Scientific Foundation SPIROSKI, Skopje, Republic of North Macedonia
South East European Journal of Immunology. 2023 Jun 06; 6(1):18-23.
https://doi.org/10.3889/seejim.2022.6033
eISSN: 1857-9388
Category: *Review article*
Section: *Basic Immunology*

OPEN ACCESS

The Potential of Fractional Exhaled Nitric Oxide as a Biomarker in Predicting and Optimizing Use of Treatment in Asthma

Hirowati Ali[1,2,3]*, Salsabila Faiha Wiendra Rasya[1], Muhammad Abi Ghoffari Siregar[1]

[1]Department of Biochemistry, Faculty of Medicine, Andalas University, Indonesia; [2]Graduate School of Biomedical Sciences, Faculty of Medicine, Andalas University, Indonesia; [3]Biomedical Laboratory, Faculty of Medicine, Andalas University, Indonesia

Edited by: Slavica Hristomanova-Mitkovska
Citation: Ali H, Rasya SFW, Siregar MAG. The Potential of Fractional Exhaled Nitric Oxide as a Biomarker in Predicting and Optimizing Use of Treatment in Asthma. SEE J Immunol. 2023 Jun 06; 6(1):18-23. https://doi.org/10.3889/seejim.2023.6033
Keywords: Asthma; Biomarkers; Fractional Exhaled Nitric Oxide
***Correspondence:** Hirowati Ali, Department of Biochemistry, Faculty of Medicine, Andalas University, Kec. Pauh, 25175, Padang, Indonesia. E-mail: hirowatiali@med.unand.ac.id.
Received: 15-Mar-2023
Revised: 30-Apr-2023
Accepted: 19-May-2023
Copyright: © 2023 Hirowati Ali, Salsabila Faiha Wiendra Rasya, Muhammad Abi Ghoffari Siregar
Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist
Open Access: This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

Abstract

Asthma affects the respiratory system and causes airway inflammation. The indication of asthma includes a triad of airway inflammation, hyperresponsiveness, and obstruction. Nitric Oxide (NO) is a gas that is exhaled and is a sign of airway inflammation. NO levels in the exhaled breath of patients with type 2 asthma are elevated, and fractional exhaled nitric oxide (FeNO) is an objective biomarker of airway inflammation. Measurements of FeNO are noninvasive, require minimal patient effort, and are easy to collect in clinical settings. The current review is a systematic review performed using PubMed, Science Direct, and Google Scholar according to The Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocol (PRISMA-P) guidelines. This review discusses the entanglement of understanding FeNO measurement and supplementing existing diagnostic and assessment tools for inflammatory lung diseases. Monitoring FeNO can also help identify different asthma phenotypes within the asthma syndrome and suggest the optimal administration of inhaled corticosteroids (ICS) as elevated FeNO levels indicate ICS response. Non-adherence to ICS is a significant contributor to the failure of asthma treatment. A FeNO suppression test can be done to determine non-adherence. FeNO levels should be used with a careful history, conventional spirometric testing with bronchodilator reversibility, measures of bronchial hyperreactivity using methacholine, and other measures of eosinophilic inflammation, such as a peripheral blood eosinophil cell count. FeNO is more sensitive and specific when paired with other lung function tests.

Introduction

Asthma is the most common significant non-communicable disease in children and adults, with high mortality and morbidity in severe cases. Globally, asthma is ranked 28th among the leading causes of burden diseases and 16th among the leading causes of disability each year. Around 300 million people have asthma worldwide; an additional 100 million more might be affected in 2025. Geographical variations influence asthma in its severity, prevalence, and mortality [1]. Asthma is included in the SDGs 2030 by the United Nations and the WHO Global Action Plan for preventing and controlling NCDs. Risk factors that cause asthma include genetic predispositions, lifestyle factors, overweight or obesity, exposure to tobacco smoke, low birth weight, prematurity, and other sources of air pollution (house dust mites, molds, and occupational exposure to chemicals, fumes, or dust [2].

