits of exhaled biomarkers and to highlight those areas where further research is required (42).

Nevertheless, the major disadvantage and shortcoming of the EBC is lack of reliable and reproducible standardization of the method (37, 70, 54).

The aim of this study was to introduce our own method, to search for possible standardization and to obtain our own initial experience in healthy volunteers.

METHODS

SUBJECTS

Thirty healthy volunteers between 25–69 years of age were included in the study. The volunteers were the staff of the Faculty Hospital and the Faculty of Medicine of the Charles University in Hradec Králové. The exclusion criteria were the history of chronic lung disease or other serious chronic illness or respiratory infection within 2 weeks preceding the study. The group contained 14 smokers and 16 non-smokers. The project was carried out according to the Declaration of Helsinki, and was approved by the Ethics Committee (201706S11P) of the University Hospital in Hradec Králové. All participants signed an informed consent.

CONDENSATION OF EXHALED BREATH

The condenser EcoScreen (Jaeger, Hoechberg, Germany) is a system in which a mouthpiece with a one-way valve and a refrigerated collecting system are connected to a power supply by an extendable arm. Due to the function of the valve (which is connected to the mouthpiece), inspiratory and expiratory air are separated. The collecting system is connected to the valve block and is placed in a cooling thermoblock. During exhalation, air flows through the lamellar condenser, becomes liquid, and drops into the collecting vial.

All parts of the collection kit were rinsed with ethanol and deionised water and were air dried before each use. The subjects rinsed their mouth with infant water (Cl^- < 5 mg/L) with a defined composition.The subject did not smoke, did not eat and did not drink coffee or sweet lemonade at least one hour before sampling. The subjects were not exposed to increased physical activity for at least 30 minutes before sampling. The sampling occurred between 8:00 and 11:00 a.m. The ECoScreen was cooled to –10 °C, based on the information provided by the manufacturers. EBC was collected during 10 minutes of exhalation. After 10 minutes of quiet breathing, 1–3 mL of EBC were collected from adult individuals. Volume of EBC is proportional to the total exhaled volume and breathing frequency. EBC samples were immediately transferred to polypropylene tubes and were frozen at –80 °C.

In order to examine repeatability of the measurements over a moderate period of time, the collection of EBC was repeated after two weeks.

DENSITY DETERMINATION

Total amount of 500 µL of EBC was transferred by a validated pipette to a polypropylene tube of defined weight. Thereafter, the condensate tube was weighed on Sartorius analytical balances (Germany). Accuracy of measurement was at 0.01 mg and was repeated three times. The measurement was run at 25 °C.

PROTEIN DETERMINATION

Protein content was determined with a diagnostic reagent for quantitative *in vitro* determination of Bio-La-Test Total Protein kit (Erba Lachema, Czech Republic). Named values were verified by determination Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich). The measurement was run at 37 °C and was repeated three times.

UREA DETERMINATION:

Urea content was determined with a diagnostic reagent for quantitative *in vitro* determination of Bio-La-Test Urea kit (Erba Lachema, Czech Republic). The measurement was run at 37 °C and was repeated three times.

CHLORIDE DETERMINATION

Chloride content was determined with a Ion-selective chloride electrode (Fisher Scientific, Czech Republic). The measurement was run at 25 °C and was repeated three times.

PH DETERMINATION

All samples were deaerated in an ultrasonic bath. pH were determined with a pH electrode (Fisher Scientific, Czech Republic). The measurement was run at 25 °C and was repeated three times.

STATISTIC ANALYSIS

Results had normal distribution and therefore are presented as mean ± SD or median (interquartile range). Data obtained were tested statistically by means of non-paired t-test. All statistics was performed using SigmaStat software (Jandel Scientific, Eckhardt, Germany, Version 3.1).

RESULTS

SUBJECTS

Thirty healthy volunteers, 24 females and 6 males, were included in the study. The mean age of the female and male subjects was comparable (40.4 years, range: 25–58 and 39.7 years, range: 28–69, P = 0.93). The group included 14 smokers and 16 non-smokers. The proportions of smokers among females and males were 10/24 (42%) and 4/6 (67%), respectively (P = 0.38). The mean age of non-smokers and smokers did not differ (40.8 years, range: 25–58 vs. 39.6 years, range: 25–69, P = 0.93).

TWO-WEAK REPEATABILITY OF EBC MEASUREMENTS

The repeated measures analysis of variance was used to calculate the average intra-individual and inter-individual coefficients of variation of duplicate measurements performed two weeks apart as well as the intraclass correlation coefficient (Table 1). The mean intra-individual variabilities are considerable for the concentration of total protein, chloride and urea. On the contrary, the measurements of density and pH shows the lowest $\%CV_{intra}$. The intraclass correlation coefficient was good for pH (ICC = 0.81) and moderate for urea measurement (ICC = 0.52), whereas its negative values indicated that the test-retest variability ($\%CV_{intra}$) of density and total protein was higher than the interindividual variability ($\%CV_{inter}$) (Table 1). The repeatability of EBC pH and urea measurements is visualized with the help of Bland-Altman plots in Figure 1.

DENSITY

Density of EBC sample was 1007.2 ± 4.7 g/L (Table 1). Density of EBC was not dependent on smoking, gender or age of subjects. There was no statistically significant differ-

Tab. 1 Descriptive statistic of measured analytes and physical quantity.

