**Are levels of IL-13 and IL-4 in exhaled breath condensates (EBC) predictive for the formation of chronic inflammation in children with asthma?**

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**Abstract**

**Introduction:** It is difficult to detect asthma in young children. There are no standard prognostic methods for diagnosis. The aim of the study was the clinical and prognostic evaluation of IL-4 and IL-13 concentrations in children with recurrent wheezing.

**Material and Methods:** 96 children with recurrent wheezing were enrolled. 81 patients had transit wheezing, 15 had asthma. 25 healthy children were selected as controls. The concentration of IL-4 and IL-13 was analyzed in exhaled breath condensate (EBC) by enzyme-linked immunosorbent assay (ELISA). Data analysis was performed using Statsoft Statistica Version 8 (Tulsa, OK) and MedCalc statistical software version 17.2.

**Results:** Increased concentrations of IL-4 (p <0.001) and IL-13 (p <0.001) were significantly higher than controls when clinical manifestations occurred. IL-4 (H = 41.3862; p = 0.0000) and IL-13 (H = 29.9101; p = 0.0000) were significantly high and depended on the patient's affiliation with a particular group. The correlation between the concentration of IL-4 and IL-13 was proved (p <0.001). The prognostic value of the concentration of anti-inflammatory cytokines IL-4 and IL-13 (p <0.001) was performed.

**Conclusions:** An increase in the concentration of anti-inflammatory cytokines directly at the inflammatory locus in children with recurrent wheezing was determined. The dependence of the concentration of IL-4 and IL-13 on the formation of asthma has been proved.

**Keywords:** asthma, inflammation, IL-4, IL-13, children

**Introduction**

Asthma is one of the most common chronic diseases [1-5]. The number of severe and uncontrolled forms of asthma has increased, leading to greater disability and mortality among patients [1-2]. The debut of the disease takes place in childhood [3-5]. But at present there are difficulties in diagnostics of formation of asthma [1, 3, 6]. Firstly, the clinical signs of wheezing in children can be caused by many diseases, among which besides asthma there are hereditary and birth defects with metabolic processes, pathology of the gastroesophageal zone, congenital and acquired defects of the heart and main vessels and others [3-5, 7]. Secondly, in children under 6 years of age, there are limitations in the study of lung function. Therefore, at this stage of diagnosis in children under 6 years is based mainly on clinical and anamnestic data [6-8]. So, the global medical problem is to expand the diagnostic possibilities of early detection of asthma in children.

The pathogenesis of the disease, namely chronic inflammation, is a systemic process, but mainly it damages the respiratory tract [8, 9]. At the beginning of the formation of inflammation, the mechanisms do not dependent on the type of the damage (allergic, infectious, etc.) and have general signs of action of cytokine complexes [10-13]. After synthesizing proinflammatory interleukins (IL1, IL-6, IL-8, TNF-α, GM-CSF), anti-inflammatory (IL-4, IL-10, IL-13, TGF-b) are synthesized [11-13]. It is known that IL-4 function is to induce increased concentration of IgE, and IL-13 is a unique Th2-linked cytokine that interacts with B cells and thus regulates inflammatory and immune responses [14-16]. Recent studies have found an increase in serum concentration of these anti-inflammatory cytokines not only induce the inflammatory process but also support it, leading to chronic process. These anti-inflammatory interleukins that characterize chronic inflammation lead to airway remodeling and hyperplasia of the muscular system of the bronchopulmonary system [17]. Thus, it is advisable to study the concentration of these indicators directly in the inflammation locus.

The study of mediators and markers of inflammation released by damage of the respiratory system is possible due to the procedure of bronchoalveolar lavage or obtaining induced phlegm [18]. However, because of the fact that these studies are related to invasive intervention, they cannot be repeated in a short period of time, especially in pediatric patients. Considering non-invasive methods that can be used in this contingent of patients and able to reflect the state of local inflammation of the respiratory system, the receiving of exhaled breath condensate (EBC) is on the first place [18]. It is a promising source of biomarkers of lung disease. EBC is not a biomarker, but rather a matrix in which biomarkers can be identified [18, 19]. At present anti-inflammatory interleukins due to non-invasive techniques, namely their concentration in EBC, have been poorly understood. This is especially true for a cohort of young children. In this study, we conducted clinical and prognostic evaluation of interleukin 4 and interleukin 13 concentration in EBC children with recurrent wheezing episodes.

