Review

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Clinical Application of Exhaled Breath Condensate in Diagnosis of Diseases

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Respiratory diseases are highly prevalent in the general population, and the morbidity, mortality, and healthcare burden on society at large have been on the rise worldwide. For example, lung cancer is a major contributor to cancer-related mortality around the globe, and identifying clinically relevant biomarkers for lung cancer detection at both early and metastatic stages has been a pressing need. Human metabolism is complicated and may vary with different individuals. Despite advances in the treatment and the early screening of respiratory diseases, most diagnoses are established at a late stage, i.e., when genetic and epigenetic changes have developed. A promising source of biomarkers indicative of the pathogenesis of respiratory diseases is exhaled breath condensate (EBC), a biological fluid and a natural matrix of the respiratory tract. Molecules, such as DNAs, RNAs, proteins, metabolites, and others, are found in EBC, and their presence/absence or changes in concentrations can serve as biomarkers. This review discusses the exhaled breath composition, candidate EBC biomarkers, and the potential to use EBC for diagnosing diseases, therapeutic monitoring, and screening high-risk individuals.

Keywords: E-Nose; exhaled breath condensate (EBC); volatile organic compounds

Introduction

Over the past decades, researchers have been developing non-invasive methods of examining inflammatory markers of diseases. Exhaled breath condensate (EBC) collection is a relatively new technique for harvesting pulmonary specimens. EBC is a liquid form of exhaled gases and vapors that can be safely collected from patients who can breathe on their own or those on mechanical ventilation simply by placing a collecting device in the expiratory circuit of the ventilator [1].

The collection of EBC was first described in 1980 in the former Soviet Union but has recently gained renewed interest as a non-invasive means of analyzing the status of the lung [2]. Investigations on EBC have been conducted under different diseases, including chronic obstructive pulmonary disease (COPD), lung cancer, acute respiratory distress syndrome (ARDS), and asthma, among others. These studies have identified some patterns of change in an array of biomarkers that are appreciable in EBC [3,4]. The biomarkers include but are not limited to, nitric oxide (NO), eicosanoids, such as leukotrienes, prostanoids and isoprostanes, products of lipid peroxidation, hydrogen peroxide (H_2O_2), and inflammatory proteins [4]. By assessing the inflammatory biomarker profiles of specific conditions, clinicians might use EBC findings as prognosticators and indicators that guide treatment. This paper reviews the studies and clinical applications of metabolomics in EBC in the diagnosis, prognosis, and treatment of diseases.

EBC

The gas expelled from the lungs after gas exchange is composed of nitrogen, oxygen, carbon dioxide, a small amount of inert gas, and water vapor. Additionally, it also contains trace but a wide variety of endogenous and exogenous compounds [5]. The production and operation of EBC *in vivo* are shown in Fig. 1. EBC consists of a wide array of water-soluble volatile and non-volatile substances. Some valuable substances are listed in Table 1 (Ref. [5,6]). The substances' properties fall into three categories: exhaled volatile organic compounds (VOCs), exhaled nitric oxide, and exhaled non-volatile compounds [6].

VOCs stem from endogenous metabolites of the human body or bacteria from the digestive and respiratory tracts and may be influenced by smoking, medication, food intake, etc. [7]. In addition, the exhaled VOCs also contain inhaled VOCs from the external environment. Common endogenous VOCs include isoprene and acetone. Ingestion of some precursors, such as valproate or ^{13}C-dextromethorphan, can lead to the presence of 3-heptanone or $^{13}CO_2$ in VOCs [8]. Currently, acetone, isoprene, acetaldehyde, and other substances have been the major targets of active research [5].

F_{ENO} emerged as a non-invasive marker of airway inflammation in asthma in the 1990s and has since been studied as a marker of many other diseases [9]. Some studies