	Density	pH	Total Protein	Chlorides	Urea
	(g/L)		(µg/mL)	(mmol/L)	(µmol/L)
Mean ± SD	1007 ± 4.7	6.8 ± 0.8	1.6 ± 1.2	3.1 ± 1.8	199 ± 156
SD/Mean (%)	0.5	11.5	74.8	58.0	77.2
Median	1007	7.0	1.3	3.4	173
IQR	1004–1010	6.1–7.4	0.7–2.4	1.2–4.6	83.9–291
Range	996–1016	5.5–8.1	0.1–4.5	0.4–6.2	1.0–610
CV_{intra} (%)	1.5	5.3	61.2	57.7	61.3
CV_{inter} (%)	1.2	12.0	52.0	62.4	88.6
ICC	–0.06	0.81	–0.19	0.20	0.52

The descriptive statistics of measured analytes and physical quantity were calculated from the results of second measurements; SD – standard deviation, IQR – interquartile range. The CVintra (%) and CVinter (%) reffer to the mean intra-individual and inter-individual coefficients of variation of duplicate measurements performed two weeks apart; ICC – intraclass correlation coefficient of repeated measurements.

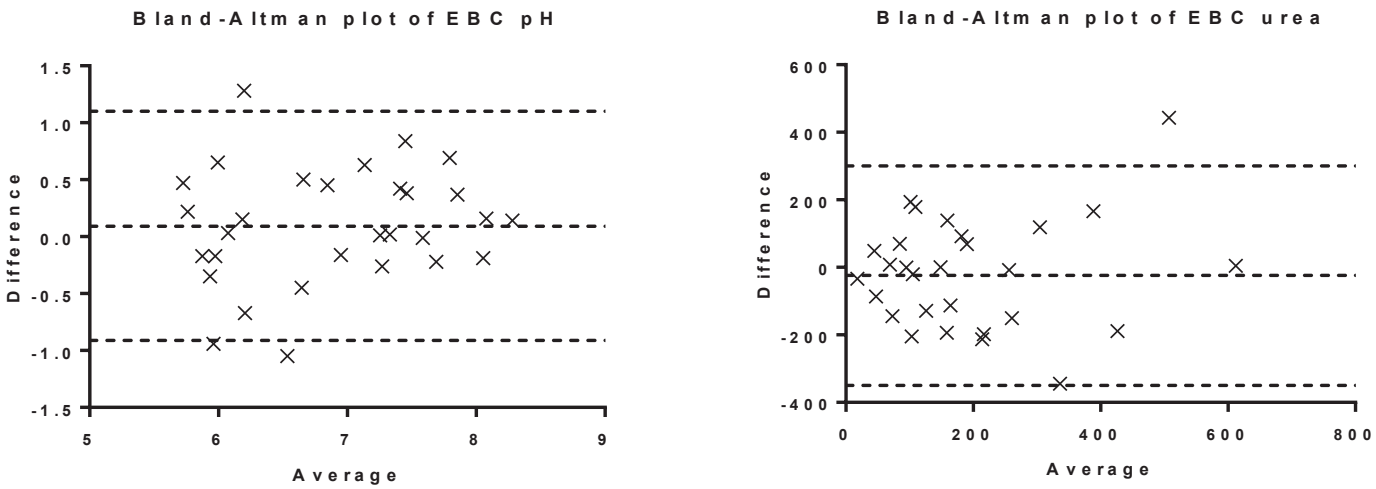

Fig. 1 The repeatability of EBC pH and urea measurements between visits 1 and 2 separated by two weeks. Bland Altman difference vs. average plots with the mean difference and 95% limits of agreement visualized as the broken lines.

Tab. 2 Pearson Product Moment Correlation between variables of interest.

		Age	Density	Chlorides	pH	TP	Urea
Age	r2	x	0.12	0.12	0.10	-0.05	-0.02
	P	x	0.52	0.54	0.58	0.78	0.80
Density	r2	–	x	0.07	-0.19	0.11	0.05
	P	–	x	0.73	0.31	0.55	0.41
Cl-	r2	–	–	x	0.16	-0.18	-0.22
	P	–	–	x	0.40	0.36	0.25
pH	r2	–	–	–	x	-0.33	0.013
	P	–	–	–	x	0.08	0.95
TP	r2	–	–	–	–	x	0.013
	P	–	–	–	–	x	0.95

Coefficients of determination and two-tailed P-values are listed. The existence of correlation was not proved between any of the pairs of variables (P > 0.05).

Tab. 3 Univariate analyses of the effects of smoking, age and gender on measured analytes and physical quantity.

	Density	pH	Total Protein	Chlorides	Urea
	(g/L)		(µg/mL)	(mmol/L)	(µmol/L)
Smokers, N = 14	1008 ± 3.4	6.2± 0.5	1.6 ± 1.5	2.8 ± 2.0	229 ± 178
Nonsmokers, N = 16	1006 ± 5.7	7.4 ± 0.5	1.9 ± 1.2	3.4 ± 1.7	172 ± 134
P	0.32	< 0.001	0.13	0.41	0.33
Younger (25–40 yr), N = 14	1008 ± 4.9	6.9 ± 0.8	1.6 ± 1.5	3.2 ± 2.1	227 ± 181
Older (41–70 yr), N = 16	1007 ± 4.8	7.0 ± 0.8	1.3 ± 1.2	3.0 ± 1.7	174 ± 131
P	0.60	0.37	0.82	0.79	0.36
Women, N = 24	1007 ± 5.0	6.9 ± 0.8	1.5 ± 0.9	3.1 ± 1.9	164 ± 125
Men, N = 6	1009 ± 2.8	6.5 ± 0.7	2.1 ± 2.1	3.2 ± 1.7	336 ± 201
P	0.19	0.25	0.26	0.95	< 0.02

ence between non-smokers and smokers, between females and males and between age groups (Table 3). There were no significant relationships between density and other investigated parameters (Table 2).