Adult asthma is more prevalent in women than men, whereas childhood asthma is more prevalent in men, and this difference is thought to be caused by sex hormones [3]. Asthma prevalence remains high in adults and children, particularly in low- and middle-income nations, though it has decreased in some developed nations. Classifications of asthma based on its clinical, physiological, and biological characteristics are called phenotypes. Endotype asthma is more inclined to its pathophysiologic mechanisms at a cellular and molecular level. There are three classifications of asthma phenotypes in children: transient wheeze, atopic wheeze, and non-atopic wheeze. In adults, it also includes eosinophilic, early-onset allergic, AERD asthmatic phenotype, and neutrophilic, although these are still not well understood [4], [5].

Based on its airway inflammation, asthma can be classified into two groups, eosinophilic (T2-high), where sputum eosinophils, blood eosinophils, serum-specific IgE, and FeNO are its biomarkers and non-eosinophilic (T2-low), where its clinically applicable biomarkers are still not fully understood [6]. Interleukin-4, interleukin-13, and interleukin-5 are inflammatory cytokines involved in T2-high asthmas [6], [7]. Nitric oxide synthesis from airway epithelia is stimulated by interleukin-4 and interleukin-13, which are produced at low levels and function as a vasodilator, bronchodilator, and an inflammatory mediator, making FeNO a biomarker of T2-high [8], [9].

FeNO reflects T2-high asthma and is a non-invasive tool for ruling in asthma. The testing can be done by inhaling the patient's total lung capacity, followed by a constant exhalation for 6–10 s, with a result ready within 1 minute. Diagnosis for asthma cannot be made using only the scores of FeNO. However, it should be used alongside detailed history taking, spirometric testing (before and after bronchodilator responses), a methacholine challenge test, and a blood count indicating high eosinophil levels to assess lung mechanics and its hyperactivity [5], [9].

Methods

This literature review was done by collecting, reviewing, and citing related journals from search engines such as PubMed, Science Direct, and Google Scholar. Keywords for English considered papers were asthma, FeNO measurement, asthma biomarker, and asthma detection. The selection process according to PRISMA-P guidelines is shown in a flow diagram (Figure 1). Inclusion criteria were screened by journals that were used within the seven years of range, titles, and abstracts and published in English.

Papers excluded if articles were not written in English, review articles, meta-analyses, and did not focus on asthma, FeNO measurement, asthma biomarker, and asthma detection. Collected papers, organized and further analyzed. A total of 22 article journals were gathered in this literature review.

Results and Discussion

Pathogenesis of asthma

In general, asthma is distinguished into its phenotype, observable characteristics and also its endotype, which gravitates more into its underlying mechanisms. Cellular components that are involved in asthma are eosinophils, mast cells and to some extent, neutrophils, epithelial cells, and macrophages. The indication of asthma includes a triad of airway inflammation, hyperresponsiveness, and obstruction.

