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**Review Article**

# Exhaled Breath Condensate (EBC) analysis: Latest non-invasive practical method for diagnosing atopic dermatitis and monitoring response to corticosteroid therapy

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## Abstract

***Background*** Patch tests and blood tests are the most commonly used methods for diagnosing atopic dermatitis. However, these methods are invasive and time-consuming, hence limit their benefits. The practical and non-invasive method with a high level of sensitivity and specificity is required to find specific biomarkers as a novel method in diagnosing atopic dermatitis.

***Methods*** A search was conducted in Google Scholar, PubMed, and SAGE. Inclusion criteria were the following: English literature, using experimental methods, and clinical studies. The keywords used in the search were "exhaled condensate" OR "EBC" AND "breath metabolism" OR "respiration" AND "electronic nose" OR "E-nose" OR "enose" AND "atopic dermatitis" OR "eczema" OR "atopic" OR "atopy" OR "atopic".

***Results*** The breathomics concept included detection of changes in the profile of exhaled breath condensates (EBC) or volatile organic compounds (VOC) in the exhaled breath. Specific biomarkers were found during gas chromatography-mass spectrometry (GC-MS) analysis on EBC: an increase in NO, IL-4, leukotrienes, and 8-isoprostane, as well as a decrease in IFN-γ and pH of EBC which were strong indications in diagnosing atopic dermatitis and monitoring the response to corticosteroid therapy in patients.

***Conclusion*** EBC-specific biomarker analysis is a practical and non-invasive method with high sensitivity and specificity. This method may assist clinicians to diagnose atopic dermatitis and monitor the response to corticosteroid therapy, particularly in a mass clinical setting.

***Key words***
Exhaled breath condensate, breath metabolomics, electronic nose, atopic dermatitis.

## Introduction

Atopic dermatitis is a non-communicable disease characterized by recurrent and chronic inflammation of the skin. This allergic disease results in an itchy rash and dry skin in the predilection area (forehead, cheeks, neck, elbow creases and knee creases). It is triggered by certain foods containing allergens, exposure to dust, etc. In general, this dermatitis occurs in infancy and childhood, but may also affect adults. About 50% of atopic dermatitis patients encounter an "atopic march" where the symptoms of asthma and rhinitis coexist.[1]

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Currently, atopic dermatitis is diagnosed based on clinical symptoms and the patient's family history. Laboratorium studies like blood tests to evaluate IgE level and inflammatory mediators such as leukotrienes, histamines, prostaglandins, and 8-isoprostane may be helpful, but those testings commonly are unavailable at primary healthcare facilities e.g. primary clinics. Patch test is a tool to determine the cause of dermatitis. The discomfort caused by an allergic reaction may limit its use, and it may take several days to conclude the results. A less invasive, rapid, and simple screening test is required to increase the effectiveness and efficiency in diagnosing atopic dermatitis. Early diagnosis prevents severe symptoms, improves the quality of life, and reduces the economic burden.[2,3]

Technological advances offer a novel technique: the metabolomic test for the study of biological samples, including breath samples. Currently, non-invasive diagnostic tests using samples from expired breath, known as exhaled breath condensates (EBC), are being developed. The benefits include rapid results and less discomfort for the patient.[4] Peroni *et al*.[3] observed that EBC samples could be used to diagnose patients with atopic dermatitis.[2] This method is suitable for both pediatric and HIV/AIDS patients who are at high risk of developing opportunistic infections.[3]

This literature review was aimed to describe and evaluate EBC as a sample in the analysis of breath metabolomics (breathomics) for diagnosing atopic dermatitis. This analysis was performed implementing an electronic nose (E-Nose) as a practical non-invasive method, particularly in children. Also, this diagnostic method can also be used in monitoring the response to corticosteroid therapy. It is expected to become a further reference for conducting clinical trials.

## Methods

The design of this study was a literature review based on previously published studies sourced from Google Scholar, PubMed, and SAGE. Inclusion criteria were the following: English literature, using experimental methods, and clinical studies. Literature review, systematic literature, and meta-analysis were excluded. The search keywords were "exhaled breath condensate" OR "EBC" AND "breath metabolomics" OR "breathomics" AND "electronic nose" OR "E-nose" OR "enose" AND "atopic dermatitis" OR "eczema" OR "atopic march" OR "atopy" OR "atopic".

