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Review

A Review of the Usefulness of Non-invasive Exhaled Breath Condensate pH Analysis for Diseases Diagnosis with Elements of Meta-analysis: An Update from 2012

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Abstract: Exhaled breath condensate (EBC) sample analysis is an entirely non-invasive novel sample collection method that is fast, easy to perform, and effort-appeared independent. EBC samples can be very useful in identifying the biomarkers of many diseases. This review provides an updated overview of EBC pH disturbances in different disorders as well as physiological levels among healthy individuals since 2012. Our meta-analysis addresses some of the key questions related to sample processing before pH measurement and discusses various methods of condensate standardization that can be employed prior to conducting a pH assay. Given the recent widespread interest in research into the use of EBC to identify biomarkers, it is necessary to establish a pathway leading from analytical methods for biomarker evaluation using EBC pH to clinical applications of this technology. This review fills a gap in the literature and attempts to connect theory to practical analytical approaches to analyzing EBC samples and making critical treatment-related decisions next to the patient's bed.

Keywords: Exhaled Breath Condensate (EBC) Samples; Noninvasive Colleting Method; Biomarker, Diseases, pH, acidification

1. Introduction

Exhaled breath condensate (EBC) is a new type of biological sample that is a source of numerous biomarkers, which may potentially be useful in helping clinicians diagnose various illnesses. Although respiratory disorders alter the chemical compounds in EBC, which makes it possible to detect them, these compounds can also be affected by systemic diseases. Therefore, EBC can also be used to detect both respiratory and systemic diseases. EBC sample collection is an entirely non-invasive and highly simple process that can be performed on spontaneously breathing patients, as well as on those who are being assisted by mechanical ventilators.

EBC sample analysis has gained much attention in recent years, with many studies showing its numerous possible clinical applications. Specifically, EBC sample analysis can be useful for diagnosing a number of conditions, as these conditions cause chemical changes within EBC. Some examples of such conditions include: asthma, chronic obstructive pulmonary disease (COPD), lung cancer, mechanical ventilation, idiopathic pulmonary fibrosis (IPF), cystic fibrosis, pulmonary arterial

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hypertension (PAH), obstructive sleep apnea (OSA), sarcoidosis, systemic lupus erythematosus (SLE), and chronic renal disease (CRD). In addition, EBC may also be useful in pharmacokinetics [1].

Since many conditions can be detected via disturbances in the pH of EBC samples, this simple test could have numerous potential clinical applications. Given this potential, we have compiled a summary of previous studies that examine EBC pH analysis in order to provide researchers with a useful tool that can assist them in introducing this analytical approach into their everyday clinical practice.

There are a few new reviews of EBC analysis [1-4] in which the authors discuss the methodological problems associated with EBC pH analysis, as well as its possible clinical applications for different compounds. Furthermore, the number of trials that have identified new biomarkers and levels of known markers in different conditions has been increasing rapidly. Indeed, it seems to be impossible to adequately detail all of these articles in one paper. Changes in concentrations of non-volatile as well as volatile compounds in EBC are properly described but we find a gap as far as pH disturbances are taken under consideration. Finally, we have elected to examine EBC acidification, as it seems simple to measure in principle, but is much harder to quantify in practice (especially if it may serve as clinical tool).

2. Meta-analysis

2.1 Methods

In order to estimate EBC pH values and examine the possible influence particle factors, we used the data in the below-cited articles (and a few more [5-7]) to perform a simple meta-analysis of the average EBC pH values in healthy controls. In total, 47 articles were taken into consideration. Studies with incomplete data (e.g. lack of de-aeration time, lack of collection device name) or results that were not presented using mean ± SD (e.g. media ± IQR) were excluded from the statistics. Once these articles had been eliminated, we were left with 27 articles (Figure 1) and a total sample of 472 individuals for analysis (Table 1). We conducted our analysis using Statistica 2013 software. In order to properly strenghten data obtained from trials with greater study group we decided to introduce such parameter as number of participants. For this reason, the weights of the examples were used in all statistics. As a weight for the data number of individuals in each particular trial were set. As such, our results are weighted means and SD.

The analysis contains comparisons (performed with t student test) of the mean EBC pH levels between trials differing in parameters, such as: gas-standardization technique (CO_2-loading vs. de-aeration with CO_2-free gas); type of CO_2-free gas (argon vs. nitrogen); type of collection device (R-Tube vs. EcoScreen); time of de-aeration (10 – 15 minutes—which is acknowledged by many authors to be enough for equilibrium—and 20 minutes); and the correlation measured between the mean pH of the samples and the time required for the de-aeration process.