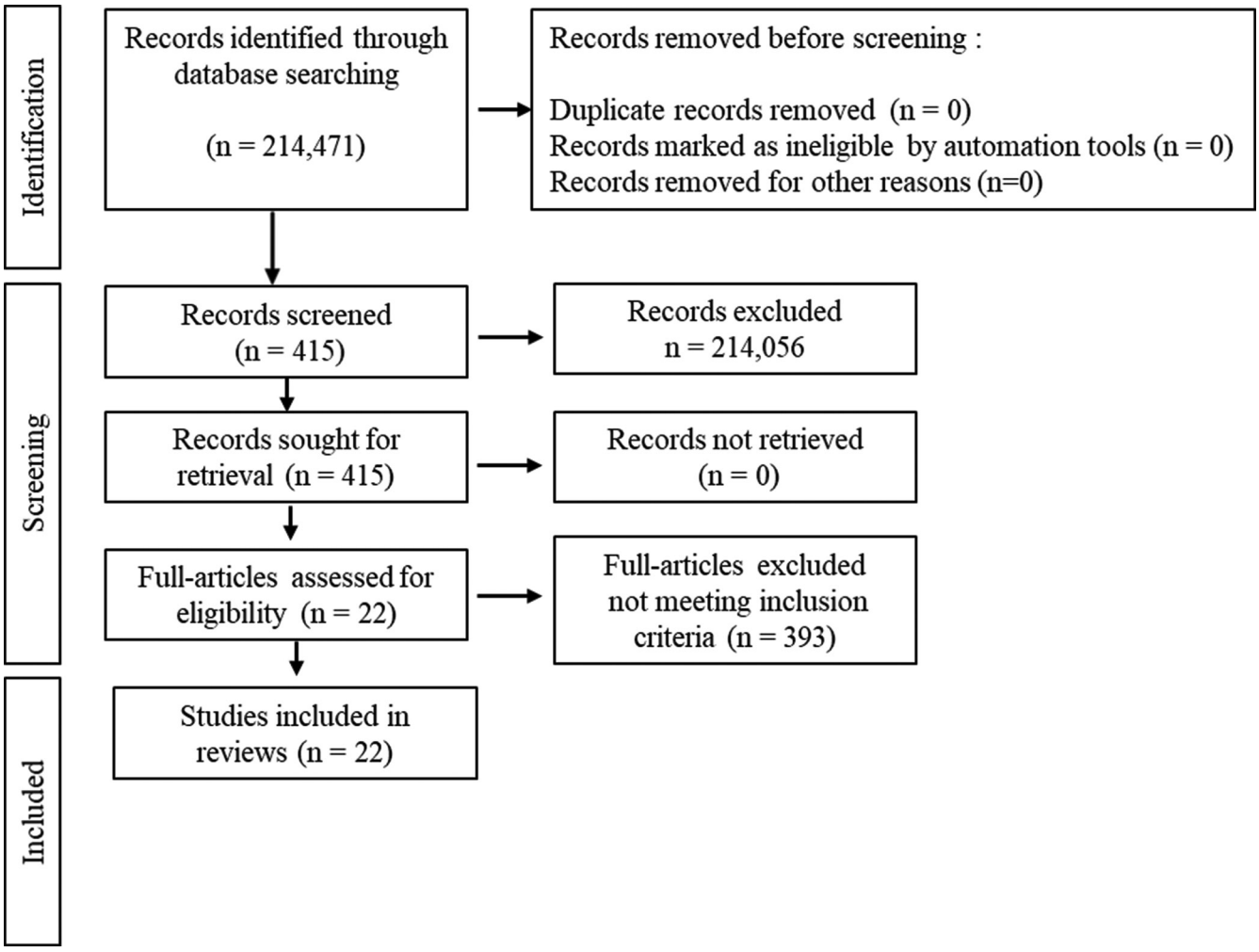

Figure 1: Selection of papers included in current review. Relevant papers were included asthma, FeNO measurement, asthma biomarker, and asthma detection

The pathogenesis of asthma is intricate and the causes of it are mostly due to genetics and environmental [10]. Genetics factors that play in asthma include atopy and gene abnormalities that affects the immune responses and epithelial barrier function that contributes to asthma[7], [11], [12], [13].

Knowledge of the heterogeneity of immunology in asthma has been advanced (Table 1). Allergic eosinophilic asthma occurs in at least 50% of patients with asthma, where interleukin-4 or interleukin-13, and interleukin 5 are the cytokines that are involved in this inflammatory process. These cytokines are produced due to allergic sensitization and simulations by dendritic cells with the help of coactivators such as adaptive T helper 2 cells and epithelium-derived thymic stromal lymphopoietin. Interleukin-4 has the role to activate mast cells. The recruitment of eosinophils in this type of asthma is mediated by eosinophil chemoattractants, namely prostaglandin D2. Where interleukin-5 is responsible for the maturation and survival of eosinophils. In non-allergic eosinophilic asthma, a response to prostaglandin D2, interleukin-33, interleukin-25, and thymic stromal lymphopoietin, is a production of interleukin-5 and interleukin-13, which are released after epithelial damage [14], [15].

