Pediatr Pol 2024; 99 (1): 61-65
DOI: https://doi.org/10.5114/polp.2024.135829
Submitted: 30.05.2023, Accepted: 13.08.2023, Published: 04.03.2024

PEDIATRIA Polska
Polish Journal of PAEDIATRICS

REVIEW PAPER

There's something in the air – the role of volatile organic compounds in exhaled air in the diagnosis and treatment of asthma in children

Katarzyna Gmachowska[1,2], Aleksandra Jarek[3], Joanna Jerzyńska[1], Paweł Majak[4], Daniela Podlecka[1]

[1]Department of Pediatric and Allergy, Korczak Pediatric Center, Copernicus Hospital, Medical University of Łódź, Poland
[2]International Doctoral Scholl, Medical University of Łódź, Poland
[3]Medical University of Łódź, Poland
[4]Department of Pulmonology, Korczak Pediatric Center, Copernicus Hospital, Łódź, Poland

ABSTRACT

Bronchial asthma, which is the most common chronic disease of the respiratory system, causes significant diagnostic problems in younger children due to the difficulty of performing typical respiratory function tests, including spirometry. This problem is especially important for paediatricians, allergists, and pulmonologists. A new, promising method of objectifying allergic inflammation in the respiratory tract is the measurement of volatile organic compounds (VOCs) in exhaled air. They are formed during the metabolic processes of the body's cells and the microorganisms that inhabit it. Clinical benefits of the VOC profile have been proven in differentiating asthma phenotypes, monitoring its course, predicting exacerbations, and responding to treatment. However, further studies are needed to standardise this process and establish reference standards. The aim of this study is to review the latest literature on the usefulness of VOC profiles in exhaled air and the composition of the microbiome in children with bronchial asthma.

KEY WORDS:

volatile organic compounds (VOCs), asthma, children's allergic diseases.

INTRODUCTION

Bronchial asthma is the most common chronic respiratory disease in children and adolescents [1]. In recent years, an increase in the incidence of asthma and the occurrence of asthma symptoms in increasingly younger age groups has been observed all over the world [2]. According to World Health Organisation data, in 2019, bronchial asthma was present in 262 million people worldwide, causing 455,000 deaths [2]. In the paediatric population, asthma affects 5–20% of children in Europe [1].

The diagnosis of asthma is significantly complicated in the paediatric population, especially in children under 5 years of age, due to the difficulty in performing respiratory function tests, including spirometry. According to the Standards for the Diagnosis and Treatment of Asthma of the Polish Society of Allergology, the Polish Society of Lung Diseases, and the Polish Society of Family Medicine (STAN3T) guidelines, emphasis is placed on the objectification of asthma-confirming tests, which, both in children < 5 years old and those aged 6–11 years old, can be very difficult due to the inability to perform spirometry correctly [3]. According to the Global Initiative for Asthma 2023 guidelines, the diagnosis of asthma in children under 5 years of age is made on the basis of the presence of episodes of wheezing without other features of in-

ADDRESS FOR CORRESPONDENCE:
Joanna Jerzyńska, Department of Pediatric and Allergy, J. Korczak Pediatric Center, Copernicus Hospital, 71 Piłsudskiego St., 90-329 Łódź, Poland, phone: +48 42 207 47 26, e-mail: alergol@kopernik.lodz.pl

fection in the history, the presence of allergic diseases, and the confirmation of symptom relief after treatment and their recurrence after discontinuation of drugs [1].

The diagnosis of asthma in younger children is largely based on medical history, indicating recurrent wheezing [1]. For this reason, for several years researchers have been searching for new methods that are useful in diagnosing asthma, assessing its severity, controlling treatment, and predicting exacerbations. A promising new diagnostic method for diagnosing asthma and predicting its exacerbations, both in adults and children, appears to be the measurement of volatile organic compounds (VOCs) in exhaled air.

VOLATILE ORGANIC COMPOUNDS

Volatile organic compounds are a heterogeneous group of gases that includes several thousand organic chemicals with high volatility, poor water solubility, and low boiling points [3, 4]. Regarding the origin of the compounds, 2 groups are distinguished: endogenous and exogenous.

Exogenous VOCs found in the air are produced by industrial plants during the production process of paints, finishing materials, and fuel production, among others. Exogenous VOCs also include vehicle exhaust and gases produced during volcanic eruptions. Volatile organic compounds, through interactions with other chemical compounds, form tropospheric ozone, primary and secondary pollutants, including pathogenic acetyl peroxide [5].