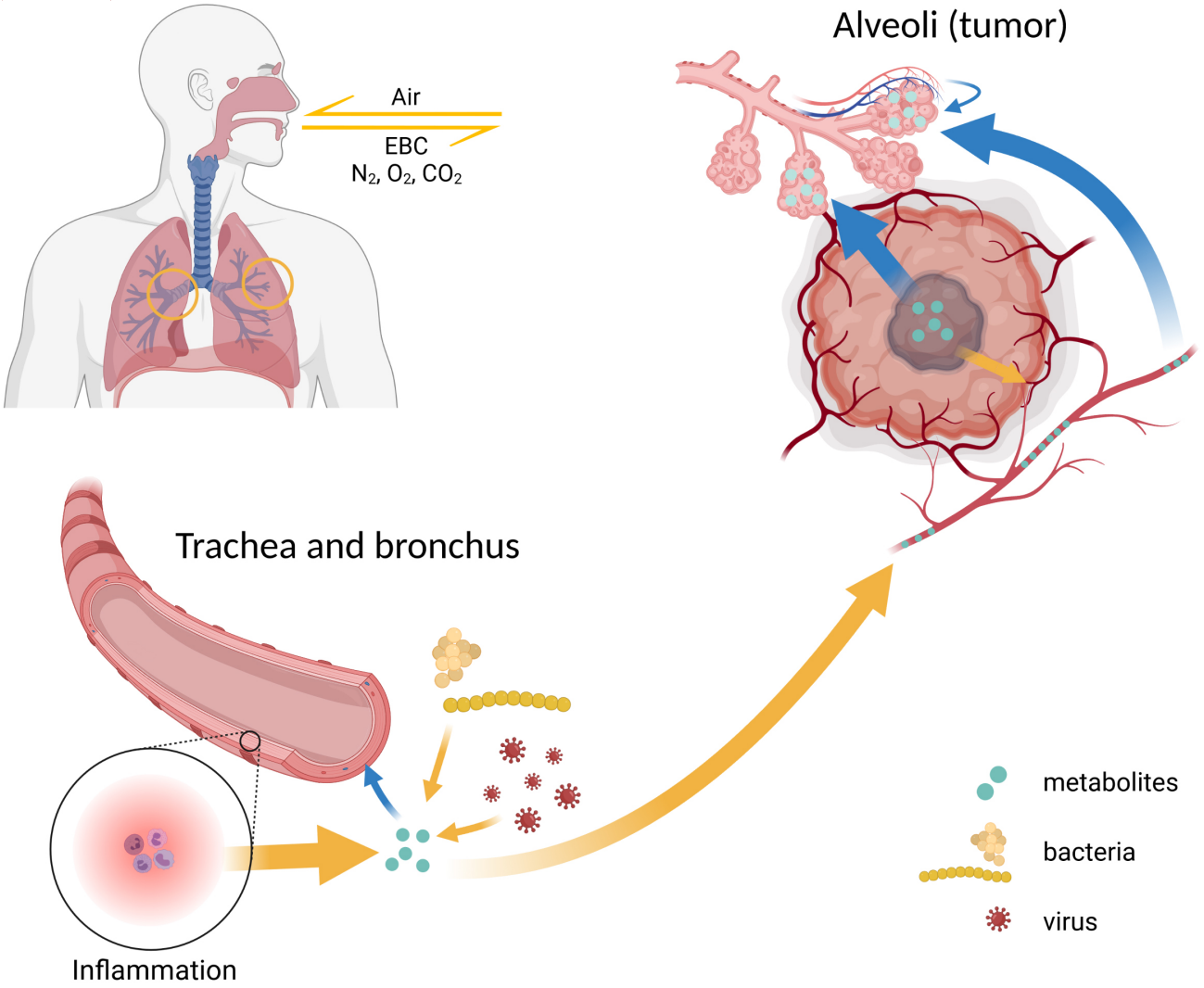

Fig. 1. A schematic diagram showing the production and transport of metabolic substances in EBC. The yellow arrows denote the production of the metabolites and the movement in the body, and the blue arrow indicates the passage of the metabolites into the respiratory tract. *Created with BioRender.com. EBC, exhaled breath condensate.

exhibited that F_{ENO} was also of some value in the research of environmental exposure and respiration [10,11].

The exhaled non-volatile compounds in EBC are minimal but include a wide variety of substances, including proteins, surfactants, macromolecules, microorganisms, small molecules, ions, and so on [4]. Common substances, such as prostaglandins, leukotrienes, oxidative stress metabolites, glutathione, adenosine, and H_2O_2 [4,12,13], have been covered by a great many studies.

Examination of EBC

EBC contains a large number of substances, and an abundant amount of technology has been developed for examination. Samples are usually tested directly when biomarker information is available. For example, when detecting alcohol driving, we can detect ethanol in EBC by infrared spectroscopy. When exploring new biomarkers, omics analysis can often be considered. Metabolomics and proteomics widely detect metabolites and proteins in samples and find valuable markers through data analysis. These two methods have been applied to the analysis of EBC [13–15]. Although there is no relevant application of transcriptomics at present, relevant detection achievements of nucleic acids have been obtained, and the application and development of transcriptomics can be expected in the future [16]. In addition, there is a faster way to detect EBC substances quickly, namely the electronic nose (e-nose).

By mimicking a mammal's nose, the e-nose uses a vast array of sensors that interact nonselectively with odor molecules to produce a signal sent to a computer for data processing, to analyze valuable information. There are different types of electronic nose sensors, including organic polymers, metal oxides, quartz crystal microbalances, and even selective detection of substances in combination with gas chromatography (GC) or mass spectrometry (MS) [17]. In detecting VOCs or other substances in EBC, e-nose can

Table 1. Categories of biomarkers in EBC [5,6].

Compounds	Representative substances
Amino acids	lysine, tyrosine, aspartic acid
Lipids	monostearin, monopalmitin, fatty acid
Amine	glutamine, 11-eicosenamide
Organic acids	formic acid, acetic acid, propionic acid
Alcohol	methanol, ethanol, propanol
Aldehydes	acetaldehyde, propionaldehyde
Ketones	acetone
Hydrocarbons	acetoin, isoprene, squalene
Others	F_{ENO}, p-cresol, indole, formate, 1-methylimidazole, PGE2

EBC, exhaled breath condensate; PGE2, Prostaglandin E2.

be used to reduce the demand for samples and shorten the inspection time, further improving the degree of convenience.

Respiratory Diseases

Since EBC comes from breathing gases, respiratory diseases are the most suitable for the use of EBC for research. Diseases associated with airway inflammation, such as COPD and asthma, can be found in patients' EBC due to inflammation. EBC changes have also been observed in lung cancer and cystic fibrosis due to changes in local tissue activity. Recent advances in the use of EBC in respiratory diseases will be covered in the following section.