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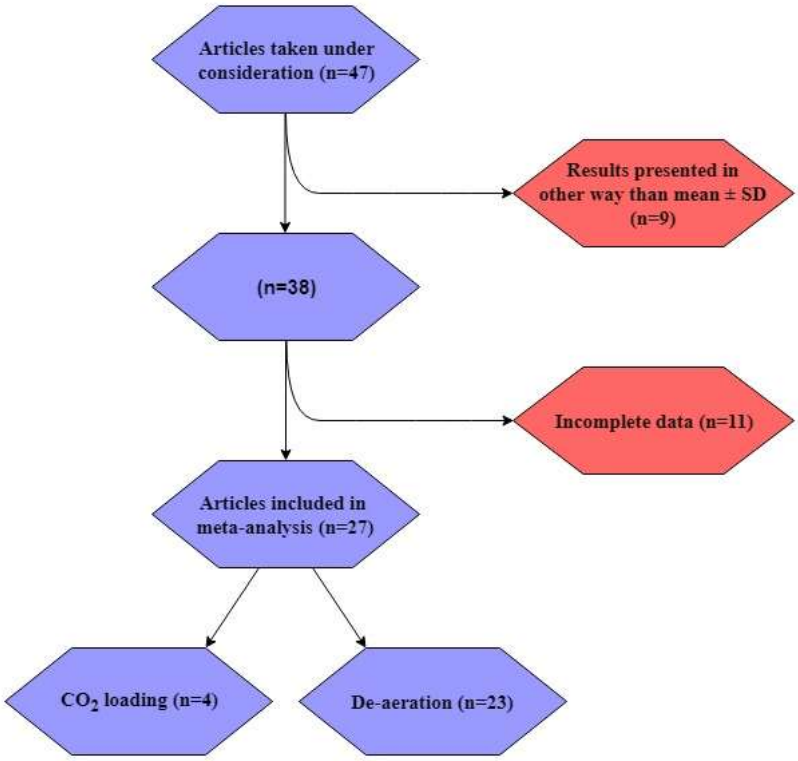

Figure 1. Selection of articles included in meta-analysis

Table 1. Articles included in meta-analysis.

	Mean EBC pH	Number of participants (weight in the statistics)	Gas standardisation method	Time of de-aeration [minutes]	Collection device
[55]	6.39 6.02	28	CO_2 – loading	- - - - -	ES RT
[56]	6.63	20	CO_2 – loading	- - - - -	ES
[8]	6.54 6.19	12	CO_2 – loading	- - - - - - - - - -	ES RT
[88]	6.45 6.10	12	CO_2 – loading	- - - - - - - - - -	ES AN
[68]	7.50	20	Argon de-aeration	0,33	O
[54]	7.61	12	Argon de-aeration	5	ES
[87]	8.20	15	Nitrogen de-aeration	6	RT
[50]	7.22	35	Argon de-aeration	8	RT
[26]	7.85	15	Argon de-aeration	10	ES
[31]	7.50	26	Nitrogen de-aeration	10	ES
[24]	7.40	20	Argon de-aeration	10	TD
[30]	8.12	28	Argon de-aeration	10	TD
[8]	8.04	12	Argon de-aeration	10	ES

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[20]	7.77	10	Argon de-aeration	10	ES
[21]	7.99	10	Argon de-aeration	10	ES
[37]	7.98	20	Argon de-aeration	10	ES
[29]	7.58	19	Argon de-aeration	10	ES
[19]	7.75	23	Argon de-aeration	10	RT
[35]	7.88	16	Argon de-aeration	10	RT
[5]	7.85	15	Argon de-aeration	10	ES
[72]	7.46	12	Argon de-aeration	10	ES
[7]	8.26	16	Argon de-aeration	10	ES
[39]	7.65	19	Argon de-aeration	10	O
[41]	7.57	10	Argon de-aeration	10	O
[83]	8.16	32	Argon de-aeration	15	O
[14]	8.27	5	Argon de-aeration	20	RT
[6]	8.38 8.41	10	Argon de-aeration	20	RT ES

RT (R-tube), ES (EcoScreen), AN (Anacon), TD (TURBO DECCS), O (other; e.g. glass tubes surrounded with wet ice, dry ice or liquid Nitrogen).

2.2 Results

First, we compared the EBC pH values calculated after CO_2 loading (as described by Kullmann et al [8]) with those obtained after de-aeration with CO_2-free gas (argon or nitrogen). Since CO2 loading significantly decreased pH results (6.45 ± 0.15 vs. 7.78 ± 0.32, $p<0.001$, total participants number of included articles: 72 vs. 400), further analysis was performed on the results obtained after CO_2-free gas standardization (n=400). The resultant calculations showed that the sample collection device and the type of gas used for standardization had no effect on average EBC pH (Table 2). However, it was found that de-aeration time and EBC pH are statistically significantly (weighted) correlated ($r=0.50$, $p<0.05$) (Figure 2). The mean pH values obtained after 20 minutes of de-aeration were significantly higher than those obtained after 10 – 15 minutes of gas standardization (8.34 ± 0.05 v.s 7.83 ± 0.26, $p<0.001$) (Table 2).