Chronic airway diseases, including bronchial asthma, are characterised by inflammation, the monitoring of which is extremely helpful in the management of these diseases. Currently available techniques to directly measure the presence of inflammation and oxidative stress in the airways include fractioned exhaled nitric oxide (FENO), bronchoscopy, bronchoalveolar lavage, and biopsy [6]. However, these techniques are invasive examinations and are therefore not recommended for routine monitoring of the patient's condition.

It has been shown that many diseases with acute or chronic inflammation involve the production of specific VOCs that can be identified in exhaled air by mass spectrometry (endogenous VOCs). Studies have observed the usefulness of a profile of endogenous VOCs in the diagnosis and monitoring of diseases such as bronchial asthma [4], chronic obstructive pulmonary disease [7], inflammatory bowel disease [8], and cystic fibrosis [7].

Analysis of exhaled air can help detect pathological metabolic processes taking place in the body in a non-invasive manner. The usefulness of assessing the profile of volatile respiratory compounds is based on changes in metabolic processes associated with a disease [4]. Volatile organic compounds are produced as a result of cell metabolism in the respiratory tract and by microorganisms living there [4]. Exhaled air samples are assessed for the presence of the compounds in question at different concentrations in the breath of a given group of patients compared to healthy control subjects [4].

VOLATILE ORGANIC COMPOUNDS IN EXHALED AIR

Endogenous VOCs are produced by the cells of the human body and the microorganisms that inhabit it through metabolic processes. People with bronchial asthma exhibit excessive production of reactive oxygen species, which react with lipid membrane structures, causing degradation of polyunsaturated fatty acids and resulting in the formation of VOCs, including hydrocarbons [9]. Volatile organic compounds are also produced by microorganisms in the human body. Once VOCs are formed, they are oxidised to smaller components by increased enzyme activity, including cytochrome P450 oxidase, or they pass directly into the bloodstream [9]. This is followed by their excretion in the exhaled air.

The airway microflora has been shown to be associated with the risk of bronchial asthma by influencing inflammatory processes [10]. The airway microbiome is influenced by mode of delivery, pH, temperature, mucus quality, local immune response of the mucosa, exposure to pathogens, and antibiotic treatment, among other factors. In early childhood, colonisation with *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and/or *Haemophilus influenzae* within one month has been shown to be a predictor of subsequent wheezing and the diagnosis of asthma at 5 years of age [10].

Patients with severe asthma, compared to healthy subjects or those with mild or moderate asthma, had significantly higher numbers of *Actinobacteria*, although the largest observed differences were in the genus *Klebsiella* (7.8-fold increase in severe asthma) [11].

The profile of VOCs in exhaled air can differentiate between bronchial asthmatic and healthy subjects. Dallinga *et al.* [12] analysed the results of VOCs in exhaled air performed in 63 asthmatic and 57 healthy children, identifying 8 promising organic compounds, mainly hydrocarbons, that differentiated the results of asthmatic and healthy children (sensitivity 89% and specificity 95%).

Currently, the presence of a specific profile of VOCs in asthma exacerbation is increasingly emphasised. In a study by Brinkman *et al*., monitoring of exhaled metabolites (methanol, acetonitrile, bicyclo-octan-1-ol, 4-methyl-C9H16O were observed to be significantly statistically increased) was shown to distinguish between loss of asthma control and clinically stable episodes [13]. The accuracy of distinguishing baseline, loss of control, and recovery was 68–77% for chromatography-mass spectrometry (GC-MS) and 86–95% for electronic nose (eNose) [14]. Significant associations between exhaled metabolites captured by GC-MS, and the presence of eosinophilia in sputum were also demonstrated (Pearson $r \geq 0.46$, $p < 0.01$) [13].

Due to the non-invasive nature of the examination of VOCs in exhaled air, the search for profiles that may correspond to inflammation in the airways, useful in the diagnosis and differentiation of asthma, is ongoing [4]. Paredi *et al.* [14] demonstrated that a profile of VOCs can assess asthma severity and control. The study reported elevated breath ethane concentrations in asthmatic patients not previously treated with steroids (n = 12, 2.06 ±0.30 ppb) compared to those treated with corticosteroids (n = 14, 0.79 ±0.10 ppb, $p < 0.01$). In addition, ethane levels were found to be higher in patients with severe asthma ((2.86 ±0.37 ppb) compared to patients with mild asthma (1.26 ±0.12 ppb, $p < 0.05$), suggesting that ethane may be a useful marker for detecting asthma [14].