PH

EBC pH was 6.8 ± 0.8 (Table 1). pH was dependent on smoking (Figure 2). EBC pH in smokers was lower than in non-smokers. Gender and age of subjects did not influ-

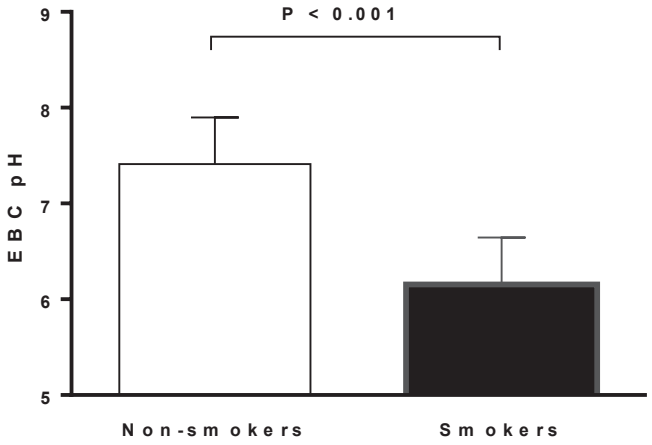

Fig. 2 Exhlaled breath condensate pH *in non-smokers and smokers.* Each bar represents mean ± SD.

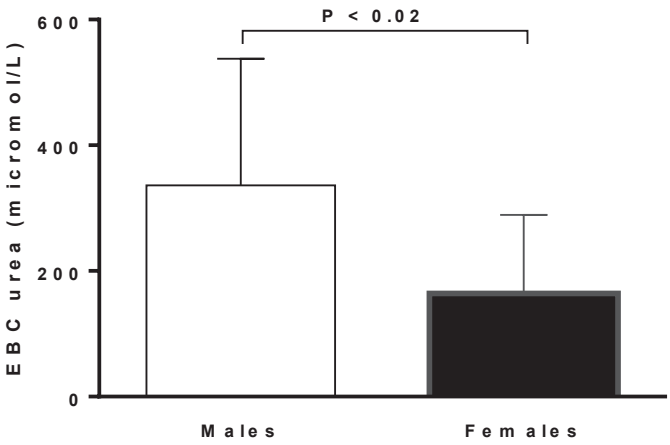

Fig. 3 Concentration of urea in the breath condensate exhaled by men and women. Each bar represents mean ± SD.

ence pH values (Table 3). There were no significant relationships between pH and other investigated parameters or physical quantity (Table 2).

TOTAL PROTEIN

Total protein concentration were 1.6 ± 1.2 μg/mL (Table 1). Our results show that total protein level is not dependent on smoking, gender and age of subjects. There were no statistically significant differences between non-smokers and smokers, between females and males and between age groups (Table 3). There were no significant relationships between total protein levels and other investigated parameters (Table 2).

CHLORIDE

Chloride ion concentration in EBC samples was 3.1 ± 1.8 mmol/L (Table 1). Chloride content was not dependent on smoking, gender and age of subjects. There were no statistically significant differences between non-smokers and smokers, females and males and between age groups (Table 3). There were no significant relationships between chloride ion levels and other investigated parameters (Table 2).

UREA

Urea concentration was 199 ± 156 μmol/L (Table 1). Urea levels were dependent on gender (Figure 3). Urea concentration in females was lower than in males. Smoking and age do not have any impact on urea levels (Table 3). There were no significant relationships between urea levels and other investigated parameters or physical quantity (Table 2).

DISCUSSION

First attempts on breath diagnostics go back to Hippocrates who described foetor ex ore and foetor hepaticus in his treatise on breath smell analysis. The modern era of breath testing was initiated in 1971, when Pauling analyzed volatile organic compounds from breath trapped in a cooled stainless steel tube and found out that normal human breath contains more than 250 different volatile organic compounds (73, 54). At present, breath analysis is divided into two main directions. The first area of breath analysis deals with the detection of volatile organic compounds. The second areas looks into the aqueous part of breath, which contains mainly non-volatile compounds and water soluble volatiles. There is a lot of studies with both exhaled air analysis and/or, EBC (8, 72).

EBC represents one of the most accessible biological materials, which can be obtained by a non-invasive way. Yet, lack of reliable and reproducible standardization of the method is the major problem at present. Despite multiple articles having been published on EBC, there is no clear consensus at present on whether the analysis of EBC can provide a definite diagnosis of the diseases. There seems to be a high risk of pre-analytical and analytical errors and based on this, interpretation of EBC biomarkers should be taken with a lot of precaution.

There are are numerous possible source of pre-analytical errors can be miscellaneous. Collection devices are an important source of variability of EBC biomarkers (86, 40, 21). The other principal factors of variability include cooling temperature (26) and condenser materials (86). Diet, smoking, medication and physical activity influenced EBC results (55, 15, 10, 9). Use of a nose clip is another unsolved question. When using a nose clip, the subject is forced to exhale only through the mouth, preventing thus accidental exhalation through the nose. Yet, the use of a nose clip may affect composition of EBC (95). Currently, there are devices developed by Loccioni Gruppa Humancare (Angeli di Rosora, Italy) which help to assess collection parameters, when the EBC is being obtained from human individuals. These devices provide continuous visual feedback to the subjects to control breathing patterns (99).

The method of determination may be the source of variability in the analytical phase. EBC can be analysed for example by ionex chromatography (47), plasma mass spectrometry (1), Liquid chromatography–mass spectrometry (48) or Polymerase chain reaction (62). We decided to use colorimetric assays and ion-selective electrodes in our study. The possible sources of variability errors are given for the relevant analytes and physical quantities.