**Material and Methods**

**Study design.**

This was a longitudinal cohort study of children with wheezing recurrences from February 2015 to November 2019 who were treated at a children's hospital. The study consisted of two stages. In the first phase (recruitment, February 2015 - May 2015), among the 305 children who were admitted to the hospital with wheezing, 126 children between the ages of 1.5 and 6 who had the inclusion criteria and had no exclusion criteria were included to the study. Inclusion criteria: signing informed consent by the patient's parents; patient's age from 1.5 to 6 years; current wheezing episode at recruitment is third and more. Exclusion criteria: congenital and chronic cardiopulmonary or neurological disease; hereditary diseases that lead to changes in the functioning of the respiratory tract, including cystic fibrosis; proven immune deficiency; proven or suspected acute or chronic bacterial infection, including infection of the oral cavity and respiratory tract; suspected or confirmed gastroesophageal zone diseases; previous treatment with antileucotriene drugs or systemic corticosteroids. The patients underwent specific treatment according to worldwide recommendations [20]. All patients underwent clinical medical history, physical examination and laboratory assessment. Children were also examined for IL-4 and IL-13 concentration in the exhaled breath condensate. The study of anti-inflammatory cytokines was conducted on the first day of the disease in the presence of clinical manifestations of wheezing.

The control group comprised 25 healthy children (with similar age / gender) without any signs of chronic and a acute diseases for the previous three months, presented to Hospital for routine health control or vaccination. The parents of all patients and controls were informed about the study objectives and written informed consent was obtained before inclusion in the study.

Second stage (follow-up, May 2015 - September 2019). Patients were called for examination at the age of 5-6 years. The inclusion and exclusion criteria in the study were reevaluated. In the second stage, 96 children had the inclusion criteria and had no exclusion criteria. Anamnestic data for this period were studied. An examination was carried out to verify the diagnosis of asthma at the time of reexamination. The diagnosis was based on GINA 2019 criteria and included symptoms (cough and wheezing) for more than 10 days during upper respiratory tract infection, more than 3 episodes of wheezing per year, the child coughed between episodes, and there were present atopic dermatitis or food allergy history or family history of asthma [20]. Examination of the lung function with the detection of various ventilator disorders was conducted. An objective assessment of the severity of respiratory failure and differential diagnosis of obstructive and possible restrictive pulmonary ventilation disorders were performed. Patients were divided into groups depending on the asthma diagnosed during this period or diagnosed during the visit. The first group included 81 transient wheezing (TW) patients who stopped wheezing symptom at follow-up. The second group included 15 patients with doctor-diagnosed asthma (DDA).

**Exhaled Breath Condensate: Technical and Diagnostic Aspects.**

The device for EBC receiving was designed using a portable glass tube in accordance with material collection standards. The glass tube was cooled, surrounded by a mixture of refrigerants to obtain a temperature that reached from -5 °C to -10 °C. There, exhaled air in the form of droplets is converted to EBC [18, 21]. After collection, the glass tube was detached, and the sample was stored immediately at -70 ° C. Device has a manual control of condensing temperature and cleaning requirements of the device between consequent trials. All collections were done the day before 8:30 and 09:30 a.m. and EBC specimens were immediately frozen. Subjects wore a nose clip, which prevented contamination of the material with excretions from the nasal mucosa, and prevented the possibility of inhaling or exhaling through the nasal cavity. For children over three years of age, condensate was collected through systems with a single-use mouthpiece that connected to a one-way exhalation valve to prevent the effects of inhale on the condensate. But for subjects younger than two years, an exhalation valve with an inhalation mask was used.