Ibrahim *et al.* [15] showed 13 compounds that differentiated between patients with uncontrolled asthma (asthma control questionnaire ≥ 1) with 89% accuracy (AUC 0.90) (patients with asthma compared to healthy subjects showed an increase in benzyl alcohol, 3,4-dihydroxybenzonitrile, butanoic acid, benzoic acid, cyclohexanol, benzene, pentadecane), and lower concentrations of 2-butanone, butanoic acid, dodecane, cyclohexene, and 2,5-cyclohexadiene.

Volatile organic compounds also allow differentiation of the inflammatory phenotypes of asthma (eosinophilic asthma vs. neutrophilic asthma). Schleich *et al.* [16] showed that exhaled nonanal, 1-propanol, and hexane concentrations were elevated in patients with neutrophilic asthma, while hexane, 2-hexanone and 1-propanolol concentrations were reduced in eosinophilic asthma. In contrast, Ibrahim *et al.* [17] found 11 compounds in exhaled air that distinguished the profile typical of eosinophilic asthma with 83% classification accuracy (AUC 0.98) (including camphene, cyclohexanone, cyclohexen-4-methylene). In contrast, 14 compounds distinguished neutrophilic asthma with 72% accuracy (AUC 0.90) (including cyclopentene, naphthalene, cyclohexanol, tetradecane, and decahydro-8a-ethyl-1,1,4a,6-tetramethylnaphthalene) [17].

The volatile organic compound profile may be useful to determine the potential response to treatment in children with asthma. Bannier *et al.*, using a regulated multivariate analysis of variance, showed that respiratory volatile compounds in exhaled air can predict response to inhaled corticosteroids in children with wheezing [18].

Fractioned exhaled nitric oxide measurement is used in the diagnosis of patients with eosinophilic and Th2-dependent asthma [1, 19]. However, it should be remembered that factors such as age, gender, height, air pollution, and exposure to tobacco smoke and diseases such as allergic rhinitis, eosinophilic bronchitis, and viral infections may affect the result [19]. Fractioned exhaled nitric oxide is a well-standardised technique in cooperative children, but until now, no standardised methods have been recommended in uncooperative children.

The combination of the eNose method of measuring VOCs together with the FENO measurement showed the best diagnostic performance for asthma (95.7%) [16]. The results of the eNose measurement obtained in this study showed the highest diagnostic efficacy of this method (87.5%) in diagnosing asthma, compared to that observed when spirometry (70.8%), FENO (79.2%), or the combination of FENO and spirometry (83.3%) were performed [16].

METHODS FOR MEASURING VOLATILE ORGANIC COMPOUNDS

To date, many techniques have been described for the collection, detection, and analysis of VOCs in exhaled air.

The measurement of VOCs in exhaled air is a non-invasive and technically simple test [6]. It is carried out in a sitting position, with patients breathing calm through a facemask connected to a valve of a resistance-free plastic bag which is then sent for analysis. Prior to the test, the patient should not eat or drink for 2 hours, brush their teeth, or chew gum, so as not to falsify the results.

Volatile organic compounds in exhaled air are usually measured using GC-MS or eNose technology. These methods differ in the fact that VOCs show the presence of specific chemicals, while the eNose method only provides a pattern, for example, a fingerprint characteristic of asthma [6].

In the case of gas chromatography, the exhaled air is first collected and stored in specially designed sorbent bags or tubes. The gas sample is then assessed by gas chromatography, which is usually also followed by GC-MS or flame ionisation detection [5]. Volatile organic compounds are partitioned on the basis of their chemical properties, followed by ionisation and separation according to their mass-to-charge ratio (m/z) [5].

Breath samples can also be analysed with eNose. This method consists in creating the so-called breathprint or fingerprint of the analysed sample using special nanosensors that change the electrical resistance. Each of the resulting breath prints represents a mixture of volatile respiratory compounds and is therefore used in pattern recognition algorithms for many diseases [5, 6].

A study by Farraia *et al.* [20] describes a cohort of both paediatric and adult patients with symptoms suggestive of asthma, and using the eNose, the results showed good identification of patients with severe asthma.

Abdel-Aziz *et al.* [21] used a large research cohort of asthma patients and demonstrated through internal and external validation that the eNose method can detect the presence of atopy in asthma patients and can be used to define asthma phenotypes.