COPD

A major feature of COPD is chronic inflammation of the airway. With the help of EBC, the increase in F_{ENO} can effectively mark COPD patients [18]. In addition, F_{ENO} can distinguish COPD from asthma-COPD overlap syndrome (ACOS) [19]. Metabolomics and proteomics have also made some achievements in studying the application value of EBC in COPD. One study found that the nuclear magnetic resonance (NMR) metabolic profiling of EBC can effectively distinguish asthma patients from COPD patients, even if they have a history of smoking [20]. NMR spectra of EBC can also discriminate COPD and PLCH patients who have a history of smoking from non-COPD smokers and the differences between them, and provide a clear metabolic spectrum for each category of patients [21]. Metabolomics in EBC can also help evaluate the efficacy of different drugs in treating COPD [22]. Through proteomics analysis, EBC can also identify stable COPD and non-COPD control groups [13].

Asthma

Compared with COPD, asthma is mainly characterized by acute changes in airway function. Even so, through the analysis of EBC, we can still explore a lot of useful information about asthma. In a study containing 64 subjects between 6 and 18 [23], the authors collected EBC and assessed expiratory fingerprints using an electronic nose. The results showed that a two-cluster hierarchical model based on exhaled volatile organic compounds could significantly distinguish between asthmatic and non-asthmatic patients. In another study [24], through the analysis of extracellular vesicles in EBC, the authors successfully revealed the difference in lung microbiota between patients with asthma and the control group, and proposed a diagnostic model of asthma using artificial intelligence. In contrast, the loss of ammonium reflected the decrease in ammonia synthesis caused by the down-regulation of glutaminase, resulting in acid neutralization damage.

Lung Cancer

Lung cancer is a malignant tumor in which there are a large number of pathophysiological states worth exploring, and with the help of EBC, many metabolic processes and genetic changes can be easily found. In a study of lung cancer [25], the authors found that the concentration of IL-11 in the exhaled breath of patients increased significantly and proved that IL-11 is closely related to the poor prognosis of NSCLC. In addition to COPD, proteomics in EBC can also identify biomarkers of lung cancer [26]. This method can also distinguish lung cancer from benign pulmonary nodules, which is of guiding significance for future research on related pulmonary nodules [27]. Nucleic acid and gene sequencing can also be applied to EBC. In this study of gene mutations in lung and colorectal cancer [28], by using a more sensitive amplification-based next-generation sequencing technique (NGS) to identify cancer-related mutations in DNA isolated from EBC, we can find mutations in EBC DNA in healthy subjects, which may represent very early neoplastic changes or a normal apoptotic process that eliminates damaged cells by mutation or genetic material changes.

Cystic Fibrosis

Cystic fibrosis is a rare multiple-organ hereditary disease caused by mutations in the CF transmembrane conduction regulator (*CFTR*) gene [29]. The lungs are the most common site of involvement, and respiratory failure is the leading cause of death in patients with cystic fibrosis [30].

In a study, by analyzing the metabolic profile of EBC, it was found that asymmetric dimethylarginine and related metabolites could be used as biomarkers in pediatric patients with CF [31].

Other Respiratory Diseases

In addition to the above diseases, many respiratory diseases can be studied by EBC, such as obstructive sleep apnea syndrome (OSAS) [32,33] and ARDS [34]. Targeted analysis of VOCs in EBC can also distinguish children with and without pseudomonas aeruginosa-positive cultures from adults with clinically acceptable sensitivity values [35].

Digestive Diseases

It has been mentioned in the introduction that various substances can reach the lungs through blood circulation and participate in the formation of EBC, so respiratory diseases and other diseases may also be studied through EBC. The digestive system has rich blood circulation and shares part of the channels with the respiratory tract. Therefore, many studies on digestive diseases with the help of EBC will be covered below.

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic intestinal inflammation that significantly impacts patients' quality of life. In a study of CD, the author evaluated the composition of microflora by pyrophosphate sequencing of 16S rRNA V1-V3 gene region and analysis of EBC by gas chromatography-mass spectrometry (GC-MS) [36]. They found that 17 volatile metabolites were associated with 17 species of bacteria, which provided a new clue for targeted intervention. In another study [37], the authors found that VOCs can be used as a new, non-invasive, rapid, and relatively inexpensive tool for diagnosing IBD, with high sensitivity and specificity. VOCs can also separate healthy controls, active patients, and remission CD patients in a real-life cohort [38]. As a diagnostic marker of IBD, EBC can also accurately distinguish healthy people, CD, and UC [39]. Even for similar diseases, such as UC, EBC can effectively distinguish active UC from remission, and the characteristics of UC are significantly different from those of patients with non-UC colitis [40].

Malignant Tumor

Digestive tract tumors are a significant contributor to digestive system diseases. As a component of EBC, VOCs have been able to effectively distinguish patients with colorectal cancer from healthy people, providing an effective non-invasive examination program for the disease [41,42]. In addition, VOCs can also distinguish the metabolic fingerprints of cancer tissues from normal colonic mucosa of the same patient, such as the contents of benzaldehyde, phenylethyl and indole are significantly different from those of normal colonic mucosa [43]. In addition to colorectal cancer, different expiratory VOC curves can also distinguish patients with esophageal and gastric adenocarcinoma from non-cancer controls [44].