The aim of our current study was to search for markers that could serve as a standard that several markers could be related to. Investigated the following parameters: density density, pH, total protein level, chloride level and urea level. Some authors suggested that data normalization to the internal control is not necessary, because the levels of analytes during the disease increase many times (17, 23). Carter and colleagues have different view on normalization. Their study showed that standardization should be specific for each biomarker, as a more general model would not optimize collection of all compounds (21).

Our results showed that the density of EBC could be used for standardization. Density meets the requirements in the sense, that it reaches almost the same value across the across the different EBC samples as well as a good test-retest reproducibility of the result. It is not influenced by smoking, gender or age of subjects. Condensed water vapour enriched with trace amounts of volatile and non-volatile components is the major component of the EBC (99, 3). Therefore, the EBC density is close to 1 g/mL and the other dissolved minor components do not change the density. The larger content of dissolved volatiles compounds such as ammonia (53) or air trapping in the sample would decrease density. Thus, density could help to compensate for the influence of such factors during sampling but it will unlikely fulfill all expectations for an internal standard. Namely, density is unrelated to the efficiency of the transfer of a particular molecule/ion into exhaled air and, to the completeness of its trapping during cooling.

The interpretation of EBC pH values is complex. EBC pH reflects acidity of the airway lining fluid. However, its value is from a part affected by volatile airway acids (CO_2) and bases (NH_3) which show a variable content upon standing of the EBC sample. The attempts to eliminate CO_2 as a confounding factor included either its removal from the sample by deaeration using an inert gas or saturation of the sample with CO_2 to a stable pH (41). In our study, we used deaeration using ultrasonic baths that secures that CO_2 was removed. Vaughan et al were able to document that EBC pH reflects lung homeostasis. The pH of the airway in health has been reported to be slightly alkaline while acidification of EBC occurs during disease states (96). The pH also depends on temperature of sampling and volume of NH_4^+. EBC collected at –70 °C were more acidic than those collected at –20 °C (26) We assume that we eliminated this variability by using Ecosreen and followed the same conditions in all sampling. The literature shows that pH is influenced by diet (55). Ćalušić et al showed that consuming a standardized meal constituting 101.3 g of water, 34 g of protein, 11.2 g of fat, and 47.3 g of saccharides has no effect on pH (15). Hunt et al. found EBC pH decreased significantly after 50 ml sweet limonade and returned to baseline after 15 min (Hunt et al. 2006). We have eliminated the effect of food and beverages by avoiding least one hours before the sampling. The subjects were also not exposed to increased physical activity for at least 30 minutes before sampling. Bikov et al. found that following exercise EBC pH decreased in exercise-induced bronchoconstriction positive group although in healthy subjects this decline was not demonstrated (11). Thus, amount of NH_4^+ found in the EBC reflects the contamination with salivary NH_3, some of which is derived from bacterial degradation of urea (29). Volume NH_4^+ can be reduced by washing the mouth with infant water.

Smoking is another factor which influences pH. According to several studies, EBC pH may be lower in smokers compared to non-smokers (4, 51, 40). We confirmed this, too. The measurement of pH with a glass electrode is technically simple. The intraclass correlation coefficient of EBC pH was the highest of all analytes examined in this study. However, the influence of general factors on the EBC pH including the unstable content of volatile acids/bases, hydration of the airways, smoking, exercise-induced bronchoconstriction etc., precludes its use for standardization of the EBC measurements.

We also revealed, that there was a substatial variability in total protein concentration among investigated subjects. Our results are consistent with a study by Bloemen et al. Their study showed that total protein concentration of EBC differed significantly between healthy individuals. Unlike we did, she showed a correlation between the age of subjects and total protein concentration (12). The variability in total protein values might be caused by protease activity (49). The addition of protease inhibitors may further prevent degradation of proteins, however, protease inhibitors can interfere with protein analysis. EBC storage also plays its role. Storage in polyethylene tubes minimizes protein adhesion. (22). Elevated inflammatory markers in respiratory disease increase total protein levels in EBC (68). Type of material of the inner part of the condensers may also play its role. Ecosreen had greater albumin retention compared to glass or Teflon coatings (86).

Our results showed that chlorides are not suitable for standardization. This observation is consistent with the hypothesis that EBC chloride correlates with plasma chloride levels (30). Physical exercise can alter ion regulation by increasing chloride secretion to a significant and similar degree compared to the individuals with cystic fibrosis (97). EBC of patients with chronic persistent cough is more acidic and has lower chloride levels than EBC of non-coughing healthy subjects (71). We did not find any correlation between pH and chloride levels.

Urea concentrations in the EBC are quite low and more variable than the other evaluated parameters, i.e. density and pH. It is believed that the EBC urea value correlates with plasma urea levels (32) as well as chloride levels. NH_4^+ can also interfere with urea analysis (31).

CONCLUSIONS

EBC analysis has a potential become a safe and a non-invasive diagnostic test, not only for lung diseases. In the future it could replace some of more invasive methods. In order to address the issues regarding standardization, it is necessary to consider the matter of collection devices and techniques, modes and conditions. Standardization needs to be specific for each individual biomarker, with future investigations focusing on optimal collection.

ACKNOWLEDGEMENTS

This work was supported by the project PROGRES Q40-15 and Q40-01 (from Charles University).