It should be emphasised that the profile of VOCs in exhaled air is influenced by exogenous factors such as food consumed prior to testing, environmental pollutants, smoking, detergents, and chronic non-pulmonary diseases such as liver failure or diabetes [13, 19]. The presence of bacteria alters the patterns of exhaled VOCs, so

breath analysis can be used to diagnose infections of bacterial aetiology [22, 23].

The amount and type of VOCs in exhaled air is also influenced by the pharmacological treatment used [24]. Lysine, glycolic acid, 4-carene, and octanal have shown a positive correlation with trace amounts of asthma medication in the urine of people with severe asthma, confirming that VOCs are associated with asthma medication [24].

ASSOCIATION OF MICROBIOME VARIABILITY WITH BRONCHIAL ASTHMA AND THE PROFILE OF VOLATILE ORGANIC COMPOUNDS

The total number of micro-organisms colonising the mucous membranes forms a complex ecosystem called the microbiota, interacting closely with the mucosa-associated lymphoid tissue (MALT) system. In light of current knowledge, any alteration of this ecosystem can induce and sustain chronic local inflammation with numerous systemic consequences [15].

Identification of the airway microbiota may facilitate the assessment of asthma status or asthma phenotype. Identification of the airway microbiome may help to explore its role in the development of asthma or small airway function [24].

Huang *et al.* [22] demonstrated by 16S rRNA analysis of sputum samples a higher bacterial diversity and increased abundance of *Proteobacteria* in asthmatic patients compared to non-asthmatic subjects. In addition, by analysing the bronchial microflora, they identified differences in microbial composition that showed a correlation with asthma severity. Compared to control patients, *Actinobacteria* and *Klebsiella* species were found in patients with severe bronchial asthma. A number of *Actinobacterium* taxa and reduced abundance of *Proteobacteria* were evidenced in studies in patients with severe asthma [22].

The importance of dysbiotic processes in the upper airways in the development of asthma in preschool children was confirmed by the response of the airway in sinusitis and asthma (RAISE) study. In a group of 133 pre-schoolers with chronic rhinosinusitis, a specific nasopharyngeal dysbiosis with a reduced *Patescibacteria*/*Actinobacteria* ratio was described [25]. This different ratio increased the risk of asthma independently of atopy and, as indicated by as yet unpublished data, was associated with a specific profile of VOCs [25].

The cited studies demonstrate a potential link between the presence of specific non-pathogenic microorganisms in the airways with an increased risk of developing asthma. Furthermore, airway dysbiosis influences the increased frequency of airway infections and the subsequent development of asthma.

Testing the microbiome inhabiting an organism is quite difficult and expensive, so new, easier ways of assessing it are being sought. Volatile organic compound testing provides an indirect assessment of the composition of the microbiome by analysing the profile of exhaled gases, which reflect biochemical reactions in the body and the metabolic activity of microorganisms.

Individual gases detected by VOC analysis are not indicative of microbiome disorders; only by considering their profile in exhaled air can the existence of a disease be presumed.

One of the problems in VOC analysis is the presence in the exhaled air of gases produced by microorganisms living in the oesophagus and stomach. It is therefore recommended that VOC testing be performed under reproducible conditions with appropriate recommendations. The current approach to VOC diagnosis is to analyse the concentration of VOCs of the different phases of exhalation.

CONCLUSIONS

There is a worldwide increase in the incidence of bronchial asthma and other allergic diseases and an increased interest in the problem of dysbiosis in the body. Consequently, new, simple, non-invasive, and low-cost methods are being sought for the diagnosis and treatment of common conditions. The testing of VOCs is a new promising method in the diagnosis and course monitoring of many diseases, including bronchial asthma. It has been shown that the VOC profile can reflect the type of inflammation in the airways, help to identify the asthma phenotype, and predict exacerbations. Volatile organic compounds are formed not only in the metabolic processes of the body's cells but are also produced by the microorganisms inhabiting it. For this reason, a change in the microbiota profile may be the reason for the development of asthma or its exacerbation.