Table 2. Results of meta-analysis

Compared parameters		N	EBC pH of healthy individuals Weighted means ± SD	p
Gas standardisation method*	CO_2 loading	72	6.45 ± 0.15	<0.001
	De-aeration with CO_2 – free gas	400	7.78 ± 0.32	
CO_2 – free gas used for de-aeration	Argon	359	7.78 ± 0.32	0.55
	Nitrogen	41	7.76 ± 0.34	
Collection device[1]	R-tube	104	7.74 ± 0.42	0.37
	EcoScreen	157	7.78 ± 0.25	
De-aeration time*	10 – 15 minutes	303	7.83 ± 0.26	<0.001
	20 minutes	15	8.34 ± 0.05	

*statistically significant differences

[1] Two of most common devices were compered. Rest of authors used other devices or glass tubes with different kinds of cooling (wet ice, dry ice, liquid Nitrogen).

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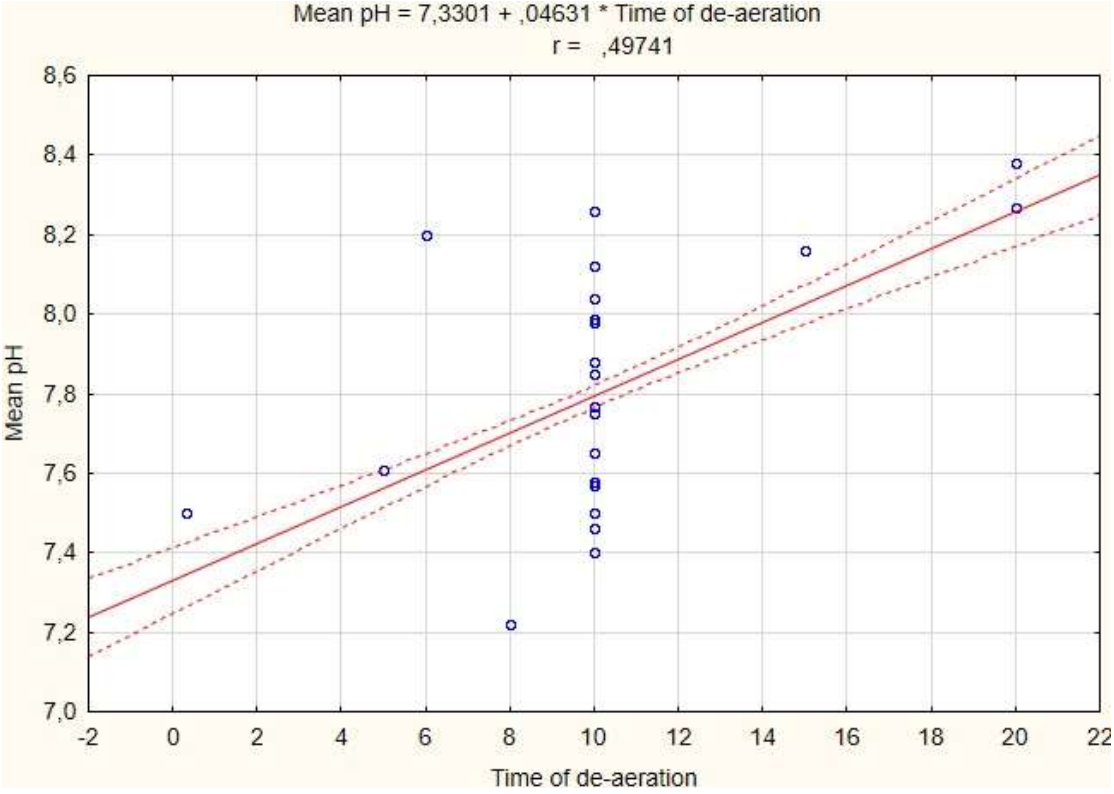

Figure 2. Correlation between mean EBC pH values obtained from analyzed articles and time of de-aeration (minutes).

3. Sample issues

3.1 CO_2 issue

Carbon dioxide represents one of the most important problems in pH analysis. The level of CO_2 in EBC is highly important because it is a precursor of carbonic acid. As such, there is a critical need to reduce the impact of CO_2 on EBC pH, as the acidic pH of EBC containing CO_2 does not contribute to the real acidification of airways. Fortunately, the effects of CO_2 can be reduced via gas standardization, which is particularly necessary for samples that are not immediately analyzed following collection. There are 2 methods of gas standardization: CO_2-free gas (e.g. argon) de-aeration and CO_2 – loading. The main problem with CO_2-free gas standardization is that such gasses, which shouldn't contain carbon dioxide, are not really CO_2 free. Since the gas being used to standardize the EBC is contaminated by an unknown amount of CO_2, the results of pH assays using this method tend to have poor reproducibility and may differ between laboratories [9-12]. One potential solution to this issue is by carbon dioxide loading the EBC samples and performing further statistical analysis, as this can help ensure better inter-laboratory comparison and repeatability [8]. While there are a number of CO_2-free gas standardization techniques, argon-gas standardization is the most prevalent. Using this technique, the median pCO_2 can be reduced from 4.08 kPa to 0.36 kPa and the median pH can be increased from 6.15 to 7.40 with 15 minutes of bubbling. EBC pH and pCO_2 have a statistically significant correlation ($r=-0.72$, $p<0.0001$) in neat samples, which is eroded by the argon de-aeration process ($r=0.26$, $p=0.11$). This proves that carbon dioxide considerably influences EBC acidification. After CO_2 loading and calculating EBC pH values at pCO_2 =5.33 kPa, the median pH was 1.3 lower than it was with argon standardization. Despite this, there is a strong positive correlation between the respective pH results following argon and CO_2 standardization ($r=0.88$, $p<0.0001$) [13].