REFERENCES

1. Aherrera A, Olmedo P, Grau-Perez M et al. The association of e-cigarette use with exposure to nickel and chromium: A preliminary study of non-invasive biomarkers. Environmental Research 2017; 19: 313–20.
2. Ahmed N, Bezabeh T, Ijare OB et al. Proton Magnetic Resonance Spectroscopy (1H MRS) of Sputum and Exhaled Breath Condensate: A Noninvasive Tool for Lung Cancer Screening. Traditional Journal of Radiation Oncology 2016; 96: E432.
3. Aksenov AA, Zamuruyev KO, Pasamontes A et al. Analytical methodologies for broad metabolite coverage of exhaled breath condensate. Journal of Chromatography B 2017; 1061–1062: 17–25.
4. Antus B, Barta I, Kullmann T et al. Assessment of exhaled breath condensate pH in exacerbations of asthma and chronic obstructive pulmonary disease: A longitudinal study. American Journal of Respiratory and Critical Care Medicine 2010; 182: 1492–7.
5. Aprea E, Cappellin L, Gasperi F et al. Application of PTR-TOF-MS to investigate metabolites in exhaled breath of patients affected by coeliac disease under gluten free diet. Journal of Chromatography B 2014; 966: 208–13.
6. Augusto VS, Rodrigues AJ, C Silveira AP et al. Exhaled and plasma nitrite: a comparative study among healthy, cirrhotic and liver transplant patients. Arquivos De Gastroenterologia 2014; 51: 16–20.
7. Baldwin SR, Grum CM, Boxer LA et al. Oxidant activity in expired breath of patients with adult respiratory distress syndrome. The Lancet 1986; 4: 11–3.
8. Balint B, Donnelly LE, Hanazawa T, Kharitonov SA, Barnes PJ. Increased nitric oxide metabolites in exhaled breath condensate after exposure to tobacco smoke. Thorax 2001; 56: 456–61.
9. Barreto M, Villa MP, MD, Olita C et al. 8-Isoprostane in Exhaled Breath Condensate and Exercise-Induced Bronchoconstriction in Asthmatic Children and Adolescents. Chest Journal 2009; 135: 66–73.
10. Biernacki WA, Kharitonov SA, Barnes PJ. Increased leukotriene B4 and 8-isoprostane in exhaled breath condensate of patients with exacerbations of COPD. Thorax 2003; 58: 294–8.
11. Bikov A, Galffy G, Tamasi L et al. Exhaled breath condensate pH decreases during exercise-induced bronchoconstriction. R espirology 2014; 19: 563–9.
12. Bloemen K, Lissensa G, Desagerb K, Schoetersa G. Determinants of variability of protein content, volume and pH of exhaled breath condensate. Respiratory Medicine 2007; 101: 13311–7.
13. Bodini A, Tenero L, Sandri M et al. Serum and exhaled breath condensate leptin levels in asthmatic and obesity children: a pilot study. Journal of Breath Research 2017; 11: 046005.
14. Caglieri A, Goldoni M, Acampa O et al. The Effect of Inhaled Chromium on Different Exhaled Breath Condensate Biomarkers among Chrome-Plating Workers. Environmental Health Perspectives 2006; 114: 542–46.
15. Ćalušić AL, Varnai VM, Macan J. Acute effects of smoking and food consumption on breath condensate pH in healthy adults. Experimental Lung Research 2011; 37: 92–100.
16. Cap P, Maly M, Pehel F, Pelikan Z. Exhaled leukotrienes and bronchial responsiveness to methacholine in patients with seasonal allergic rhinitis. Annals of Allergy, Asthma & Immunology 2009; 102: 103–9.
17. Carpagnano GE, Carratú P, Gelardi M et al. Increased IL-6 and IL-4 in exhaled breath condensate of patients with nasal polyposis. Monaldi Archives for Chest Disease 2009; 71: 3–7.
18. Carpagnano GE, Spanevello A, Sabato R et al. Exhaled pH, exhaled nitric oxide, and induced sputum cellularity in obese patients with obstructive sleep apnea syndrome. Translational Research 2008; 151: 45–50.
19. Carpenter CT, Price PV, Christman BW. Exhaled Breath Condensate Isoprostanes Are Elevated in Patients With Acute Lung Injury or ARDS. Chest 1998; 114: 1653:59.
20. Carraro S, Giordano G, Reniero F et al. Asthma severity in childhood and metabolomic profiling of breath condensate. Allergy 2013; 68: 110–17.
21. Carter SR, Davis CS, Kovacs EJ. Exhaled breath condensate collection in the mechanically ventilated patient. Respiratory Medicine 2012; 106: 601–13.
22. Conrad DH, Goyette J, Thomas PS. Proteomics as a Method for Early Detection of Cancer: A Review of Proteomics, Exhaled Breath Condensate, and Lung Cancer Screening 2008; 23: 78–84.
23. Corradi M, Folesani G, Andreoli R et al. Aldehydes and glutathione in exhaled breath condensate of children with asthma exacerbation. American Journal of Respiratory and Critical Care Medicine 2003; 167: 395–9.
24. Corradi M, Gergelova P, Mutti A. Use of exhaled breath condensate to investigate occupational lung diseases. Current Opinion in Allergy and Clinical Immunology 2010; 10: 93–8.
25. Cunningham S, McColm JR, Pei Ho L, Greening AP, Marshal TG. Measurement of inflammatory markers in the breath condensate of children with cystic fibrosis. The European Respiratory Journal 2000; 15: 955–7.
26. Czebe K, Barta I, Antus B et al. Influence of condensing equipment and temperature on exhaled breath condensate pH, total protein and leukotriene concentrations. Respiratory Medicine 2008; 102: 720–25.
27. Dalaveris E, Kerenidi T, Katsabeki-Katsafli A et al. VEGF, TNF-α and 8-isoprostane levels in exhaled breath condensate and serum of patients with lung cancer. Lung Cancer 2009; 64: 219–25.
28. Edmé JL, Tellart AS, Launay D et al. Cytokine concentrations in exhaled breath condensates in systemic sclerosis. Inflammation Research 2008; 57: 151–6.
29. Effros RM, Casaburi R, Su J et al. The effects of volatile salivary acids and bases on exhaled breath condensate pH. American Journal of Respiratory and Critical Care Medicine 2006; 173: 386–92.
30. Effros RM, Hoagland KW, Bosbous M et al. Dilution of respiratory solutes in exhaled condensates. American Journal of Respiratory and Critical Care Medicine 2002; 165: 663–9.
31. Effros RM. Do low exhaled condensate NH4+ concentrations in asthma reflect reduced pulmonary production? American Journal of Respiratory and Critical Care Medicine 2003; 167: 91–2.
32. Esther CR Jr, Boysen G, Olsen BM et al. Mass spectrometric analysis of biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis. American Journal of Physiology. Lung Cellular and Molecular Physiology 2009; 296: 1987–93.
33. Fulcher YG, Fotso M, Chang CH et al. Noninvasive Recognition and Biomarkers of Early Allergic Asthma in Cats Using Multivariate Statistical Analysis of NMR Spectra of Exhaled Breath Condensate. PLOS ONE 2016; 11: e0164394.
34. García-Gómez D, Bregy L, Nussbaumer-Ochsne Y et al. Detection and Quantification of Benzothiazoles in Exhaled Breath and Exhaled Breath Condensate by Real-Time Secondary Electrospray Ionization–High-Resolution Mass Spectrometry and Ultra-High Performance Liquid Chromatography. Environmental Science & Technology 2015; 49: 12519–24.
35. Gasparič J, Hyšpler R, Tichá A. Exhaled breath and metabolism disorders (in Czech). Vesmír 2004; 83: 283–285.
36. Goldoni M, Caglieri A, Andreoli R et al. Influence of condensation temperature on selected exhaled breath parameters. BMC Pulmonary Medicine 2005; 5: 1–9.
37. Grob NM, Aytekin M, Dweik RA. Biomarkers in exhaled breath condensate: a review of collection, processing and analysis. Journal of Breath Research 2008; 2: 1752–55.
38. Guillen-Del Castillo A, Sánchez-Vidaurre S, Simeón-Aznar C, et al FRI0442 Prognostic Role of Exhaled Breath Condensate in Patients with Pulmonary Involvement Associated to Systemic Sclerosis. Annals of the Rheumatic Diseases 2015; 74: 587.
39. Heffler E, Crimi C, Brussino L et al. Exhaled breath condensate pH and cysteinyl leukotriens in patients with chronic cough secondary to acid gastroesophageal reflux. Journal of Breath Research 2017; 11: 016002.
40. Hoffmeyer F, Raulf-Heimsoth M, Harth V, Bünger J, Brüning T. Comparative analysis of selected exhaled breath biomarkers obtained with two different temperature-controlled devices. BMC Pulmonary Medicine 2009; 9: 48.
41. Horváth I, Barnes PJ, Loukides S, et al. A European Respiratory Society technical standard: exhaled biomarkers in lung disease. Eur Respir J 2017; 49(4).
42. Horváth I, Hunt J, Barnes PJ. Exhaled breath condensate: methodological recommendations and unresolved questions. The European Respiratory Journal 2005; 26: 523–48.
43. Hryniuk A, Ross BM. A preliminary investigation of exhaled breath from patients with celiac disease using selected ion flow tube mass spectrometry. Journal of Gastrointestinal and Liver Diseases 2010; 19: 15–20.
44. Huang Y, Lemberg DA, Day AS et al. Markers of inflammation in the breath in paediatric inflammatory bowel disease. Gastroenterology 2014; 59: 505–10.