There is a need for further studies of volatile compounds and microbiota profiles to develop standards useful in clinical practice.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Global Initiative for Asthma. Global Strategy for asthma management and prevention, 2023. Available from: http://ginasthma.org/.
2. Available from: https://www.who.int/news-room/fact-sheets/detail/asthma, Published 11.03.2022.
3. Pawliczak R, Emeryk A, Kupczyk M, et al. Standardy rozpoznawania i leczenia astmy Polskiego Towarzystwa Alergologicznego, Polskiego Towarzystwa Chorób Płuc i Polskiego Towarzystwa Medycyny Rodzinnej (STAN3T). Alergol Pol 2023; 10: 1-14.
4. Smolinska A, Klaassen EMM, Dallinga JW, et al. Profiling of volatile organic compounds in exhaled breath as a strategy to find early predictive signatures of asthma in children. PLoS One 2014; 9: e95668.
5. Pinthong N, Thepanondh S, Kondo A. Source identification of VOCs and their environmental health risk in a petrochemical industrial area. Aerosol Air Qual Res 2022: 22: 42-49.

6. Van de Kant KD, van der Sande LJ, Jöbsis Q, et al. Clinical use of exhaled volatile organic compounds in pulmonary diseases: a systematic review. Respir Res 2012; 13: 117.
7. Van Berkel JJ, Dallinga JW, Moller GM, et al. A profile of volatile organic compounds in breath discriminates COPD patients from controls. Resp Med 2010; 104: 557-563.
8. Kokoszka J, Nelson RL, Swedler WI, Skosey J, Abcarian H. Determination of inflammatory bowel-disease activity by breath pentane analysis. Dis Colon Rectum 1993; 36: 597-601.
9. Kinnula VL. Production and degradation of oxygen metabolites during inflammatory states in the human lung. Curr Drug Targets Inflamm Allergy 2005; 4: 465-470.
10. Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. N Engl J Med 2007; 357: 1487-1495.
11. Huang YJ, Nariya S, Harris JM, et.al. The airway microbiome in patients with severe asthma: Associations with disease features and severity. J Allergy Clin Immunol 2015; 136: 874-884.
12. Dallinga JW, Robroeks CMHT, van Berkel JJB, et.al. Volatile organic compounds in exhaled breath as a diagnostic tool for asthma in children. Clin Exp Allergy 2010; 40: 68-76.
13. Brinkman P, van de Pol MA, Gerritsen MG, et al. Exhaled breath profiles in the monitoring of loss of control and clinical recovery in asthma. Clin Exp Allergy 2017; 47: 1159-1169.
14. Paredi P, Kharitonov SA, Barnes PJ. Elevation of exhaled ethane concentration in asthma. Am J Respir Crit Care Med 2000; 162: 1450-1454.
15. Ibrahim B, Basanta M, Cadden P, et al. Non-invasive phenotyping using exhaled volatile organic compounds in asthma. Thorax 2011; 66: 804-809.
16. Schleich FN, Zanella D, Stefanuto PH, et al. Exhaled volatile organic compounds are able to discriminate between neutrophilic and eosinophilic asthma. Am J Respir Crit Care Med 2019; 200: 444-453.
17. Ibrahim W, Natarajan S, Wilde M, et.al. A systematic review of the diagnostic accuracy of volatile organic compounds in airway diseases and their relation to markers of type-2 inflammation. ERJ Open Res 2021; 7: 00030-2021.
18. Bannier MAGE, Kienhorst S, Jöbsis Q, et al. Exhaled breath analysis for investigating the use of inhaled corticosteroids and corticosteroid responsiveness in wheezing preschool children. J Clin Med 2022; 11: 5160.
19. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005; 171: 912-930.
20. Farraia M, Cavaleiro Rufo J, Paciência I, et al. Human volatilome analysis using eNose to assess uncontrolled asthma in a clinical setting. Allergy 2020; 75: 1630-1639.
21. Abdel-Aziz MI, Brinkman P, Vijverberg SJH, et al. eNose breath prints as a surrogate biomarker for classifying patients with asthma by atopy. J Allergy Clin Immunol 2020; 146: 1045-1055.
22. Huang YJ, Nariya S, Harris JM, et.al. The airway microbiome in patients with severe asthma: associations with disease features and severity. J Allergy Clin Immunol 2015; 136: 874-884.
23. Dallinga JW, Robroeks CMHT, van Berkel JJB, et.al. Volatile organic compounds in exhaled breath as a diagnostic tool for asthma in children. Clin Exp Allergy 2010; 40: 68-76.
24. Brinkman P, Ahmed WM, Gómez C, et.al. U-BIOPRED Study Group. Exhaled volatile organic compounds as markers for medication use in asthma. Eur Respir J 2020; 55: 1900544.
25. Majak P, Molińska K, Latek M, et al. Upper-airway dysbiosis related to frequent sweets consumption increases the risk of asthma in children with chronic rhinosinusitis. Pediatr Allergy Immunol 2021; 32: 489-500.