Esophageal Diseases

EBC can also be used in the study of esophageal diseases. In a study on the treatment of esophageal atresia (EA) [45], the authors found that the level of antioxidant Glutathione in EBC was significantly decreased in patients with EA, cysteinyl-leukotriene (Cys-LT) in the PPI group, and 8-iso in the gastric fundus folding group. Another study analyzed the microRNAs (miRNAs) in EBC and revealed that the decreased level of miRNA-21 in EBC in patients with EA suggested the problem of airway hyperresponsiveness, which may be related to gastroesophageal reflux (GER) and its surgical treatment [16].

Other Digestive Diseases

In addition to the above diseases, other disease studies also involve EBC. The e-nose, which can analyze VOCs, has a certain potential in distinguishing chronic pancreatitis, pancreatic cancer, and healthy controls, and may be of good significance for the early detection of pancreatic lesions [46]. For liver cirrhosis, a study has proved that expiratory ammonia can effectively distinguish between patients with liver cirrhosis and healthy people [47]. Another study demonstrated the ability to evaluate human health and disease status using non-invasive screening tools based on breath and urine VOC analysis at a moderate level of abstraction through data fusion of the two systems [48]. The metabolic analysis of EBC [49] shows that the changes in glycerol phospholipid metabolism, sphingolipid metabolism, arachidonic acid metabolism, and amino acid metabolism can predict the poor prognosis of patients with liver failure.

Breast and Thyroid Diseases

Although the breast and thyroid are superficial tissues, EBC still has the opportunity to use its talents, such as convenient, non-invasive, and can help understand the pathophysiological changes in the disease process. In a study that containing patients with lung, colon, breast, and prostate cancer by using a tailor-made array of cross-reactive nanosensors based on organically functionalized gold nanoparticles and GC-MS, VOCs was able to distinguish respiratory differences between healthy people and cancer patients [50]. Moreover, each type of cancer may have a unique VOC pattern. Similarly, with the help of chemical resistive gas sensors and stoichiometric analysis, VOCs can form characteristic chemical fingerprints, so they can also be used as a valuable tool for lung cancer, breast

cancer, and COPD screening testing [51]. In a study of 899 subjects, the results showed that the prediction accuracy was 91%, the sensitivity was 86%, the specificity was 97%, the positive predictive value was 97%, the negative predictive value was 97%, and the area under the working curve of the subjects (AUC) was 0.99, and the Kappa value was 0.83 [52]. In a more detailed study, it was found that there were significant differences in seven substances ((S)-1,2-propanediol, cyclopentanone, ethylene carbonate, 3-methoxy-1,2-propanediol, 3-methylpyridine, phenol, and tetramethylsilane) in VOCs between breast cancer patients and healthy people, as well as between breast cancer patients and gastric cancer patients [53]. In a similar study on thyroid cancer, there were significant differences in VOCs between patients with nodular goiter and normal controls, patients with papillary thyroid cancer and normal controls, patients with papillary thyroid cancer, and patients with nodular goiter; 7,7 and 3 characteristic metabolites played a decisive role in sample classification [54].

Genitourinary Diseases

Renal disease may affect renal function, leading to changes in blood composition, and changes in the contents of EBC may occur at this time. In fact, the studies of EBC in renal disease are not uncommon. One study has confirmed that the concentration of 45 volatile organic compounds exhaled by critically ill patients with acute renal injury is higher than that of patients with normal renal function [55]. During dialysis, the concentration of 2/3 of the substances will gradually drop. This suggests that EBC can reflect the severity of acute renal injury and can be used to evaluate the efficacy of dialysis. Chronic nephropathy can also cause changes in VOC components in EBC, such as ammonia, ethanol, isoprene, valeraldehyde, and heptanal [56]. In end-stage renal disease, 2-propanol, ammonia, and acetaldehyde contained in EBC can also distinguish between individuals with and without renal failure [57]. In addition to renal disease, prostate disease can also be reflected in changes in EBC. Prostate biopsy is the gold standard for the diagnosis of prostate cancer, but it is an invasive test. In the study, hand-held electronic nose devices trained by neural networks can effectively distinguish between untreated, histologically confirmed primary prostate cancer patients and control patients [58]. In the future, we can try to conduct more similar studies to explore changes in local physiological activity in the disease.

Other Diseases

In addition to the previously listed diseases, other organ and tissue lesions can also cause changes in the composition of EBC. The distance between the heart and the lung is the closest; their functions are closely related and influence each other. Therefore, there are also associated studies on EBC in patients with cardiovascular disease. Through the analysis of VOC data, elderly patients with congestive heart failure can be well distinguished from healthy people and COPD patients [59].

In addition to cardiovascular diseases, there are some research results on neural system diseases. In a study of Parkinson's disease, the authors used an electronic system to detect volatile molecules (sensor arrays) in EBC to distinguish between *de novo* PD and control subjects [60]. Compared with midbrain ultrasound (93%, 90%, 92%) and olfactory detection (62%, 89%, 73%), the sensitivity, specificity, and accuracy of the sensor array in detecting normal Parkinson's disease were 79%, 84%, and 81%, respectively. This confirms the application potential of this detection technology for EBC in diseases. In another study on multiple sclerosis, the authors used an electronic nose to analyze VOCs in EBC [61]. After training the model through a neural network and adding the effect of drug intake, the results confirmed that an electronic nose could distinguish between MS patients and healthy control subjects, which may create a rapid screening test tool for MS.