45. Hunt J, Yu Y, Burns J et al. Identification of acid reflux cough using serial assays of exhaled breath condensate pH. Cough 2006; 2: 3.
46. Chen JL, Lv XD, Ma H2, Chen JR, Huang JA. Detection of cancer embryo antigen and endothelin-1 in exhaled breath condensate: A novel approach to investigate non-small cell lung cancer. Molecular and Clinical Oncology 2016; 5: 124–8.
47. Chladkova J, Krcmova I, Chladek J et al. Validation of nitrite and nitrate measurements in exhaled breath condensate. Respiration 2006; 73: 173–9.
48. Jackson TC, Zhang YV, Sime PJ, Phipps RP, Kottmann RM. Development of an accurate and sensitive method for lactate analysis in exhaled breath condensate by LC MS/MS. Journal of Chromatography B 2017; 1061–2: 468–73.
49. Karakoc GB, Inal A, Yilmaz M, Altintas DU, Kendirli SG. Exhaled breath condensate MMP-9 levels in children with bronchiectasis. Pediatric Pulmonology 2009; 44: 1010–6.
50. Kim KH, Jahan SA, Kabir E. A review of breath analysis for diagnosis of human health. Trends in Analytical Chemistry 2012; 33: 1–8.
51. Koczulla AR, Noeske S, Herr C et al. Acute and chronic effects of smoking on inflammation markers in exhaled breath condensate in current smokers. Respiration. 2010; 79: 61–7.
52. Krenke K, Peradzyńska J, Lange J et al. Inflammatory cytokines in exhaled breath condensate in children with inflammatory bowel diseases. Pediatric Pulmonology 2014; 49: 1190–5.
53. Krishnan ST, Devadhasan JP, Kim S. Recent analytical approaches to detect exhaled breath ammonia with special reference to renal patients. Analytical and Bioanalytical Chemistry 2017; 409: 21–31.
54. Kubáň P, Foret F. Exhaled breath condensate: Determination of non-volatile compounds and their potential for clinical diagnosis and monitoring. A review. Analytica Chimica Acta 2013; 805: 1–18.
55. Kullmann T, Barta I, Antus B, Horváth I. Drinking Influences Exhaled Breath Condensate Acidity. Lung 2008; 186: 263–68.
56. Kurada S, Alkhouri N, Fiocchi C, Dweik R, Rieder F. Review article: breath analysis in inflammatory bowel diseases. Alimentary Pharmacology and Therapeutics 2015; 41: 329–41.
57. Ladva CN, Golan R, GreenwaldR, et al. Metabolomic Profiles of Plasma, Exhaled Breath Condensate, and Saliva are Correlated with Potential for Air Toxics Detection. Journal of Breath Research 2017; JBR-100601.R2.
58. Lee AL, Button BM, Denehy L et al. Exhaled Breath Condensate Pepsin: Potential Noninvasive Test for Gastroesophageal Reflux in COPD and Bronchiectasis. Respiratory Care 2015; 60: 244–50.
59. Lee JS, Shin JH, Hwang J-H, Baek JE, Choi B-S. Malondialdehyde and 3-Nitrotyrosine in Exhaled Breath Condensate in Retired Elderly Coal Miners with Chronic Obstructive Pulmonary Disease. Safety and Health at Work 2014; 5: 91–6.
60. Leese E, Staff1 JF, CarolanVA, Morton J. Exhaled Breath Condensate: A Novel Matrix forBiological Monitoring to Assess Occupational Exposure to Respirable Crystalline Silica. Annals of Work Exposures and Health 2017; 61: 902–6.
61. Li Y, Chongsuvivatwong V, Geater A, Liu A. Exhaled breath condensate cytokine level as a diagnostic tool for obstructive sleep apnea syndrome. Sleep Medicine 2009; 10: 95–103.
62. Lin X, Wu Z, Fan Y et al. Correlation analysis of surfactant protein A and surfactant protein D with lung function in exhaled breath condensate from lung cancer patients with and without COPD. Molecular Medicine Reports 2017; 16: 4948–54.
63. Liu D, Luo G, Luo C et al. Changes in the Concentrations of Mediators of Inflammation and Oxidative Stress in Exhaled Breath Condensate During Liver Transplantation and Their Relations With Postoperative ARDS. Respiratory care 2015; 60: 679–88.
64. Loukides S, Horvath I, Wodehouse T, Cole PJ, Barnes PJ. Elevated Levels of Expired Breath Hydrogen Peroxide in Bronchiectasis. American Journal of Respiratory and Critical Care Medicine 1998; 158: 991–994.
65. Łuczyñska M, Szkudlarek U, Dziankowska-Bartkowiak B, Waszczykowska E, Kasielski M, Sysa-Jedrzejowska A, Nowak D. Elevated exhalation of hydrogen peroxide in patients with systemic sclerosis. Eur J Clin Invest 2003; 33: 274–279.
66. Mahairidou A, Rodopoulou S, Tomos I et al. Exhaled Breath Condensate Acidification Occurs During Surgery for Abdominal Cancer. Anticancer Research 2017; 37: 3315–21.
67. Marie-Desvergne C, Dubosson M, Touri L et al. Assessment of nanoparticles and metal exposure of airport workers using exhaled breath condensate. Journal of Breath Research 2016; 10: 036006.
68. Moloney ED, Mumby SE, Gajdocsi R et al. Exhaled breath condensate detects markers of pulmonary inflammation after cardiothoracic surgery. American Journal of Respiratory and Critical Care Medicine 2004; 169: 64–69.