**Measurement of IL-4 and IL-13 in exhaled breath condensate.**

IL-4 in EBC were analyzed by the ELISA technique using commercial kits (Human IL-4, “Vector Best Ukraine”, catalog number: А-8754), and IL-13 concentration were analyzed by the ELISA technique using commercial kits (Human IL-13, eBioscience (Bender MedSystems), catalog number: BMS231-3, USA) according to the manufacturer's instructions.

**Statistical analysis**

All statistical analyses were performed using StatSoft STATISTICA Version 8 package program (Tulsa, OK) and MedCalc statistical Software Version 17.2. Shapiro-Wilk’s test was used, and histogram and q-q plots were examined to assess the normality. As the sample distribution was different from the normal, the median (Me) and the interquartile range were determined (Lq - lower quartile; Uq - upper quartile). Fisher's exact test, χ2 was used to calculate two relative indicators. Non-parametric Mann-Whitney test (MW) was used to compare the two samples and Wilcoxon (T) nonparametric test was used to compare the two dependent samples. The difference between the two parameters was considered statistically significant at p <0.05. When comparing indicators that were compared by more than 2 points, H used the Kruskal — Wallis (KW) test of variance, and the differences were considered significant with the Bonferroni correction. The calculation of p was by two formulas. The first when equalizing between groups. The second when equalizing with a control group. The first formula was p = 0.05 / n, where n is the number of group. The second formula was p = 0.05 / n-1, where n is the number of groups. The correlation between parameters was determined using the Spearman rank correlation analysis (r); p < 0.05 was considered to indicate a statistically significant difference. Receiver operating characteristic (ROC) curves were drawn for variables to determine the optimal «cut-off» values to predict an endpoint. The endpoint of this study is the formation of chronic inflammation in children with recurrent wheezing. Statistical «cut-off» values were calculated by minimizing the distance between the point with specificity=1 and sensitivity=1 and various points on the ROC curve. For ROC analysis, an area under the curve (AUC) of 1.0 indicates perfect discrimination, whereas an area of 0.5 indicates that the test discriminates no better than chance. The cutoff point of each variable and sensitivity, specificity, positive likelihood ratio (+LR), negative likelihood ratio (LR) of this «cut-off» point was obtained using the Youden index. To determine the most reliable screening tool among these four variables, pairwise comparison of these variables was performed by determining the differences between area under the curve using the Hanley and McNeil method.

**Ethics approval and consent to participate**

The planned clinical studies were carried out after receiving approval by the local ethics committee (date: February 1, 2015; number: 2015/01) and were conducted in accordance with the principles of the Helsinki Declaration, amended in October 2013.

**Results**

**General information**

Of the 96 children with recurrent wheezing who enrolled to the study, the majority of children with transient wheezing and stopped wheezing symptom at follow-up, and a minority of children developed asthma. These groups accounted 84.38% and 15.62% respectively. No significant difference was found when comparing age and gender groups. Anamnestic factors such as pregnancy, cesarean delivery, and frequent manifestations of viral infections of the upper respiratory tract, presence of pets, passive smoking were statistically unreliable. When assessing environmental factors, such as the, were not statistically different when comparing groups. In allergic history, atopic dermatitis, food allergy, allergic rhinitis, the presence of allergic diseases and asthma in relatives, wheezing in the first year of life were likely to be more frequent in patients with DDA (table 1).

Such laboratory parameters as elevated eosinophil level and high IgE level were also significantly increased in patients with DDA (table 1).

**IL-4 and IL-13 concentration**

We found that in clinical manifestations in children with recurrent wheezing, a significantly increased concentration of IL-4 was observed. Statistical analysis revealed that IL-4 and IL-13 concentrations were significantly high. That is, the statistical characteristics of the respective indicators of different groups differed significantly, and the concentration of these indicators depended on the patient's belonging to a particular group. It was found that in children of both groups the concentration of IL-4 and IL-13 was increased compared to the control group, and the highest rates were observed in children with diagnosed asthma (table 2).