The mouth, oropharynx, and glottis are all part of the upper respiratory tract, so lesions in these locations can also be reflected in changes in EBC. A study of 91 patients with head and neck squamous cell carcinoma (HNSCC) showed that the diagnostic accuracy, sensitivity, and specificity of using VOCs in EBC were 72%, 79%, and 63%, respectively [62]. In the three groups of models used to distinguish oral, oropharyngeal, and glottic sub-loci and healthy controls, the AUC was 0.85, 0.82, and 0.83, respectively. This shows that this method can effectively distinguish HNSCC patients from healthy controls.

Hernia recurrence and aortic aneurysm are related to impaired collagen metabolism. Preventive options will be greatly enhanced if patients at risk of hernia recurrence and aortic aneurysms can be identified in a reliable, low-cost, non-invasive manner. A three-arm proof-of-concept study conducted in three hospitals used electronic noses to detect VOCs in EBC [63]. Neural network analysis showed the ability to distinguish between patients with recurrent hernia and controls (AUC = 0.74, sensitivity = 0.79, specificity = 0.65) and between patients with aortic aneurysm and healthy controls (AUC = 0.84, sensitivity = 0.83, specificity = 0.81). This proves that EBC can distinguish patients at risk of recurrent hernia and aortic aneurysms from healthy controls and suggests that many similar exploratory studies can be carried out in the future.

Discussion and Conclusion

Although great progress has been made in diagnosing and treating various diseases, a comprehensive understanding of the metabolic process in the occurrence and development of diseases is far from enough. EBC can provide more biological information about the lesions involved in blood

circulation and ventilation as a biological sample from the lower respiratory tract. In addition, as a non-invasive detection method, EBC has the advantages of safety and convenience.

This review describes the practical results of EBC in several systems. Some systematic research results are very rich. For these diseases, future research should focus on metabolomics, proteomics, and transcriptome, which can explain the changes of the disease in depth and detail, which can not only help the research and development of EBC but also help us to understand the disease better, to improve clinical decision-making. For some systems with fewer achievements, we should reflect on whether there are lessons to be learned from the existing research to improve the research methods and find a suitable starting point to carry out more research and promote the development of technology and the progress of disease awareness.

More basic research and clinical trials are warranted to improve EBC metabolomics performance, develop standardized procedures, and provide reference data. Many studies have demonstrated that EBC analysis promises to function as an essential tool in future scientific research and clinical practice.

Abbreviations

EBC, exhaled breath condensate; COPD, chronic obstructive pulmonary disease; ARDS, acute respiratory distress syndrome; NO, nitric oxide; H_2O_2, hydrogen peroxide; VOCs, volatile organic compounds; e-nose, electronic nose; GC, gas chromatography; MS, mass spectrometry; ACOS, asthma-COPD overlap syndrome; NMR, nuclear magnetic resonance; NGS, next-generation sequencing technique; CFTR, CF transmembrane conduction regulator; OSAS, obstructive sleep apnea syndrome; EA, esophageal atresia; Cys-LT, cysteinyl-leukotriene; CD, Crohn's disease; UC, ulcerative colitis; GC-MS, gas chromatography-mass spectrometry; IBD, inflammatory bowel disease; miRNAs, microRNAs; CDA, canonical discriminant analysis; AUC, area under the working curve of the subjects; CRPS, complex regional pain syndrome; HNSCC, head and neck squamous cell carcinoma.

Availability of Data and Materials

Not applicable.

Author Contributions

WYM made substantial contributions to conception and design. ZLZ searched most of the references. ZLZ and WYM wrote the main manuscript text. ZLZ wrote the introduction, EBC, Examination of EBC, Respiratory Diseases, Digestive Diseases, Breast and Thyroid Diseases, Genitourinary Diseases, Other Diseases. WYM wrote the Abstract, Discussion and Conclusion. ZLZ prepared Fig. 1. WYM revised the manuscript critically for important intellectual content. Both authors reviewed the manuscript and gave final approval of the version to be published. Both authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Walsh BK, Mackey DJ, Pajewski T, Yu Y, Gaston BM, Hunt JF. Exhaled-breath condensate pH can be safely and continuously monitored in mechanically ventilated patients. Respiratory Care. 2006; 51: 1125–1131.

[2] Sidorenko GI, Zborovskiĭ EI, Levina DI. Surface-active properties of the exhaled air condensate (a new method of studying lung function). Terapevticheskii Arkhiv. 1980; 52: 65–68.

[3] Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. American Journal of Respiratory and Critical Care Medicine. 2001; 163: 1693–1722.

[4] Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, *et al*. Exhaled breath condensate: methodological recommendations and unresolved questions. The European Respiratory Journal. 2005; 26: 523–548.

[5] Vasilescu A, Hrinczenko B, Swain GM, Peteu SF. Exhaled breath biomarker sensing. Biosensors & Bioelectronics. 2021; 182: 113193.

[6] Horváth I, Barnes PJ, Loukides S, Sterk PJ, Högman M, Olin AC, *et al*. A European Respiratory Society technical standard: exhaled biomarkers in lung disease. The European Respiratory Journal. 2017; 49: 1600965.