69. Mosquera-Restrepo SF, Caro AC, García LF, Peláez-Jaramillo CA, Rojas M. Fatty acid derivative, chemokine, and cytokine profiles in exhaled breath condensates can differentiate adult and children paucibacillary tuberculosis patients. Journal of Breath Research 2017; 11: 016003.
70. Mutlu GM, Garey KW, Robbins RA et al. Collection and Analysis of Exhaled Breath Condensate in Humans. American Journal of Respiratory and Critical Care Medicine 2001; 164: 731–7.
71. Niimi A, Nguyen LT, Usmani O, Mann B, Chung KF. Reduced pH and chloride levels in exhaled breath condensate of patients with chronic cough. Thorax 2004 Jul; 59: 608–12.
72. Ojoo JC, Mulrennan SA, Kastelik JA, Morice AH, Redington AE. Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis. Thorax 2005; 60: 22–6.
73. Pauling L, Robinson AB, Teranishi R, Cary P. Quantitative Analysis of Urine Vapor and Breath by Gas-Liquid Partition Chromatography. Proceedings of the National Academy of Sciences 1971; 68: 2374–6.
74. Peel AM, Crossman-Barnes CJ, Tang J et al. Biomarkers in adult asthma: a systematic review of 8-isoprostane in exhaled breath condensate. Journal of Breath Research 2017; 11: 016011.
75. Pelclova D, Barosova H, Kukutschova J et al. Raman microspectroscopy of exhaled breath condensate and urine in workers exposed to fine and nano TiO2 particles: a cross-sectional study. Journal of Breath Research 2015; 9: 036008.
76. Pelclová D, Fenclová Z, Kacer P et al. Increased 8-isoprostane, a marker of oxidative stress in exhaled breath condensate in subjects with asbestos exposure. Industrial Health 2008; 46: 484–9.
77. Pelclová D, Fenclová Z, Kačer P et al. 8-isoprostane and Leukotrienes in Exhaled Breath Condensate in Czech Subjects with Silicosis. Industrial Health 2007; 45: 766–74.
78. Pelclova P, Zdimal V, Kacer P et al. Oxidative stress markers are elevated in exhaled breath condensate of workers exposed to nanoparticles during iron oxide pigment production. Journal of Breath Research 2016; 10: 016004.
79. Pleil JD. Breath biomarkers in toxicology. Archives of Toxicology 2016; 90: 2669–82.
80. Radauceanu A, Grzebyk M, Edmé JL et al. Effects of occupational exposure to poorly soluble forms of beryllium on biomarkers of pulmonary response in exhaled breath of workers in machining industries. Toxicology Letters 20016; 263: 26–33.
81. Reder NP, Davis CS, Kovacs EJ, Fisichella PM. The diagnostic value of gastroesophageal reflux disease (GERD) symptoms and detection of pepsin and bile acids in bronchoalveolar lavage fluid and exhaled breath condensate for identifying lung transplantation patients with GERD-induced aspiration. Surgical Endoscopy 2014; 28: 1794–800.
82. Rindlisbacher R, Strebel C, Guler S et al. Exhaled breath condensate as a potential biomarker tool for idiopathic pulmonary fibrosis – a pilot study. Journal of Breath Research 2017; 12(1): 016003.
83. Robroeks CMHHT, van de Kant KDG, Jöbsis Q et al. Exhaled nitric oxide and biomarkers in exhaled breath condensate indicate the presence, severity and control of childhood asthma. Clinical and Experimental Allergy 2007; 37: 1303–11.
84. Rolla G, Fusaro E, Nicola S, Bucca C, Peroni C, Parisi S, Cassinis MC, Ferraris A, Angelino F, Heffler E, Boita M, Brussino L. Th-17 cytokines and interstitial lung involvement in systemic sclerosis. J Breath Res 2016; 10: 046013.
85. Romero PV, Rodríguez B, Martínez S et al. Analysis of Oxidative Stress in Exhaled Breath Condensate From Patients With Severe Pulmonary Infections. Archivos De Bronconeumologia 2006; 42: 113–9.
86. Rosias PP, Robroeks CM, Kesker A et al. Biomarker reproducibility in exhaled breath condensate collected with different condensers. The European Respiratory Journal 2008; 31: 934–42.
87. Sack U, Scheibe R, Wotzel M et al. Multiplex Analysis of Cytokines in Exhaled Breath Condensate. International Society for Analytical Cytology 2006; 69A: 169–72.
88. Shahid SK, Kharitonov SA, Wilson NM, Bush A, Barnes PJ. Increased Interleukin-4 and Decreased Interferon-γ in Exhaled Breath Condensate of Children with Asthma. American Journal of Respiratory and Critical Care Medicine 2002; 165: 1290–93.
89. Schwarz EI, Engler A, Kohler M. Exhaled breath analysis in obstructive sleep apnea. Journal Expert Review of Respiratory Medicine 2017; 11: 631–9.
90. Soyer OU, Dizdar EA, Keskin O, Lilly C, Kalayci O. Comparison of two methods for exhaled breath condensate collection. Allergy 2006; 61: 1016–18.
91. Soyer T, Soyer OU, Birben E et al. Pepsin levels and oxidative stress markers in exhaled breath condensate of patients with gastroesophageal reflux disease. Journal of Pediatric Surgery 2013; 48: 2247–50.