Non-eosinophilic asthma is not fully understood yet. High levels of neutrophils are produced from the release of cytokines from T helper 17 cells, T helper 1 cells, or type 3 innate lymphoid cells that are activated by macrophages and neutrophil chemokines like C-X-C motif chemokine ligand (CXCL)-8. In bronchiectasis, a common comorbidity of severe asthma in adults, an increased amount of neutrophils indicates that a bacterial colonization exists or is an effect of corticosteroids which promotes the survival of neutrophils and suppresses type 2 immunity [14], [15], [16], [17].

Nitric oxide synthesis

Nitric oxides (NO) concentrations are high in the respiratory and cardiovascular systems and are produced in the lungs from a group of enzymes, nitric oxide synthases, through the oxidation process from L-arginine to L-citrulline [10], [11], [12]. Nitric oxide synthases (NOS) are expressed in three isoforms, which were initially referred to either constitutive or inducible forms of NOS. Categorization of the constitutive forms is based on their expression site, neuronal or endothelial, where both are produced by pulmonary cells, namely NOS1 and NOS3, respectively. NOS1 works as a muscle relaxant while NOS3 is assumed to act primarily through modulating the frequency of ciliary beats. As NOS_1 and NOS_3 are both corticosteroid resistant, steroids have no effect on NO release. Under normal settings, the inducible form (iNOS or NOS_2) is presumably produced continuously by human airway epithelial cells and is induced by pro-inflammatory cytokines. In contrast to constitutive nitric oxide synthase (cNOS), NOS_2 is glucocorticoid sensitive. Once NOS_2 is activated, NO production climbs to nanomolar concentrations within several hours, levels that are substantially greater than the levels caused by cNOS NO production [18].

As a bronchodilator and an inflammatory mediator, nitric oxide is essential in lung biology. The bronchodilator nature of NO is supported by animal data at low concentrations. On the other hand, nitric oxides appear to cause inflammation in high quantities. Exhaled nitric oxide levels that are elevated are thought to be the outcome of oxidative pathway overactivity, which results in pro-inflammatory cytokines and the subsequent stimulation of NOS_2 activity. Its qualities have allowed it to be quantified in exhaled breath and used as a diagnostic and management tool for respiratory disease [18], [19].

Measurement of fractional exhaled nitric oxide (FeNO)

Nitric oxide (NO) is a gas that can be exhaled through breathing. Measuring the fraction of NO during a steady-state respiration, called fractional exhaled NO (FeNO), is a standard and quantitative method for assessing the NO level in exhaled breath. The source of FeNO is derived from the action of several different nitric oxide synthase (NOS) enzymes, but the main source of increased NO levels identified in asthma comes from the inducible nitric oxide synthase-2 (iNOS2) which is associated with induction

Table 1: Characteristic and pathological features of asthma immunopathology [7], [14], [15], [16], [17]

Characteristic pathological features of asthma	Etiology	Sputum cytology	Transcriptomic profiles
Allergic eosinophilic inflammation	Allergens	Eosinophil++Neutrophil – Epithelial damage++Mucus+Reticular basement membrane thickening++Airway smooth muscle mass ++	Production of IL-4, IL-5, IL-13, prostaglandin D2 (PGD2), upregulation of Muc5ac expression, synthesis IgE
Non-Allergic eosinophilic inflammation	Pollutants, microbes	Eosinophil++Neutrophil – Epithelial damage++Mucus+Reticular basement membrane thickening++Airway smooth muscle mass ++	Production of IL-5, IL-33, ILC2, prostaglandin D2 (PGD2), synthesis IgE
Non-eosinophilic asthma/ paucigranulocytic	Pollutants, oxidative stress	Eosinophil - Neutrophil – Epithelial damage+Mucus +/- Reticular basement membrane thickening +/- Airway smooth muscle mass +	IL-1β, IL-6, IL-8, IL-17 and interferon γ (IFN-γ)
Mixed granulocytic asthma	Pollutants, oxidative stress, microbes	Eosinophil+Neutrophil+Epithelial damage++Mucus++Reticular basement membrane thickening+Airway smooth muscle mass +	Production of hCRP, IFN-γ, IL-6, PGD2
Type 1 and type 17 neutrophilic inflammation	Pollutants, oxidative stress, microbes	Eosinophil - Neutrophil++Epithelial damage++Mucus++Reticular basement membrane thickening+Airway smooth muscle mass +	Activation of Th1 and Th7, production of ILC 3, IL-17, IL-23, CXCL-8