[7] Pleil JD, Stiegel MA. Evolution of environmental exposure science: using breath-borne biomarkers for "discovery" of the human exposome. Analytical Chemistry. 2013; 85: 9984–9990.

[8] Mattison LK, Fourie J, Hirao Y, Koga T, Desmond RA, King JR, *et al*. The uracil breath test in the assessment of dihydropyrimidine dehydrogenase activity: pharmacokinetic relationship between expired $^{13}CO_2$ and plasma [2-^{13}C]dihydrouracil. Clinical Cancer Research : an Official Journal of the American Association for Cancer Research. 2006; 12: 549–555.

[9] American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. American Journal of Respiratory and Critical Care Medicine. 2005; 171: 912–930.

[10] Flamant-Hulin M, Caillaud D, Sacco P, Penard-Morand C, Annesi-Maesano I. Air pollution and increased levels of frac-

tional exhaled nitric oxide in children with no history of airway damage. Journal of Toxicology and Environmental Health. Part A. 2010; 73: 272–283.

[11] La Grutta S, Ferrante G, Malizia V, Cibella F, Viegi G. Environmental effects on fractional exhaled nitric oxide in allergic children. Journal of Allergy. 2012; 2012: 916926.

[12] Teng Y, Sun P, Zhang J, Yu R, Bai J, Yao X, *et al*. Hydrogen peroxide in exhaled breath condensate in patients with asthma: a promising biomarker? Chest. 2011; 140: 108–116.

[13] Sun C, Zhou T, Xie G, Fu S, Gao L, Liao J, *et al*. Proteomics of exhaled breath condensate in stable COPD and non-COPD controls using tandem mass tags (TMTs) quantitative mass spectrometry: A pilot study. Journal of Proteomics. 2019; 206: 103392.

[14] Maniscalco M, Cutignano A, Paris D, Melck DJ, Molino A, Fuschillo S, *et al*. Metabolomics of Exhaled Breath Condensate by Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry: A Methodological Approach. Current Medicinal Chemistry. 2020; 27: 2381–2399.

[15] Maniscalco M, Fuschillo S, Paris D, Cutignano A, Sanduzzi A, Motta A. Clinical metabolomics of exhaled breath condensate in chronic respiratory diseases. Advances in Clinical Chemistry. 2019; 88: 121–149.

[16] Soyer T, Birben E, Türer ÖB, Kahveci M, Tuğcu GD, Soyer ÖU, *et al*. MicroRNA Levels in Exhaled Breath Condensate of Patients with Esophageal Atresia. Diseases of the Esophagus: Official Journal of the International Society for Diseases of the Esophagus. 2021; 34: doaa082.

[17] Baldwin EA, Bai J, Plotto A, Dea S. Electronic noses and tongues: applications for the food and pharmaceutical industries. Sensors (Basel, Switzerland). 2011; 11: 4744–4766.

[18] Donohue JF, Herje N, Crater G, Rickard K. Characterization of airway inflammation in patients with COPD using fractional exhaled nitric oxide levels: a pilot study. International Journal of Chronic Obstructive Pulmonary Disease. 2014; 9: 745–751.

[19] Chen FJ, Huang XY, Liu YL, Lin GP, Xie CM. Importance of fractional exhaled nitric oxide in the differentiation of asthma-COPD overlap syndrome, asthma, and COPD. International Journal of Chronic Obstructive Pulmonary Disease. 2016; 11: 2385–2390.

[20] Maniscalco M, Paris D, Melck DJ, Molino A, Carone M, Ruggeri P, *et al*. Differential diagnosis between newly diagnosed asthma and COPD using exhaled breath condensate metabolomics: a pilot study. The European Respiratory Journal. 2018; 51: 1701825.

[21] de Laurentiis G, Paris D, Melck D, Montuschi P, Maniscalco M, Bianco A, *et al*. Separating smoking-related diseases using NMR-based metabolomics of exhaled breath condensate. Journal of Proteome Research. 2013; 12: 1502–1511.

[22] Vignoli A, Santini G, Tenori L, Macis G, Mores N, Macagno F, *et al*. NMR-Based Metabolomics for the Assessment of Inhaled Pharmacotherapy in Chronic Obstructive Pulmonary Disease Patients. Journal of Proteome Research. 2020; 19: 64–74.

[23] Cavaleiro Rufo J, Paciência I, Mendes FC, Farraia M, Rodolfo A, Silva D, *et al*. Exhaled breath condensate volatilome allows sensitive diagnosis of persistent asthma. Allergy. 2019; 74: 527–534.

[24] An J, McDowell A, Kim YK, Kim TB. Extracellular vesicle-derived microbiome obtained from exhaled breath condensate in patients with asthma. Annals of Allergy, Asthma & Immunology: Official Publication of the American College of Allergy, Asthma, & Immunology. 2021; 126: 729–731.

[25] Wu J, Chen J, Lv X, Yang Q, Yao S, Zhang D, *et al*. Clinical value of serum and exhaled breath condensate inflammatory factor IL-11 levels in non-small cell lung cancer: Clinical value of IL-11 in non-small cell lung cancer. The International Journal of Biological Markers. 2021; 36: 64–76.