## Results and Discussion

***The Role of EBC in Breath Metabolomics*** The non-invasive, fast, and safe nature of exhaled breath condensate (EBC) enables this method to be used as a diagnostic tool. A temperature control device is usually required to collect and condense the breath sample for analysis as EBC.[5] The EBC is a matrix complex that has been shown to have a chemical composition similar to that of blood and extracellular lung fluid. This biological sample is enriched by a wide variety of compounds including non-volatile molecules and water-soluble volatile compounds. Also, lipids, antibodies and carbohydrates could be detected in the breath sample. In general, human EBC contains up to 2000 various compounds.[6]

The metabolomic analysis of EBC needs to be carried out very carefully, especially during the sample preparation phase due to the low concentration of the EBC components to be studied. Besides, there are immense amounts of confounding agents that could potentially impair the sample if the breath sampling is not carried out adequately and the tools are uncleaned properly. Sample preparation was based on the

principles of protein precipitation and extraction. There are various extraction methods: solid-phase extraction (SPE), liquid-liquid extraction, and lyophilization. SPE uses hydrophilic and lipophilic absorbent substances, while liquid-liquid extraction is typically conducted with the help of tertiary butyl methyl ether to extract toxins. Following the extraction process, the lyophilization system can identify analytical samples better and more accurately. This system can reconstitute the EBC in the volume required and can double the number of compounds detected compared with other alternatives.[7]

EBC analysis may not accurately measure solute concentrations in blood or extracellular lung fluid but the concentrations of certain compounds would have significant differences between healthy and ill individuals, hence the EBC analysis can be a potential diagnostic tool. Several compounds can reflect the state of disease and offer the potential to become biomarkers. Currently, nitric oxide (NO), hydrogen peroxide ($H_2O_2$), and acetone measured from breath samples are the three most frequently studied compounds for the inflammatory response in the respiratory system.[8]

Lipid concentrations can also be measured from the EBC, including fatty acids, steroids, eicosanoids, and their subclasses such as prostaglandins or isoprostanes. Amino acids, oxoanionic components, imidazoles, hydroxy acids and aliphatic acids can also be analyzed from the EBC using mass spectrometry.[3,7,8] Low volatility compounds such as enzymes can also be considered as effective biomarkers for disease diagnosis. Apart from compounds, the level of acidity or pH can serve as a simple but significant biomarker to detect an abnormality. Volatile organic compounds (VOC) in EBC are not only limited to the respiratory tract but also represent some from the blood involved in the various systemic process.[8]

***Analysis of the EBC Composition in Atopic Dermatitis*** Studies regarding EBC as a tool for diagnosing atopic disease are being developed particularly in inflammatory respiratory diseases.[9] Any dysfunction in the upper and lower respiratory tract is associated with the presence of atopic rhinitis and atopic dermatitis symptoms, and a risk factor for allergen sensitization to asthma, the so-called as "atopic march". Atopic is a genetic predisposition for developing allergic diseases such as eczema, allergic rhinitis, and asthma. Epidemiological studies suggested that the proportion of atopic-correlated asthma in the general population was usually less than 50%, and most atopic patients do not have asthma. One study demonstrated that NO level was elevated in atopic individuals with or without asthma. This suggests that increasing NO levels not only can be applied to asthma patients but also be applied to other atopic patients including eczema.[1,10]

EBC acidification is one of the characteristics found in patients with the atopic march. The level of acidity in EBC has a strong correlation with the occurrence of eosinophilia and neutrophilia. Lower airway is the dominant determinant of EBC pH, so that measurement of EBC pH is one of the main factors to predict the disease severity. There was a significant decrease in airway pH in the group of patients with atopic rhinitis and atopic dermatitis without any clinical signs of airway inflammation compared with the control group.[11-13]

Peroni *et al.* assessed and compared changes in inflammatory mediators and pH of breath samples in the 2 study groups. The first group consisted of 33 pediatric patients with atopic dermatitis compared with 23 pediatric patients who were in good health. First, both study

groups were asked to exhale through the mouth into a tube. The breath sample was then collected using a condenser at -4°C. The sample was streamed into a mass spectrometry (Perkin Elmer Sciex, Thornhill, ON, Canada) to assess changes in inflammatory mediators. Mass spectrometry charged a neutral molecule to detect it. The gaseous sample was exposed to high-energy electrons so that the sample molecules will be ionized and move rapidly into a magnetic field. Also, the acidity level of the breath samples was tested using a pH meter (Crison Instruments, Barcelona, Spain). The results were then analyzed with statistical calculations.[2] After analyzing the results of the mass spectrometry test, it was found that the leukotriene and 8-isoprostane significantly increased compared to the healthy group. Meanwhile, the pH test showed a decrease in acidity levels in the atopic group compared to the healthy group. These results suggested that inflammatory mediators such as leukotriene and 8-isoprostane can be used as parameters to diagnose atopic dermatitis.[2]