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When selecting a gas-standardization technique, it is important to consider all relevant factors. There are 2 CO_2-free gas standardization methods. The first method, bubble degassing, enables shorter standardization times (usually 60 seconds is enough according to Lin et al.), but it also results in higher EBC volume loss compared to surface degassing. In contrast, the second method, surface de-aeraetion, requires 300 seconds in order to reach pH equilibrium. Bubble gas standardization's shorter standardization times and its use of an open container help to reduce the risk of contamination and the degradation of unstable compounds in the EBC sample [12]. In opposition to these results, Koczulla et al. suggest that at least 20 minutes of de-aeration is required in order to stabilize EBC pH levels [14], which was confirmed by the results of our simple meta-analysis; the mean results obtained after 10 – 15 minutes of de-aeration (which is acknowledged by most of authors as sufficient to achieve equilibrium) are significantly lower than those obtained after 20 minutes of de-aeration (Table 2.). Furthermore, it is also possible to expose the sample to atmospheric air for 2 hours. However, while this approach successfully produces CO_2 equilibrium between the EBC sample and the air, it can also cause the degradation of some of the unstable compounds. This drawback notwithstanding, this method offers one great advantage: no sample volume loss [12].

The European Respiratory Society provides the following recommendations for EBC pH measurements: minimizing storage time and measuring pH before and after sample processing or performing pH measurements by calculating its value at CO_2 level of 5.33 kPa after carbon dioxide loading [15] as introduced by Kullmann et al [8].

3.2. Ammonia issue

Ammonia is volatile basic compound that is produced by the bacteria in the upper airways. When present in high concentrations, ammonia can inhibit the ability to measure acidity in a subject's airways. This problem tends to be more relevant in oral EBC collection due to the large amounts of bacteria in the oral cavity. However, this issue may also be somewhat beneficial, as it can prevent pH assays from being overly sensitive in some cases. While low NH_3 levels are often observed in populations with higher EBC acidity, this is not a necessary condition. People with normal EBC pH can have low concentrations of ammonia as well [10].

3.3 Thawing issue

Since some volatile acids can undergo sublimation during thawing, it is recommended that samples be thawed in a closed container, which should be shaken before opening [10].

3.4 Device issue

According to most authors, the type of commercially available collection device (EcoScreen vs. R-tube) does not influence EBC pH [16]. This assertion was confirmed by our meta-analysis (Table 2). PET devices do not affect exhaled breath condensate acidification, while the use of glass devices generally results in lower pH values [16]. Collection based on the exhaled CO_2 signal (the collection process starts when a signal increase of 50% or more is detected) results in lower EBC pH values than are recorded with traditional collection approaches ($p=0.011$) [17].

4. Physiological pH of EBC

The physiological pH of gas-standardized EBC in most studies is between 7.5 and 8.1. Values below 7.4 should be considered abnormal, as they are only found in 6% of population. Such values may occur due to conditions such as viral infections and proximal gastroesophageal reflux (GERD), which may be asymptomatic [10].

It is important to note that EBC pH is not affected by the following factors: age (except very elderly); gender; time of day; volume collected; duration and temperature of collection; acute

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albuterol/salbutamol usage; methacholine induction of airway obstruction; and hypoventilation and hyperventilation [10, 16, 18]. Although gender does not influence EBC pH, it is noteworthy that pregnant women have higher pH values than non-pregnant individuals [19]. In addition, obesity does not influence EBC pH unless it is connected with obstructive sleep apnea [20], [21]. Furthermore, storage conditions such as time and temperature do not change the pH of the sample [10]; indeed, EBC results remain stable even after 6 months of storage [18]. With respect to race, it has been found that African Americans tend to exhibit lower pH more often than other groups. Moreover, pH can be altered due to alcohol intake, recent smoking, and the oral ingestion of food or fluids. As such, it is recommended that patients abstain from smoking, drinking, or eating for 30 minutes before EBC collection [10], [16]. Finally, since EBC pH can be reduced by the common cold [14], many authors elect to exclude patients who have been infected by this virus within the weeks leading up to the study.

As can be seen in Table 1, the range of "normal" EBC pH levels cited in the reviewed studies is very large (2.39 of difference between the highest and the lowest result). These differences are attributable to the different techniques that were used to measure EBC, as well as the de-aeration times that were used. Consequently, it is very difficult to form strong comparisons of the pH values between the control groups in the various studies.