MCP4=Monocyte chemoattractant protein 4; ILC2=Type 2 innate lymphoid cells; CXCL8=C-X-C motif chemokine ligand 8; ILC3=Type 3 innate lymphoid cells.

in the airway epithelium of inflammation. Functions that have been associated with NO include action as a neurotransmitter, bronchodilator, vasodilator, and mediator of inflammation. FeNO is well connected with airway eosinophilic inflammation as measured in induced sputum, a non-invasive way to measure T2 airway inflammation in asthma. There is strong evidence that FeNO levels are associated with T2 inflammatory features, specifically eosinophilia levels in induced sputum and peripheral blood [18], [20], [21].

The FeNO test is a point-of-care test that assesses the concentration of nitric oxide gas in a patient's exhaled breath sample. The patient is asked to breathe steadily and slowly into a mouthpiece that is connected to a device that performs the measurement in order to obtain this sample. This test can determine if there is an airway inflammation and whether additional treatment is needed or not. After careful review of the medical history, conducting an examination, and using the common lung function test known as spirometry, the healthcare practitioner may recommend a FeNO test for children aged 5 or older if the diagnosis of asthma is not certain. If spirometry is not available, a FeNO test is an advice [20], [21].

Association of high levels of fractional exhaled nitric oxide (FeNO) to asthma

In detecting asthma, many factors are considered to comprehensively diagnose it. Biomarkers that are often used in detecting asthma are increased fractional exhaled nitric oxide (FeNO), high blood eosinophil count, and high levels of serum total IgE. FeNO is the preferable biomarker used whether or not airway inflammation is present [18], [19]. In adults, the normal level of FeNO is <25 parts per billion (ppb) and is considered elevated when the score is greater than 50 ppb. The levels of FeNO ranges between 20 and 35 ppb where a score above 35 is deemed increased in children according to the American Thoracic Society Guidelines [20].

FeNO is a biomarker of the T2-high phenotype in association with other biomarkers. If a patient is diagnosed with asthma with an increased biomarker other than FeNO (high level of serum total IgE, normal or mild blood eosinophilia), then the risk of developing severe asthma will increase [20], [21]. Science is now focusing on the typical features of T2-low asthma and T2-high asthma that extends to mild-severe asthma. A study demonstrates that multiple T2-high phenotypes can occur. Three biomarkers (FeNO, peripheral blood eosinophils, and allergen-specific IgE) identified seven combinations: four overlapping and three homogeneous (Figure 2). Increased levels of FeNO are proven to be a biomarker that can detect T2-high asthma that can lead to asthma exacerbations with addition of increased levels of other biomarkers other than FeNO [18], [19], [20], [21].

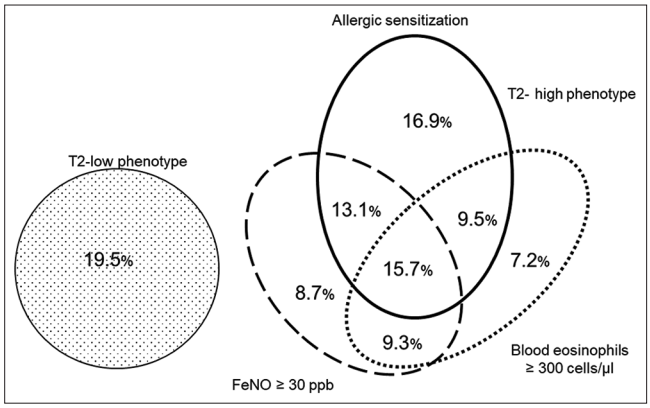

Figure 2: Clusterization of T2-low phenotype and seven subgroups in T2-high phenotype [18]