92. Syslová K, Kačer P, Kuzma M et al. Determination of 8-iso-prostaglandin F2 in exhaled breath condensate using combination of immunoseparation and LC–ESI-MS/MS. Journal of Chromatography B 2008; 867: 8–14.
93. Tufvesson E, Bozovic G, Hesselstrand R et al. Increased cysteinyl-leukotrienes and 8-isoprostane in exhaled breath condensate from systemic sclerosis patients. Rheumatology 2010; 49: 2322–6.
94. Urs R, Simpson S, Pillow J, Hall H, Clarke M. Exhaled breath condensate: Measuring inflammation and oxidative stress in preterm infants. European Respiratory Journal 2016; 48: OA243.
95. Vass G, Huszár E, Barát E et al. Comparison of Nasal and Oral Inhalation during Exhaled Breath Condensate Collection. American Journal of Respiratory and Critical Care 2003; 167: 850–5.
96. Vaughan J, Ngamtrakulpanit L, Pajewski TN et al. Exhaled breath condensate pH is a robust and reproducible assay of airway acidity. The European Respiratory Journal 2003; 22: 889–94.
97. Wheatley CM, Baker SE, Morgan MA et al. Moderate intensity exercise mediates comparable increases in exhaled chloride as albuterol in individuals with cystic fibrosis. Respiratory Medicine 2015; 109: 1001–11.
98. Whitehouse A, Brugha R, Mushtaq N, Dundas I, Grigg J. S64 Eosinophil Cationic Protein And Cytokine Analysis In Exhaled Breath Condensate In Paediatric Asthma. Thorax 2014; 69: A36.
99. Winters BR, Pleil JD, Angrish MM et al. Standardization of the collection of exhaled breath condensate and exhaled breath aerosol using a feedback regulated sampling device. Journal of Breath Research 2017; 11(4): 047107.
100. Zanconato S, Carraro S, Corradi M et al. Leukotrienes and 8-isoprostane in exhaled breath condensate of children with stable and unstable asthma. Journal of Allergy and Clinical Immunology 2004; 113(2): 63.
101. Zang X, Pérez JJ, Jones CM et al. Comparison of Ambient and Atmospheric Pressure Ion Sources for Cystic Fibrosis Exhaled Breath Condensate Ion Mobility-Mass Spectrometry Metabolomics. American Society for Mass Spectrometry 2017; 28: 1489–96
102. Rosias PPR, Dompeling E, Hendriks HJE et al. Exhaled breath condensate in children: Pearls and pitfalls. Pediatr Allergy Immunol 2004; 15: 4–19.