5. EBC pH as a biomarker of clinical conditions

5.1 Allergic rhinitis and atopic dermatitis

Brunetti et al. conducted a study, which showed that atopic rhinitis leads to lower EBC pH (7.48 vs. 7.78; $p<0.005$). In this study, the authors obtained their measurements following de-aeration with argon [22]. In a different work, De Prins et al. analyzed EBC pH in allergic populations, finding that asthma, allergic rhinitis, and nasal sIgE do not influence pH levels in EBC. However, the authors of this study do not specify which gas-standardization procedure was used, if one was used at all. This is a significant point, as their results can be unreliable if gas standardization was not performed [23].

Atopic dermatitis (AD) is another condition that can cause the acidification of exhaled breath condensate. Further results have demonstrated that populations with AD exhibit statistically significant ($p<0.05$) lower levels of pH: 7.44 vs. 7.78 [22], 7.00 vs. 7.40 [24], 8.02 vs. 8.11 [25]. Changes in airways pH are often precursors to respiratory symptoms. More trials are required to assess whether EBC pH can serve as a biomarker of respiratory disorders connected with allergies (such as asthma and rhinitis) [25].

5.2 Asthma

There are numerous works describing the possible role of EBC pH in the diagnostic and therapeutic processes in asthma patients. The following results suggest that EBC acidification may serve as a biomarker of the disease (asthma group vs. control): 7.42 vs. 7.85 (pediatric patients, $p<0.0001$) [26], 7.14 vs. 7.35 ($p<0.05$) [27], 7.53 vs. 7.85 ($p<0.001$) [28], 7.28 vs. 7.59 ($p=0.04$) [29], 7.87 vs. 8.12 ($p=0.03$) [30], 7.3 vs. 7.5 ($p<0.05$) [31], 6.49 vs. 6.64 (not de-aereted, $p<0.05$) [32], [33]. However, other results do not reveal significant differences between stable asthma and control populations ($p=0.06$) [34], (7.54 vs. 7.75, $p=0.12$) [19], (7.96 vs. 7.88, $p=0.23$) [35], (7.94 vs. 7.90, $p=0.8$ [36]), [33]. Even if significant differences in EBC pH between stable asthmatic and control groups are controversial, almost all authors agree that exacerbation and poor control of the disease leads to a decrease in pH values. Moderate, severe, or badly controlled asthma is connected with lower EBC pH values than cases that are mild or well-controlled (7.36 vs. 7.49, $p<0.005$) [26], (7.54 vs. 7.73, $p<0.05$) [37], (7.33 vs. 7.23, $p=0.0028$) [38], (5.23 vs. 7.8) [39-41]; furthermore, there is no correlation between pH and FEV_1

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or FVC in adult [19, 27, 29] or pediatric population [26, 30, 32]. The acidity of the sample is normalized when the patient's exacerbation period is properly treated [42]. In addition, EBC pH measurement can be used in conjunction with the specific inhalation challenge (SIC) to distinguish between work-exacerbated asthma (WEA) and occupational asthma (OA). A decrease in EBC pH after SIC of greater than 0.4 is connected with WEA, with a specificity of 100% and a sensitivity of 79% [43]. Conversely, an increase in EBC pH between 'at work' and 'off work' periods can help diagnose OA. Changes are greater in the SIC(+) than in the SIC(-) group (Δ mean pH: 0.59 vs. 0.17) [44]. There are no statistically significant differences in EBC acidification between GERD (+) and (-) groups in pediatric asthma populations (7.10 vs. 7.05, p=0.51) [34]. However, EBC pH cannot serve as a distinguishing factor between eosiniphilic and neutrophilic asthma (7.49 vs. 7.57, $p<0.05$) [37]. EBC pH correlates positively with ammonia levels in the sample and negatively with nitrite/nitrate, FeNO, and enosinophilia among patients with both stable and unstable forms of the asthma. Such correlations do not appear in healthy volunteers [29, 41].

Furthermore, in a group of patients treated with ICS, those with stable asthma showed higher EBC pH than those whose asthma was unstable (7.32 vs. 7.10, p=0.04) [29]. While the treatment of exacerbated asthma can lead to an increase in EBC pH, the differences are not statistically significant (7.87 vs. 8.11, p=0.12) [30].

Changes in EBC pH influence the effects of asthma treatments. Acidosis and alkylosis of the airways lead to decreased airway blood flow response to inhaled albuterol (ΔQaw); thus, under such conditions, the effect of B_2-adrenergic agonists is worse than in the case of normal pH [45]. Unlike COPD patients, asthma patients show significant improvements in EBC pH following ICS treatments [28], [40], [41], [46].

Asthmatic pregnant women also have lower EBC pH than healthy pregnant controls (7.65 vs. 8.02, p=0.006), and these results are significantly correlated with neonatal birthweight (r=0.49, p=0.047) [19].

Exercise-induced bronchoconstriction (EIB) episodes among asthmatic populations are associated lowered EBC pH, as asthma patients and healthy controls who do not experience EIB show no significant changes in EBC acidity [35]. Tobacco smoke exposure in populations of asthmatic children who are using ICS does not change airway acidification in comparison to an analogous group without such exposure [47].