**Correlation between IL-4 and IL-13 parameters**

The correlation between IL-4 and IL-13 concentration of all children with recurrent wheezing was r=+0.37, p <0.001. Statistically more significant was the relationship between parameters in children with DDA, which was r=+0.74, p <0.001, in comparison with transient wheezing r=+0.17, р <0.001.

**Prognostic criteria for IL-4 and IL-13**

Statistical analysis was performed to determine the prognostic significance of the concentration of IL-4 and IL-13 anti-inflammatory cytokine (p < 0.001). The relationship between cytokine concentration during the first episodes of wheezing and the development of asthma was determined. Prognostic criteria for the concentration of IL-4 higher than 18.45 pg / ml and IL-13 higher than 20.17 pg / ml for the endpoint, namely the formation of chronic inflammation, have been identified. Critical concentration of these interleukins with significant indicators of sensitivity and specificity of the method were determined (graph 1).

**Discussion**

This study found that among children with recurrent wheezing, a greater proportion of patients had transient wheezing and only 15.62% was diagnosed asthma. There are many modern studies that describe the possible contributing factors in children with recurrent wheezing for formation of asthma. There are phenotypes that are considered as the debut of asthma. The proportion of such children ranges from 13% to 16% according to various sources [6, 22-24]. This fact has been confirmed in our study.

In our study, increased concentration of IL-4 in the exhaled breath condensate of children with recurrent wheezing in the onset of clinical manifestations were determined. The highest rates were found in children with established asthma. IL-4 is known to stimulate IgE production, promote eosinophil accumulation in peripheral blood and tissues [14-17, 25-26]. As a result, IL-4 is regarded as a cytokine that has a direct effect on the development of allergic inflammation [14-17]. Increased concentration of this cytokine has been shown to indicate the presence of allergic inflammation in patients. There are studies demonstrating changes in serum IL-4 concentration in atopic dermatitis in children [25], adults, and adolescents with asthma [14, 17]. Recent studies have proved that IL-4 concentration increase in the EBC of adult asthma patients that considered as a marker of chronic allergic inflammation [26]. In our study, children up to 6 years of age were examined, but IL-4 was also increased, which was likely to indicate an allergic nature of inflammation of the bronchopulmonary system. Increased IL-4 concentration in the exhaled breath condensate can be considered as a manifestation of an active inflammatory process, directly in the inflammation locus.

We found that in children with recurrent wheezing in the onset of clinical manifestations IL-13 concentration in exhaled breath condensate was also increased. In patients with established asthma there were highest rates also. IL-13 is a Th2-linked cytokine and regulates the body's inflammatory and immune responses. It stimulates the production of B cells and IgE, and inhibits the production of inflammatory cytokines [14-17]. Recent studies have confirmed the changes such as the increased IL-13 concentration in serum in atopic diseases in children [17, 25]. There are data that confirmed increased IL-13 concentration in the serum in adolescents and adults asthma [14, 17]. These studies suggest that changes in the concentration of this cytokine indicate the presence of allergic inflammation. We have also regarded it as a manifestation of allergic inflammation, with the activity of the process in the respiratory system, taking into account the detection of indicators in the locus of damage.

Our study evaluated the relationship between IL 4 and IL 13 in EBC children less than 6 years of age and established direct correlation between indicators. But more likely to be significant was correlative dependence in children with established asthma. The correlations of IL-13 and IL-4 were made taking into account the presence of similar pathways that are involved in the synthesis of IgE, activation of eosinophils, mucus secretion [14-16]. Recent studies have demonstrated these relationships in adult patients with allergic airway disease [17, 25], where IL-4 has been identified as the first cytokine to be produced by mast cells and is responsible for promoting the production of IL-13 of mast cells [15]. Our study confirms the relationship between these cytokines in the presence of allergic inflammation in children.