[26] Zakharova N, Kozyr A, Ryabokon AM, Indeykina M, Strelnikova P, Bugrova A, *et al*. Mass spectrometry based proteome profiling of the exhaled breath condensate for lung cancer biomarkers search. Expert Review of Proteomics. 2021; 18: 637–642.

[27] Ma L, Xiu G, Muscat J, Sinha R, Sun D, Xiu G. Comparative proteomic analysis of exhaled breath condensate between lung adenocarcinoma and CT-detected benign pulmonary nodule patients. Cancer Biomarkers: Section a of Disease Markers. 2022; 34: 163–174.

[28] Youssef O, Knuuttila A, Piirilä P, Böhling T, Sarhadi V, Knuutila S. Presence of cancer-associated mutations in exhaled breath condensates of healthy individuals by next generation sequencing. Oncotarget. 2017; 8: 18166–18176.

[29] Kelly J. Environmental scan of cystic fibrosis research worldwide. Journal of Cystic Fibrosis: Official Journal of the European Cystic Fibrosis Society. 2017; 16: 367–370.

[30] O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet (London, England). 2009; 373: 1891–1904.

[31] Lucca F, Da Dalt L, Ros M, Gucciardi A, Pirillo P, Naturale M, *et al*. Asymmetric dimethylarginine and related metabolites in exhaled breath condensate of children with cystic fibrosis. The Clinical Respiratory Journal. 2018; 12: 140–148.

[32] 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.

[33] Carpagnano GE, Spanevello A, Sabato R, Depalo A, Turchiarelli V, Foschino Barbaro MP. Exhaled pH, exhaled nitric oxide, and induced sputum cellularity in obese patients with obstructive sleep apnea syndrome. Translational Research: the Journal of Laboratory and Clinical Medicine. 2008; 151: 45–50.

[34] Kononikhin AS, Starodubtseva NL, Chagovets VV, Ryndin AY, Burov AA, Popov IA, *et al*. Exhaled breath condensate analysis from intubated newborns by nano-HPLC coupled to high resolution MS. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences. 2017; 1047: 97–105.

[35] Kos R, Brinkman P, Neerincx AH, Paff T, Gerritsen MG, Lammers A, *et al*. Targeted exhaled breath analysis for detection of Pseudomonas aeruginosa in cystic fibrosis patients. Journal of Cystic Fibrosis: Official Journal of the European Cystic Fibrosis Society. 2022; 21: e28–e34.

[36] Smolinska A, Tedjo DI, Blanchet L, Bodelier A, Pierik MJ, Masclee AAM, *et al*. Volatile metabolites in breath strongly correlate with gut microbiome in CD patients. Analytica Chimica Acta. 2018; 1025: 1–11.

[37] Monasta L, Pierobon C, Principalle A, Martelossi S, Marcuzzi A, Pasini F, *et al*. Inflammatory bowel disease and patterns of volatile organic compounds in the exhaled breath of children: A case-control study using Ion Molecule Reaction-Mass Spectrometry. PLoS ONE. 2017; 12: e0184118.

[38] Bodelier AGL, Smolinska A, Baranska A, Dallinga JW, Mujagic Z, Vanhees K, *et al*. Volatile Organic Compounds in Exhaled Air as Novel Marker for Disease Activity in Crohn's Disease: A Metabolomic Approach. Inflammatory Bowel Diseases. 2015; 21: 1776–1785.

[39] Arasaradnam RP, McFarlane M, Daulton E, Skinner J, O'Connell N, Wurie S, *et al*. Non-invasive exhaled volatile organic biomarker analysis to detect inflammatory bowel disease (IBD). Digestive and Liver Disease: Official Journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2016; 48: 148–153.

[40] Smolinska A, Bodelier AGL, Dallinga JW, Masclee AAM, Jonkers DM, van Schooten FJ, *et al*. The potential of volatile organic compounds for the detection of active disease in patients with ulcerative colitis. Alimentary Pharmacology & Therapeutics. 2017; 45: 1244–1254.

[41] van Keulen KE, Jansen ME, Schrauwen RWM, Kolkman JJ,

Siersema PD. Volatile organic compounds in breath can serve as a non-invasive diagnostic biomarker for the detection of advanced adenomas and colorectal cancer. Alimentary Pharmacology & Therapeutics. 2020; 51: 334–346.

[42] Altomare DF, Di Lena M, Porcelli F, Trizio L, Travaglio E, Tutino M, *et al*. Exhaled volatile organic compounds identify patients with colorectal cancer. The British Journal of Surgery. 2013; 100: 144–150.

[43] De Vietro N, Aresta A, Rotelli MT, Zambonin C, Lippolis C, Picciariello A, *et al*. Relationship between cancer tissue derived and exhaled volatile organic compound from colorectal cancer patients. Preliminary results. Journal of Pharmaceutical and Biomedical Analysis. 2020; 180: 113055.

[44] Kumar S, Huang J, Abbassi-Ghadi N, Mackenzie HA, Veselkov KA, Hoare JM, *et al*. Mass Spectrometric Analysis of Exhaled Breath for the Identification of Volatile Organic Compound Biomarkers in Esophageal and Gastric Adenocarcinoma. Annals of Surgery. 2015; 262: 981–990.