EBC analysis can also identify biomarkers for special sub-group of asthma patients: individuals with an EBC pH of lower than 6.5 significantly differ from those with pH values higher than 6.5. Individuals with an EBC pH of less than 6.5 have lower FeNO levels ($p<0.0001$) and exhibit decreased provocative concentrations of methacholine, which produces a 20% decline in FEV1 (PC20) (p=0.03). Patients with very low pH levels generally show allergy symptoms in spring and winter more often than others [36, 48].

5.3 COPD

Patients suffering from an exacerbation form of this disease have lower EBC pH compared to control (5.58 vs. 6.07, $p<0.05$) and stable COPD (5.58 vs. 5.97 $p<0.05$) [49] populations. Some studies have found that these differences do not persist when control and stable COPD populations are compared (5.97 vs. 6.07) [49] [50]; in contrast, other studies have found statistically significant differences between these populations, thus suggesting EBC pH's possible role as a biomarker of stable COPD [51], (p=0.0008) [52], (7.1 vs. 7.4, $p<0.01$)[53], (6.87 vs. 7.35, $p<0.01$)[27], (7.16 vs. 7.57, $p<0.0001$) [41], (6.97 vs. 7.61, p=0.03) [54]. Given these conflicting results, further trials are needed. In addition, ICS (inhaled corticosteroid) treatments were found to have no significant effect on EBC

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acidification in COPD patients [51], [41], [54]. The VC % of pred. and the EBC pH reveal a statistically significant negative correlation (r=-0.46, p=0.007), while other respiratory parameters (FEV_1 % of pred., RV % of pred., DLco % of pred., LAV%) do not correlate with pH [27, 54].

5.4 Cystic fibrosis

Cystic fibrosis (CF) is a genetic disease that mainly affects the pancreas and lungs. Antus et al. analyzed EBC pH in a CF population using CO_2 bubbling gas standardization [8]. Their results showed no statistical differences between the CF group and the control group; that is, there is no difference in EBC acidity between people who do and do not have pseudomonas aeruginosa colonistaion. In addition, the authors further found that steroid treatment (ICS) did not influence pH. As their results showed, EBC pH is dependent on the condenser type (e.g. in CF patients – EcoScreen: 6.38 vs. R-tube: 5.94; $p<0.05$) and it is not correlated with factors such as FeNO, FVC, FEV_1, and total nor differential cell counts in sputum [55].

In contrast, Carpagnano et al. discovered statistically significant differences ($p<0.05$) between their CF group and control group. In particular, they found that EBC pH was lower among patients whose condition was exacerbated: 7.85 (control) vs. 7.31 (stable CF) vs. 7.12 (exacerbation). These results were obtained via gas standardization with argon bubbling [26]. The differences between these trials suggest the necessity for further analyses of CF populations.

5.5 Lung cancer

Lung cancer, which is one of the most common neoplasms among adult populations, does not influence EBC pH. Additionally, a subgroup of these patients who also had COPD was also examined. The results of this study indicated that this particular neoplastic process also did not affect pH. Furthermore, no statistical differences in EBC pH were observed between patients with different stages of tumor growth [56, 57]. However, two publications have provided conflicting reports on the impact of tumor histology. In the first study, Bikov et al. claimed that squamus cell carcinoma (SCC) causes lower EBC pH than adenocarcinoma (7.09 vs. 7.80; $p<0.01$) [57]. In contrast, the authors of the second work argue that tumor histology has no impact EBC pH [56]. Despite this, endobronchial localization of the tumor resulted in greater EBC acidity than the endobronchial-negative group (7.29 vs. 7.76; $p=0.04$). Moreover, gastroesophageal reflux disease (GERD) was found to also lower EBC pH, especially if left untreated. Nevertheless, EBC pH levels are not a predictive factor for survival in lung cancer populations. It should also be noted that other diseases, such as chronic heart failure, diabetes, and chronic renal failure, do not influence EBC pH [57].

5.6 Tuberculosis

The acidification of exhaled breath is a feature of pulmonary tuberculosis (TBC). As some results have revealed, TBC patients have a higher EBC pH when compared to a control group of individuals who do not have any pulmonary diseases (6.93 vs. 7.88, $p<0.001$). In addition, there is a negative correlation between pH and smear positivity level ($r=-0.514$, $p<0.02$), while no correlation appears between EBC acidification and radiological extent ($p=0.795$) [58].

5.7 Obstructive sleep apnea

Obstructive sleep apnea (OSA) is connected with inflammation and oxidative stress brought about by EBC pH. The following results clearly demonstrate that OSA populations experience higher EBC acidity (lower pH) than a control group: 7.20 vs. 7.77 ($p<0.01$) [20], 7.48 vs. 7.99 ($p<0.01$) [21], 7.44 vs. 7.64 ($p<0.01$) [59]. There is a negative correlation between EBC pH and factors such as AHI (apnea-hipopnea index), TST SaO_2 < 90%, BMI, and neck circumference [21]. These correlations suggest the presence of more advanced inflammatory processes in patients whose conditions are more severe

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[60]. Additionally, there are no statistically significant differences between the EBC pHs of healthy individuals and obese population without OSA [20, 21]. Similarly, subgroups of OSA smokers and non-smokers exhibit similar EBC pH levels (7.43 vs. 7.45, p=0.7) [59]. Data on the impact of therapy are conflicting. Some suggest that CPAP plays a positive role [20] in the normalization of EBC pH, while others claim the opposite [61].