The ROC analysis determined the predictive concentrations of IL4 and IL13. The critical concentrations of IL-4 and IL-13 were determined according to the endpoint. The endpoint of this statistical analysis was defined as the formation of chronic inflammation in patients with recurrent wheezing. The criterion of IL-4> 18.45 pg/ml and IL-13>20.17 pg/ml were found to have prognostic significance in asthma formation in children with recurrent wheezing. The IL-13 criterion in comparison with IL-4 was more statistically significant. We determined that it has greater specificity and sensitivity, taking into account the ROC analysis and, therefore, is a more significant criterion

Non-invasive techniques are used to investigate the health status of adults and children. There are several non-invasive techniques for the determination of respiratory gases in exhaled air and compounds dissolved in exhaled lung secretion. There are studies using Electronic Noses (E-noses) that can play a potential role in the screening and analysis of various respiratory and systemic diseases [27-29].The EBC was chosen for our study.

This study has several limitations. Firstly, about 45% of children were with comorbid diseases, such as atopic dermatitis or food allergy, which may effect on higher concentration of IL 4 and serum IL 13. It may also effect on EBC level. Thus, the concentration of these cytokines obtained in children with asthma could have higher rates due to concomitant allergic diseases [14, 25]. However, there is no evidence based data that concentration of IL-4 or IL-13 can increase in EBC in children with comorbid allergic diseases. Substances in the EBC are in large dilution, as the excretory fluid covers both the alveolar epithelial layer and the mucous layer of the respiratory tract. There is no existing model that allows collecting all the exhalation, since the loss of moisture depends on the humidity and temperature of the environment. These factors are likely to have an influence on the obtained results [18-19, 21, 26]. Another limitation of the study is that asthma is a heterogeneous disease and has many phenotypes [9, 22, 30-31]. There are types that have neutrophil and paucigranulocytic phenotype in their mechanism of development. The most common is the eosinophilic type of inflammation, which is associated with eosinophilic cellular infiltration and thickening of the basement membrane area. The performed studies and the selected cytokines reflect just this type of inflammation. This study was conducted taking into account only the eosinophilic phenotype.

Consequently, among patients who have recurrent wheezing, approximately every sixth child transforms the disease into asthma. The concentration of the anti-inflammatory cytokines IL-4 and IL13 in exhaled breath condensate were significantly increased in children with manifestation of wheezing and the highest rates were found in asthma-forming children. Both cytokines are involved in the regulation of allergic inflammatory processes in the body, and have an influence on the respiratory tract, as it was detected basing on IL-4 and IL-13 concentration in the exhaled breath condensate. IL-4 concentration higher than 18.45 pg / ml and IL-13 concentration higher than 20.17 pg / ml in exhaled breath condensate in children with wheezing recurrence can be considered as a possible predictor of asthma formation.

Reference:

1. Jing Guo, Wenjing Zhu, Huimin Wang,et al. Risk factors and prognosis of recurrent wheezing in Chinese young children: a prospective cohort study. Allergy Asthma Clin Immunol. 2019; 15: 38. doi: 10.1186/s13223-019-0351-4

2. Nataliia Makieieva, Dmytro Butov, Yuliia Vasylchenko, et al. Endothelial dysfunction in children with clinically stable and exacerbated asthma. Adv Respir Med. 2019;87(1):7-13. doi: 10.5603/ARM.a2019.0002.

3. Ji Eun Soh, Kyung-Moon Kim, Ji-Won Kwon, et al. Recurrent wheeze and its relationship with lung function and airway inflammation in preschool children: a cross-sectional study in South Korea. BMJ Open. 2017; 7(10): e018010. doi: 10.1136/bmjopen-2017-018010

4. Mohamed A Hendaus, Fatima A Jomha, Mohammad Ehlayel. Allergic diseases among children: nutritional prevention and intervention. Ther Clin Risk Manag. 2016; 12: 361– 372. doi: 10.2147/TCRM.S98100

5. Jose A. Castro-Rodriguez, Erick Forno, Carlos E. Rodriguez-Martinez, et al. Risk and protective factors for childhood asthma: what is the evidence? J Allergy Clin Immunol Pract. 2016; 4(6): 1111–1122. doi: 10.1016/j.jaip.2016.05.003