The potential use of FeNO and its comparison to other biomarkers

A biomarker is a quantifiable sign that can be used to assess normal or pathological biological processes or the pharmacologic response to treatment intervention. The discovery of valid biomarkers is required for the use of precision medicine in the management of asthma. Only a few biomarkers are indicative of T2-high asthma and can be useful in clinical settings for its management, such as IgE, eosinophils in blood and/or sputum, Fractional Exhaled Nitric Oxide (FeNO), and periostin. Their utility in diagnosis, prognosis, and therapy is still debatable [21], [22].

Spirometry, peak flow measurements, and changes in these variables after a steroid trial have been shown to be insensitive and weak to FeNO in detecting asthma when bronchial hyperresponsiveness and bronchodilator reversibility are used as the gold standard. FeNO is a noninvasive technique and is quite simple to perform, including in patients with significant airway obstruction, children, and pregnant women. FeNO has a minimal predictive value for sputum eosinophilia and is associated with increased airway hyperresponsiveness and the likelihood of aggravation in both children and adults. Elevated FeNO levels also suggest ICS response. ICS normally decreases FeNO levels, assessing them serially can be beneficial as an indicator of adherence to treatment among asthmatics [21], [22], [23].

Although there is a variability of normal ranges, there is substantial evidence for high positive and negative predictive values for diagnosing and treating asthma at both poles of the FeNO value spectrum. High FeNO levels (>50 ppb) indicates poor control, existence of prolonged inflammation, and the need for more anti-inflammatory medication. Low values (<25 ppb) indicates low levels of inflammation and may allow anti-inflammatory medication to be discontinued. An effective biomarker should be capable of identifying the disease as well as its particular endotype or phenotype, beneficial in disease monitoring and prognosis, and should be obtained easily with minimal stress or risk to the patient.

To this date, several biomarkers were used in asthma diagnosis such as eosinophils, serum IgE, and periostin. Unfortunately, an optimal biomarker does not yet exist, and overlap among biomarkers is a reality. Using biomarker panels in the era of precision medicine could enhance the recognition of asthma endotypes [22], [23].

Sensitivity and specificity of FeNO and other pulmonary function tests

FeNO is a simple, noninvasive biomarker for evaluating eosinophilic lower airway inflammation which produces consistent and reliable results. Diagnostic tests for small airway function includes maximal mid-expiratory flow (MMEF), mid-expiratory flow at 25% (MEF25), mid-expiratory flow at 50% (MEF50), and mid-expiratory flow at 75% (MEF75). There have been a few research on the clinical utility of pairing FeNO testing with small airway function tests in children for the detection and management of cough variant asthma [22], [23].

Furthermore, FeNO ROC analysis was performed in combination with small airway parameters (MMEF75%/25%, forced expiratory flow (FEF) 50%, FEF25%, impulse oscillometry of resonance frequency (Fres), R5-R20, or X5) to improve the value of cough variant asthma (CVA) distinction from typical asthma (TA). When combined with MMEF75%/25% (combine1), the AUC of FeNO was 0.912 (0.873-0.952), when paired with FEF50% (combine2), it was 0.893 (0.849-0.936), when paired with FEF25% (combine3), it was 0.914 (0.875-0.953), when paired with Fres (combine4), it was 0.742 (0.673-0.810), and when combined with R5-R20 (com (0.640–0.782). The AUC of the combines was significantly higher than that of FeNO or the small airway variables FEF25%, Fres, R5-R20, and X5 [23], [24].