Miniello *et al*. observed the levels of ɣ interferon and IL-4 in healthy and atopic dermatitis patients using EBC samples. The EBC sample is collected using a condenser after the patient exhales into a valved tube to collect saliva. EBC sample that entered the condenser expected not to mix with saliva as much as possible. Approximately, 0.8 mL to 2.1 mL condensate was formed at -20°C in the form of ice. The collected condensate was transferred to an Eppendorf tube and stored at -80°C before further analysis. In the atopic dermatitis group, the levels of interferon gamma (IFN-ɣ) were lower than the control group. Meanwhile, IL-4 levels were higher in the atopic dermatitis group compared to the control group.[14]

Yan *et al.* analyzed NO and EBC pH levels in the atopic group compared to the control group. The study included pediatric patients aged 6 years to 13 years. Those patients were asked to exhale for 15 minutes in a sitting position. During this time, 1 mL to 3 mL of EBC was obtained. The EBC was then streamed by argon at a rate of 350 mL/ min to remove the $CO_2$ contained in the EBC. The acidity level of the EBC was analyzed using a pH meter, and the NO level was measured using a chemiluminescence analyzer (CLD 88 sp; ECO Physics; Durnten; Switzerland) internationally standardized. The results showed that there was a significant increase in NO in the atopic patient group compared to the control group.[15]

The results of the study by Wadsworth *et al.* also supported the results of the study of Yan *et al*., stating that there was a decrease in pH and an increase in NO in the EBC of atopic patients. NO is the airway inflammation biomarker mostly used for research. NO levels in expiratory breaths can be measured quickly in primary health facilities. Increased NO is often found in atopic patients, particularly in patients who have severe asthma and atopic patients who did not respond adequately to steroid administration. This increase in NO correlates with eosinophilia in both blood and airway.[10,16] Airway epithelium having increased percentage of eosinophils could induce the activity of NO synthase to release NO. However, the mechanism by which eosinophilia induces NO synthase activity in the airway epithelium is yet to be known.[10]

Apart from diagnosing atopic patients, NO can also be used as a biomarker to observe a patient's response to steroid therapy.[17] A low FeNO level of less than 25 parts per billion indicates that the patient is unlikely to suffer from eosinophilic asthma and is less likely to respond to steroids. A high FeNO (>50 parts per billion) strongly suggests airway eosinophilia and steroid responsiveness.[10] In cases of severe

asthma where the patient has used inhaled corticosteroid therapy, NO can be an indication of the patient's uncontrolled illness due to unsuccessful treatment of airway inflammation or due to non-inflammatory causes, helping clinicians in adjusting the dose of corticosteroid given.[10,17] If the therapy is inadequate, clinicians can choose to increase the dose of corticosteroid or give adjunctive anti-inflammatory therapy like the leukotriene receptor modifiers e.g montelukast. This class of drugs can help prevent allergic reactions, particularly when combined with other antiallergic drugs such as antihistamines and corticosteroids.[18] NO level was highly varied between individuals, reflecting the natural heterogeneity of baseline epithelial nitric oxide synthase activity and/or the contribution of a non-eosinophilic factor against epithelial nitric oxide synthase activity. This is the drawback of this NO level as a biomarker to detect atopic and monitor the responsiveness to steroid therapy. However, it could be overcome by using a reference value adjusted for the individual baseline NO level, rather than using a single reference value that applies to all patients.[19]

In addition to changes of EBC compounds, Carpagnano *et al.* stated that changes in the microbiota also occurred in atopic patients. Fungal colonization, which is a normal flora in the airway, was higher in 70% atopic subjects compared to the control group. This result suggested that the microbiota in EBC could also be a potential biomarker measured on EBC evaluation. However, this statement still required further study in a larger sample size.[20]

***Potential of Electronic Nose (E-Nose) in diagnosing atopic dermatitis*** The components in EBC have wide-ranged molecular weights that need further observation to determine which components in EBC are significant in the pathology of a disease. Due to the nature of sampling that is non-invasive, fast, and safe, EBC samples are potential to be diagnostic samples for monitoring health status. The use of modern high-sensitivity methods, such as mass spectrometry, enables the analysis and observation of tested EBC sample components.[21]

The compounds contained in EBC could be specific biomarkers for disease. They can be detected using various mass spectrometry techniques, such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and proton transfer reaction mass spectrometry (PTR-MS); ion mobility spectrometry; differential mobility spectrometry (DMS); electronic nose device; colourimetric tests: infrared spectroscopy (IR spectroscopy), such as selected ion flow tube-mass spectrometry (SIFT-MS), Fourier transform-infrared spectroscopy (FTIR), and ring-down cavity spectroscopy.[22,23]