[45] Soyer T, Türer ÖB, Birben E, Kahveci M, Tuğcu GD, Soyer ÖU, *et al*. The relationship between oxidative stress markers in exhaled breath condensate and respiratory problems in patients with repaired esophageal atresia. Journal of Pediatric Surgery. 2020; 55: 1516–1521.

[46] Uslu HI, Dölle AR, Dullemen HM, Aktas H, Kolkman JJ, Venneman NG. Pancreatic ductal adenocarcinoma and chronic pancreatitis may be diagnosed by exhaled-breath profiles: a multicenter pilot study. Clinical and Experimental Gastroenterology. 2019; 12: 385–390.

[47] Zaim O, Diouf A, El Bari N, Lagdali N, Benelbarhdadi I, Ajana FZ, *et al*. Comparative analysis of volatile organic compounds of breath and urine for distinguishing patients with liver cirrhosis from healthy controls by using electronic nose and voltammetric electronic tongue. Analytica Chimica Acta. 2021; 1184: 339028.

[48] Ishida J, Oikawa T, Nakagawa C, Takano K, Fujioka K, Kikuchi Y, *et al*. Real-time breath ammonia measurement using a novel cuprous bromide sensor device in patients with chronic liver disease: a feasibility and pilot study. Journal of Breath Research. 2021; 15: 026010.

[49] Jing J, Sang XX, You SL, Zhu B, Cui YF, Li CY, *et al*. Metabolomic profiles of breath odor compounds for prognostic prediction in patients with acute-on-chronic liver failure: A pilot study. Hepatology Research: the Official Journal of the Japan Society of Hepatology. 2021; 51: 490–502.

[50] Peng G, Hakim M, Broza YY, Billan S, Abdah-Bortnyak R, Kuten A, *et al*. Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. British Journal of Cancer. 2010; 103: 542–551.

[51] Rodríguez-Aguilar M, Díaz de León-Martínez L, Gorocica-Rosete P, Pérez-Padilla R, Domínguez-Reyes CA, Tenorio-Torres JA, *et al*. Application of chemoresistive gas sensors and chemometric analysis to differentiate the fingerprints of global volatile organic compounds from diseases. Preliminary results of COPD, lung cancer and breast cancer. Clinica Chimica Acta; International Journal of Clinical Chemistry. 2021; 518: 83–92.

[52] Yang HY, Wang YC, Peng HY, Huang CH. Breath biopsy of breast cancer using sensor array signals and machine learning analysis. Scientific Reports. 2021; 11: 103.

[53] Zhang Y, Guo L, Qiu Z, Lv Y, Chen G, Li E. Early diagnosis of breast cancer from exhaled breath by gas chromatography-mass spectrometry (GC/MS) analysis: A prospective cohort study. Journal of Clinical Laboratory Analysis. 2020; 34: e23526.

[54] Guo L, Wang C, Chi C, Wang X, Liu S, Zhao W, *et al*. Exhaled breath volatile biomarker analysis for thyroid cancer. Translational Research: the Journal of Laboratory and Clinical Medicine. 2015; 166: 188–195.

[55] Hüppe T, Klasen R, Maurer F, Meiser A, Groesdonk HV, Sessler DI, *et al*. Volatile Organic Compounds in Patients with Acute Kidney Injury and Changes During Dialysis. Critical Care Medicine. 2019; 47: 239–246.

[56] Obermeier J, Trefz P, Happ J, Schubert JK, Staude H, Fischer DC, *et al*. Exhaled volatile substances mirror clinical conditions in pediatric chronic kidney disease. PloS One. 2017; 12: e0178745.

[57] 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.

[58] Waltman CG, Marcelissen TAT, van Roermund JGH. Exhaled-breath Testing for Prostate Cancer Based on Volatile Organic Compound Profiling Using an Electronic Nose Device (Aeonose™): A Preliminary Report. European Urology Focus. 2020; 6: 1220–1225.

[59] Finamore P, Pedone C, Lelli D, Costanzo L, Bartoli IR, De Vincentis A, *et al*. Analysis of volatile organic compounds: an innovative approach to heart failure characterization in older patients. Journal of Breath Research. 2018; 12: 026007.

[60] Finberg JPM, Schwartz M, Jeries R, Badarny S, Nakhleh MK, Abu Daoud E, *et al*. Sensor Array for Detection of Early Stage Parkinson's Disease before Medication. ACS Chemical Neuroscience. 2018; 9: 2548–2553.

[61] Ettema AR, Lenders MWPM, Vliegen J, Slettenaar A, Tjepkema-Cloostermans MC, de Vos CC. Detecting multiple sclerosis via breath analysis using an eNose, a pilot study. Journal of Breath Research. 2021; 15: 10.1088/1752–7163/abd080.

[62] van de Goor RMGE, van Hooren MRA, Henatsch D, Kremer B, Kross KW. Detecting head and neck squamous carcinoma using a portable handheld electronic nose. Head & Neck. 2020; 42: 2555–2559.

[63] Mommers EHH, van Kooten L, Nienhuijs SW, de Vries Reilingh TS, Lubbers T, Mees BME, *et al*. Can Electric Nose Breath Analysis Identify Abdominal Wall Hernia Recurrence and Aortic Aneurysms? A Proof-of-Concept Study. Surgical Innovation. 2020; 27: 366–372.