Optimization of inhaled corticosteroid dosage

Uncontrolled T2-high inflammation is associated with a higher likelihood of severe asthma exacerbations, hence FeNO focused treatment should result in fewer exacerbations. FeNO may also be effective in reducing inhaled corticosteroid (ICS) medication in people with controlled eosinophilic asthma whose FeNO levels have reverted to normal. This tailored strategy may prevent patients from receiving steroid medication inadvertently or at higher-than-necessary doses. It explains the algorithm of treatment according to FeNO levels. The issue is establishing a reliable FeNO cut-off point for adjusting the treatment up or down. Tailoring cut-off points based on an individual's FeNO reactions to early ICS therapy, would be the best method due to the variations of asthma. This would enable specific identification of limit points for that individual, as well as therapy customization based on FeNO variation. Non-adherence to ICS is a significant contributor to asthma treatment failure, accounting for up to 65% of cases. A FeNO suppression test can be done to detect non-adherence given that FeNO levels fall in a dose-dependent manner after ICS treatment and rise after steroid withdrawal. It is made up of 7 days of observed ICS medication with daily FeNO readings, where a drop in FeNO levels at the end of the week indicates non-adherence [23], [24].

Conclusion

Asthma is a respiratory disease that affects both children and adults. Generally, It is classified into phenotypic and endotypic asthma. There are two types of asthma based on its phenotype, T2-high and T2-low. FeNO is one of the applicable biomarkers found in T2-high that is noninvasive and requires minimal patient effort. Levels of FeNO not only suggest initial diagnosis but also its severity, response to treatment, and compliance. A score >50 ppb is deemed increased in adults, while in children, it is said to have elevated when the score is >25 ppb. Sensitivity and specificity of FeNO alone is not as high as it is when combined with other pulmonary functional tests.

Authorship Contribution Statement

The authors confirm contribution to the paper as follows: Hirowati Ali: Supervision, writing-reviewing and editing original draft. Salsabila Faiha Wiendra Rasya: writing-original draft. Muhammad Abi Ghoffari Siregar: Writing-original draft. All authors reviewed the results and approved the final version of the manuscript.

References

1. Dharmage SC, Perret JL, Custovic A. Epidemiology of asthma in children and adults. Front Pediatr. 2019;7:246. https://doi.org/10.3389/fped.2019.00246
   PMid:31275909
2. World Health Organization. Asthma. Geneva: World Health Organization; 2022. Available from: https://www.who.int/respiratory/asthma/causes/en [Last accessed on 2023 Jan 14].
3. Yung JA, Fuseini H, Newcomb DC. Sex hormones, gender and asthma. HHS Public Access. 2019;176:139-48.
4. Kuruvilla ME, Lee FE, Lee GB. Understanding asthma phenotypes, endotypes, and mechanisms of disease. Clin Rev Allergy Immunol. 2019;56(2):219-33. https://doi.org/10.1007/s12016-018-8712-1