5.8 Chronic hypersensitivity pneumonitis

Ojanguren et al. proved that EBC pH measurements can be useful for diagnosing chronic hypersensitivity pneumonitis (HP). To this end, they had a group of patients suspected of suffering from HP perform a specific inhalation challenge (SIC). In the case of exposure to molds, those in the HP population showed a significant reduction in EBC pH in comparison to populations with other pulmonary diagnoses (e.g. nonspecific interstitial pneumonia, sarcoidosis, bronchiectasis, and pulmonary fibrosis). Statistical analysis showed that a reduction in EBC pH of 0.3 units is connected with 100% specificity, but with only 30% sensitivity. In the case of exposure to avian antigens, there were no statistically significant differences in pH changes between the HP populations and the population of patients with other diagnoses. Furthermore, the authors also suggested that EBC pH can be used to reduce the number of false negative results on SIC tests [62]. Further analysis will be necessary in order to fully assess the particular clinical implications of these results. A trial comparing the HP group with a healthy population will be necessary to determine whether EBC pH can serve as a biomarker of HP irrespectively of SIC.

5.9 Interstitial lung disease due to systemic sclerosis

Systemic sclerosis is a connective tissue disease that leads to interstitial lung disease due to the excessive production of collagen. Patients with ground glass opacities in the HRCT (radiology sign) have significantly lower EBC pH than people in control groups (7.4 vs. 8.0, p=0.02) [63]. Acidic ECB measurements can serve as a prognostic factor in identifying this disease. Castillo et al. conducted a study that demonstrated the correlation between lower EBC pH values and lower future DLCO % (diffusing capacity of the lung for carbon monoxide; 4 years follow-up, r=0.45, p=0.01). ROC curve analysis confirms that the chances of progression-free survival improve considerably above the pH=7.88 threshold, with a sensitivity of 0.71 and a specificity of 0.58 [63].

5.10 Pulmonary fibrosis

Pulmonary fibrosis (PF), which is a type of interstitial pneumonia that is connected with high collagen deposits, also leads to significant changes in EBC. Among many other biomarkers (8-iso, 3-NT, total protein, eCO, FeNO) EBC pH is also able to differentiate between PF and control populations (7.6 vs. 7.4, p=0.004). This parameter correlates with EBC concentrations of H_2O_2 (r=0.3, p=0.05) and 8-isoprostane (r=0.03, p=0.04), while there is no correlation with lung function factors [64]. PF is the only disease that leads to an increase in EBC pH, which makes this parameter more condition-specific than in cases where pH is lowered. Unfortunately, the samples in the above study were not de-aerated in any way, so the obtained results may not be reliable. Moreover, the cohort only consisted of 20 patients, which is somewhat of a small sample. Therefore, it would be worthwhile to perform a study that utilized a larger number of people and that also de-aerated the obtained samples.

5.11 Gastroesophageal reflux disease

Gastroesophageal reflux disease is a condition wherein gastric acid rises into the esophagus; this can lead to inflammation and acidification of the airways, which is observed as low EBC pH levels [65], (6.34 vs. 7.22, p=0.03) [50]. Heffler et al. have demonstrated that EBC pH can be used in

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differencial diagnosis of chronic cough when all other causes have been excluded and GERD is suspected. Patients with a chronic cough due to GERD tend to have lower EBC pH levels than individuals who have a chronic cough, but no reflux (7.56 vs. 7.88, p=0.028) [66]. EBC pH can also serve as a biomarker of GERD among lung cancer patients (7.09 vs. 7.73, p=0.04) [57] but not among asthmatic patients [31, 34, 67]. In the case of chronic obstructive lung disease, there are conflicting data. One group of studies suggest that there are significant differences between GERD (+) and GERD (-) groups of patients suffering from COPD [50], while a separate group of studies have yielded contradictory results [67].

PPI treatment results in the normalization of EBC pH in patients with QUEST values no lower than 4 [31].

5.12 Inflammatory Bowel Disease

There are 2 main subtypes of inflammatory bowel disease (IBD): Crohn's disease (CD) and ulceritive colitis. The mean EBC pH of pediatric CD populations is significantly lower than in the control group (6.59 vs. 7.5; $p<0.05$). In addition, there is a significant correlation between PCDAI (Pediatric Crohn's Disease Activity Index) score and EBC pH (P=0.077). There is no correlation between acidification of EBC and ESR (P=0.89), nor between EBC and CRP (P=0.27) [68].