Mass spectrometry works with ionizing molecules to produce charged molecules or molecular fragments and measures the mass to charge ratio, which can be used in conjunction with chromatographic separation techniques. Liquid chromatography-mass spectrometry (LC-MS) is an analytical method that combines the features of liquid chromatography and mass spectrometry to identify compounds. Proton transfer reaction-mass spectrometry (PTR-MS) is a very sensitive technique for online monitoring of SOV. Ion-mobility spectrometry (I-MS) is an analytical technique used to separate and identify ionized molecules in the gas phase based on their mobility in the carrier buffer gas. This technique can be combined with mass spectrometry and/ or chromatographic separation techniques.[18] Differential mobility spectrometry is distinguished by the difference between mobility in high and low electric fields since the value of ion mobility depends on the strength of the applied field. This method is easy

to use, sensitive, fast and relatively selective. However, the use of mass spectrometry has several limitations, including the large size of the instrument and is not easy to carry (non-portable). It is suggested that future device designs can be made more portable-friendly and easier to use by health workers in the field. In addition, processing the data by mass spectrometry requires multiple step processing and analysis software, as well as manual checking of the raw data. It is time-consuming, thus the researchers try to develop simpler software to process the diagnosis results from breathomics. [22,24,25]

Then, how can large-sized mass spectrometry with specific procedures be applied to health facilities? Recently a handheld version of the mass spectrometry had been developed with a simpler procedure called the electronic nose (E-nose) **(Figure 1)**.[26] This tool was often called "electronic sensing" or "e-sensing". E-nose was reported to be able to identify various types of compounds and microbiota from food to humans.[27,28] Its ability provided the possibility for researchers to use the E-nose as a research supporting tool, both in vitro and in vivo. It was predicted that the e-nose can be used as a rapid screening tool to detect various conditions from a patient's breath sample. As it was in the early stages of developing this technology, the E-nose still needed validation to meet health facility standards. The E-nose showed many advantages over competing technologies, for example, it could detect compounds related to cellular metabolism. Another technique of this method was the ability to identify the pathogen from the protein sequence it produced, regardless of the condition of the microorganism, alive or dead.[27]

**Figure 1**

The E-nose consists of three main parts: the sample delivery system, the detection system and the computing system. The breath sample is taken by asking the patient to exhale into the hole at the top of the device (mouthpiece). The results of this expiration will then flow into a tube with temperature control. In this part, the expiratory product is condensed by adjusting the temperature to below freezing point. The detection system consists of multiple sensors that will react when it comes into direct contact with a mediator in the breath sample. After that, the sample will be computed for analysis using the software.[29] Although the E-nose sensor is very sensitive to all breath sample mediators in various diseases, we can adjust which mediators we want to detect from patient samples associated with atopic dermatitis.[3,4]

The E-nose can be used multiple times, hence it is expected to reduce the cost of patient treatment and provide more accurate results. In addition, breath sampling for diagnostic tests was less invasive than blood tests and patch tests, making this method suitable for use in pediatric patients. The results of the examination could be read directly on the instrument and do not cause any discomfort to the patient.[3,30]

E-nose has the potential to be used en masse, especially for screening and monitoring therapy in atopic dermatitis patients in health facilities. This method is very practical, easy, and beneficial because it can detect the severity of the inflammatory reaction. In addition, it does not require specially trained health personnel. Changes in the composition of the mediators

such as an increase in NO, IL-4, leukotriene and 8-isoprostane, as well as a decrease in IFN-ɣ and pH in breath samples could provide relevant information to help diagnose atopic dermatitis. This practical method could be used as an alternative to blood tests and patch tests, especially for diagnostic examinations in primary health facilities. Furthermore, if the diagnosis of atopic dermatitis can be made earlier, treatment of the disease can be given earlier to prevent worsening and deterioration of the patient's quality of life. In addition, this method could also monitor the severity of atopic dermatitis helping the physicians to easily determine or adjust the therapy in follow-up evaluation. This method also minimized the risk of opportunistic infections in invasive sampling, especially in children and HIV/AIDS patients.[3,4,14,31]

## Conclusion

A practical, non-invasive method for diagnosing atopic dermatitis with a high level of sensitivity and specificity will greatly assist physicians to diagnose atopic dermatitis, particularly in a mass clinical setting. By using the EBC analysis method derived from the patient's respiratory expiration, the discovery of specific biomarkers such as an increase in NO, IL-4, leukotriene, and 8-isoprostane, as well as a decrease in IFN-ɣ and EBC pH can be strong indications for both diagnosing atopic dermatitis and monitoring the response to corticosteroid therapy. This method had the potential to be used as an alternative to patch test and blood test methods that require a longer time and causing discomfort for patients to diagnose atopic dermatitis.

## Suggestion

Standardization and optimization of this diagnostic method should be considered as it will greatly assist health workers in the field in screening and diagnosing patients suspected of having atopic dermatitis, as well as monitoring the response to corticosteroid therapy in patients with atopic dermatitis.

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