6. Abdullah Al-Shamrani, Khalid Bagais, Ayed Alenazi, et al. Wheezing in children: Approaches to diagnosis and management. Int J Pediatr Adolesc Med. 2019; 6(2): 68– 73. doi: 10.1016/j.ijpam.2019.02.003

7. Lu Liu, Yilin Pan, Yanting Zhu, et al. Association between rhinovirus wheezing illness and the development of childhood asthma: a meta-analysis. BMJ Open. 2017; 7(4): e013034. doi: 10.1136/bmjopen-2016-013034

8. Satu Kalliola, L. Pekka Malmberg, Kristiina Malmström, et al. Airway hyperresponsiveness in young children with respiratory symptoms. Ann Allergy Asthma Immunol. 2019;122(5):492-497. doi: 10.1016/j.anai.2019.02.025.

9. Agache IO. From phenotypes to endotypes to asthma treatment. Curr Opin Allergy Clin Immunol. 2013;13(3):249-56. doi: 10.1097/ACI.0b013e32836093dd.

10. Tomotaka Kawayama, Takashi Kinoshita, Kazuko Matsunaga, et al. Role of Regulatory T cells in Airway Inflammation in Asthma. Kurume Med J. 2018; 64(3):45-55. doi: 10.2739/kurumemedj.MS6430001.

11. Kubo M. Innate and adaptive type 2 immunity in lung allergic inflammation. Immunol Rev. 2017 ;278(1):162-172. doi: 10.1111/imr.12557.

12. Han Gao, Songmin Ying, and Yuanrong Da. Pathological Roles of Neutrophil-Mediated Inflammation in Asthma and Its Potential for Therapy as a Target. J Immunol Res. 2017; 2017: 3743048. doi: 10.1155/2017/3743048

13. Anuradha Ray, Jay K. Kolls. Neutrophilic Inflammation in Asthma and Association with Disease Severity. Trends Immunol. 2017; 38(12): 942–954. doi: 10.1016/j.it.2017.07.003

14. Bagnasco D1, Ferrando M, Varricchi G, et al. A Critical Evaluation of Anti-IL-13 and AntiIL-4 Strategies in Severe Asthma. Int Arch Allergy Immunol. 2016;170(2):122-31. doi: 10.1159/000447692.

15. Jamie J.A. McLeod, B.N. Baker, John J. Ryan. Mast Cell Production and Response to IL-4 and IL-13. Cytokine. 2015; 75(1): 57–61. doi: 10.1016/j.cyto.2015.05.019

16. Jinfang Zhu. T helper 2 (Th2) cell differentiation, type 2 innate lymphoid cell (ILC2) development and regulation of interleukin-4 (IL-4) and IL-13 production. Cytokine. 2015; 75(1): 14–24. doi: 10.1016/j.cyto.2015.05.010

17. Naina Gour and Marsha Wills-Karp. IL-4 and IL-13 Signaling in Allergic Airway Disease. Cytokine. 2015; 75(1): 68–78. doi: 10.1016/j.cyto.2015.05.014

18. Davis MD1, Montpetit AJ2. Exhaled Breath Condensate: An Update. Immunol Allergy Clin North Am. 2018; 38(4):667-678. doi: 10.1016/j.iac.2018.06.002.

19. Ghio AJ1, Soukup JM, McGee J, et al. Iron concentration in exhaled breath condensate decreases in ever-smokers and COPD patients. J Breath Res. 2018; 12(4):046009. doi: 10.1088/1752-7163/aad825.

20. The Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2019. <http://www.ginasthma.org/>.

21. Brett R. Winters,1 Joachim D. Pleil,2 Michelle M. Angrish, et al. Standardization of the collection of exhaled breath condensate and exhaled breath aerosol using a feedback regulated sampling device. J Breath Res. 2017; 11(4): 047107. doi: 10.1088/17527163/aa8bbc

22. Depner M, Fuchs O, Genuneit J, Karvonen AM, et al. Clinical and epidemiologic phenotypes of childhood asthma. Am J Respir Crit Care Med. 2014;189(2):129-38. doi: 10.1164/rccm.201307-1198OC.