5.13 Mechanical ventilation

There are no correlations between EBC pH and factors such as initial C Reactive Protein (CRP), APACHE score, PaO_2/FiO_2, Tidal Volume, PEEP, arterial pH, $PaCO_2$ or HCO_3^- in group of patients undergoing mechanical ventilation (MV) due to non-pulmonary disease [69]. The duration of MV before sample collection was found to influence EBC pH (reverse correlation, r=-0.636; p=0.048) [16, 70], with one lung ventilation resulting in higher EBC acidification than normal MV. Furthermore, no statistically significant correlation was found between EBC acidification and total MV duration in a group of patients without pulmonary causes of respiratory distress [69]. Thus, it is critical to remember that the impact of MV on EBC pH changes during the analysis of the results. Despite this fact, EBC pH cannot serve as predictive factor of MV duration before weaning, nor can it help predict the risks of VAP (Ventilator – associated pneumonia) or mortality [69]. Nannini et al. also analyzed whether spontaneously condensing EBC in the trap of an expiratory arm could replace the need to use a cooling chamber. Their results showed that only spontaneous EBC collection and further argon de-aeration is comparable to cooling chamber condensation and further argon de-aeration. Unless these sample-collection methods are combined with de-aeration, they will show statistically significant differences [69]. This means that spontaneous collection in the trap can be considered as a replacement for the cooling chamber, but that the results can only be compared only de-aeration was conducted for both collection methods. Acute lung injury (ALI) causes the acidification of airways, which leads to lower EBC pH. There is a negative correlation between pH and inflammatory cytokine levels in EBC. Increased acidity in exhaled breath condensate can be an indication of inflammatory processes in the airways hours before clinical symptoms are observed. EBC can also serve as a biomarker of ventilator-induced lung injury [71, 72], and it is possible to perform real-time EBC pH analysis on mechanically ventilated patients. This technique has been used for many diseases, including: acute asthma, cystic fibrosis, COPD, infections from respiratory syncytial virus, acute respiratory disease syndrome (ARDS), and bronchopulmonary dysplasia. Mean results higher than 7.4 are considered to represent relative lung health. In the case of patients with respiratory diseases, the mean results were approximately 4.0 – 6.0 [10].

5.14 Pollution

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Air pollution and exposure to certain substances are important factors that can influence airway acidity, and it is important to keep this in mind when analyzing EBC pH as a biomarker for various clinical conditions. Substances that have been proven to increase the level of hydrogen ions in exhaled breath—thus, lowering the pH value—include: O_3 [73, 74], SPM (suspended particulate matter) [73, 75, 76], black carbon [77], NO_2 [76], benzene [76], ethylbenzene [76], TiO_2 [78], PAH (polycyclic aromatic hydrocarbons) [79], wood dust [80], wood smoke [81], and cigarette smoke [82, 83]. Furthermore, living next to a major road is also associated with lower pH values [84].

5.15 Exercise

Exercise does not seem to change EBC pH values unless the sample is de-aerated. Greenwald et al. observed a statistically significant increase in EBC pH when it was measured immediately after sample collection, but did not observe such changes when the samples were standardized [85]. Tuesta et al. performed argon bubbling during their analysis. In this experiment, subjects exercised for 10 minutes on a cycle ergometer at a stable load equal to 30% of VO2 max. EBC pH levels were measured before and 80 minutes following exercise, with an increase being observed (ΔpH=0.10, p=0.051). However, such changes did not appear when 30 and 90 minutes trials were performed [86]. In addition, there are conflicting data relating to the significant mild increase of EBC pH after exercise (measured immediately and 60 minute after exercise ends) [87].

6. Summary

EBC acidity is a good, but nonspecific, biomarker of numerous diseases. Both respiratory and systemic disorders can influence EBC pH. Therefore, it would be fruitful to conduct further research aimed at assessing utility of this parameter in each particular field, as well as to determine the level of pH analysis sensitivity for all diseases. However, the differences in the results produced by analytical and collection methods remain a huge problem. This issue should be resolved by developing new trials that are able to define physiological levels and the border of pathology of EBC pH for each method and for all relevant types of equipment. There is also a huge need for a unified pH measurement technique (especially the unification of de-aeration time and gas used for standardization), as such a method would be immensely helpful in performing large-scale studies on EBC pH and comparing the results between them. In addition, in developing this unified method, it will be necessary to define pH levels that are connected with good health (reference values). Without these steps, the everyday clinical utilization of EBC pH analysis will remain impossible.

Furthermore, many problems (e.g. gas standardization, time and temperature of storage) may be eliminated if analyses are performed with blood gas analyzers immediately after sample collection. Such an approach will surely be adopted when analysis of condensate collected from patients becomes standard clinical practice.

As this review has shown, the last 6 years have seen a succession of new trials spanning right up until the most recent review of EBC pH assays [10]. The field of EBC analysis is growing at a rapid pace, and reviews such as those written by the authors of reviews [1-4] and this one can be tremendously helpful for researchers who are still developing their knowledge of EBC.

Conflicts of Interest: We declare that no conflict of interest exists in the submission of this manuscript

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