23. Granell R, Henderson AJ, Sterne JA. Associations of wheezing phenotypes with late asthma outcomes in the Avon Longitudinal Study of Parents and Children: A population-based birth cohort. J Allergy Clin Immunol. 2016; 138(4): 1060-1070.e11. doi: 10.1016/j.jaci.2016.01.046.

24. Ren CL, Esther CR Jr, Debley JS, et al. Official American Thoracic Society Clinical Practice Guidelines: Diagnostic Evaluation of Infants with Recurrent or Persistent Wheezing. Am J Respir Crit Care Med. 2016; 194(3):356-73. doi: 10.1164/rccm.2016040694ST.

25. Matsunaga MC, Yamauchi PS. IL-4 and IL-13 Inhibition in Atopic Dermatitis. J Drugs Dermatol. 2016 Aug 1;15(8):925-9.

26. Chun-Hua Chi, Ji-Ping Liao, Yan-Ni Zhao, et al. Effect of Inhaled Budesonide on Interleukin-4 and Interleukin-6 in Exhaled Breath Condensate of Asthmatic Patients. Chin Med J (Engl). 2016; 129(7): 819–823. doi: 10.4103/0366-6999.178962

# 27. [Behera B](https://www.ncbi.nlm.nih.gov/pubmed/?term=Behera%20B%5BAuthor%5D&cauthor=true&cauthor_uid=30620934), [Joshi R](https://www.ncbi.nlm.nih.gov/pubmed/?term=Joshi%20R%5BAuthor%5D&cauthor=true&cauthor_uid=30620934), [Anil Vishnu GK](https://www.ncbi.nlm.nih.gov/pubmed/?term=Anil%20Vishnu%20GK%5BAuthor%5D&cauthor=true&cauthor_uid=30620934), et al. Electronic nose: a non-invasive technology for breath analysis of diabetes and lung cancer patients. [J Breath Res.](https://www.ncbi.nlm.nih.gov/pubmed/30620934) 2019; 13(2):024001. doi: 10.1088/1752-7163/aafc77.

# 28. [Wilson AD](https://www.ncbi.nlm.nih.gov/pubmed/?term=Wilson%20AD%5BAuthor%5D&cauthor=true&cauthor_uid=30096939). Application of Electronic-Nose Technologies and VOC-Biomarkers for the Noninvasive Early Diagnosis of Gastrointestinal Diseases. [Sensors (Basel).](https://www.ncbi.nlm.nih.gov/pubmed/30096939) 2018; 8(8). pii: E2613. doi: 10.3390/s18082613.

# 29. [Behera B](https://www.ncbi.nlm.nih.gov/pubmed/?term=Behera%20B%5BAuthor%5D&cauthor=true&cauthor_uid=30620934), [Joshi R](https://www.ncbi.nlm.nih.gov/pubmed/?term=Joshi%20R%5BAuthor%5D&cauthor=true&cauthor_uid=30620934), [Anil Vishnu GK](https://www.ncbi.nlm.nih.gov/pubmed/?term=Anil%20Vishnu%20GK%5BAuthor%5D&cauthor=true&cauthor_uid=30620934), et al. Electronic nose: a non-invasive technology for breath analysis of diabetes and lung cancer patients. [J Breath Res.](https://www.ncbi.nlm.nih.gov/pubmed/30620934) 2019; 13(2):024001. doi: 10.1088/1752-7163/aafc77.

30. Ozdemir C, Kucuksezer UC, Akdis M, et al. The concepts of asthma endotypes and phenotypes to guide current and novel treatment strategies. Expert Rev Respir Med. 2018; 12(9):733-743. doi: 10.1080/17476348.2018.1505507.

31. Braido F, Tiotiu A, Kowal K, et al. Phenotypes/endotypes-driven treatment in asthma. Curr Opin Allergy Clin Immunol. 2018; 18(3):184-189. doi: 10.1097/ACI.0000000000000440.