PMid:30206782

5. Padem N, Saltoun C. Classification of asthma. Allergy Asthma Proc. 2019;40(6):385-8. https://doi.org/10.2500/aap.2019.40.4253
   PMid:31690376
6. Breiteneder H, Peng YQ, Agache I, Diamant Z, Eiwegger T, Fokkens WJ, *et al*. Biomarkers for diagnosis and prediction of therapy responses in allergic diseases and asthma. Allergy. 2020;75(12):3039-68. https://doi.org/10.1111/all.14582
   PMid:32893900
7. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. Lancet. 2018;391(10122):783-800. https://doi.org/10.1016/S0140-6736(17)33311-1
   PMid:29273246
8. Busse WW, Kraft M, Rabe KF, Deniz Y, Rowe PJ, Ruddy M, *et al*. Understanding the key issues in the treatment of uncontrolled persistent asthma with Type 2 inflammation. Eur Respir J. 2021;58(2):2003393. https://doi.org/10.1183/13993003.03393-2020
   PMid:33542055
9. Rupani H, Chauhan AJ. Measurement of FeNO in asthma: What the hospital doctor needs to know. Br J Hosp Med (Lond). 2019;80(2):99-104. https://doi.org/10.12968/hmed.2019.80.2.99
   PMid:30746998
10. Russell RJ, Brightling CE. Pathogenesis of asthma: Implications for precision medicine. Clin Sci (Lond). 2017;131(14):1723-35. https://doi.org/10.1042/CS20160253
    PMid:28667070
11. Hoyte FC, Gross LM, Katial RK. Exhaled nitric oxide: An update. Immunol Allergy Clin North Am. 2018;38(4):573-85. https://doi.org/10.1016/j.iac.2018.06.001
    PMid:30342580
12. Tiotiu A. Biomarkers in asthma: State of the art. Asthma Res Pract. 2018;4:10. https://doi.org/10.1186/s40733-018-0047-4
    PMid:30598830
13. Busse WW. Biological treatments for severe asthma: A major advance in asthma care. Allergol Int. 2019;68(2):158-66. https://doi.org.10.1016/j.alit.2019.01.004
    PMid:30792118
14. Choy DF, Hart KM, Borthwick LA, Shikotra A, Nagarkar DR, Siddiqui S, *et al*. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. Sci Transl Med. 2015;7(301):301ra129. https://doi.org/10.1126/scitranslmed.aab3142
    PMid:26290411
15. Mastalerz L, Celejewska-Wójcik N, Ćmiel A, Wójcik K, Szaleniec J, Hydzik-Sobocińska K, *et al*. Non-eosinophilic asthma in nonsteroidal anti-inflammatory drug exacerbated respiratory disease. Clin Transl Allergy. 2023;13(3):e12235. https://doi.org/10.1002/clt2.12235
    PMid:36973957
16. National Heart, Lung, and Blood Institute. What is Fractional Exhaled Nitric Oxide (FeNO) Testing? United States: National Institute of Health; 2020. p. 20-HL-8146.
17. Högman M, Lehtimäki L, Dinh-Xuan AT. Utilising exhaled nitric oxide information to enhance diagnosis and therapy of respiratory disease-current evidence for clinical practice and proposals to improve the methodology. Expert Rev Respir Med. 2017;11(12):101-9. https://doi.org/10.1080/17476348.2017.1281746
    PMid:28076986
18. Ricciardolo FL, Sprio AE, Baroso A, Gallo F, Riccardi E, Bertolini F, *et al*. Characterization of T2-low and T2-high asthma phenotypes in real-life. Biomedicines. 2021;9(11):1684. https://doi.org/10.3390/biomedicines9111684
    PMid:34829913
19. Denton E, Price DB, Tran TN, Canonica GW, Menzies-Gow A, FitzGerald JM, *et al*. Cluster analysis of inflammatory biomarker expression in the international severe asthma registry. J Allergy Clin Immunol Pract. 2021;9(7):2680-8.e7. https://doi.org/10.1016/j.jaip.2021.02.059
    PMid:33744476
20. Khatri SB, Iaccarino JM, Barochia A, Soghier I, Akuthota P, Brady A, *et al*. Use of fractional exhaled nitric oxide to guide the treatment of asthma: An official American thoracic society clinical practice guideline. Am J Respir Crit Care Med. 2021;204(10):E97-109. https://doi.org/10.1164/rccm.202109-2093ST
    PMid:34779751
21. Gao J, Wu F. Association between fractional exhaled nitric oxide, sputum induction and peripheral blood eosinophil in uncontrolled asthma. Allergy Asthma Clin Immunol. 2018;14:21. https://doi.org/10.1186/s13223-018-0248-7
    PMid:29796021
22. American Thoracic Society. New Clinical Practice Guideline on Fractional Exhaled Nitric Oxide (FeNO) to Help Guide Asthma Treatment. New York: American Thoracic Society; 2021.
23. Zhu H, Zhang R, Hao C, Yu X, Tian Z, Yuan Y. Fractional exhaled nitric oxide (FeNO) combined with pulmonary function parameters shows increased sensitivity and specificity for the diagnosis of cough variant asthma in children. Med Sci Monit. 2019;25:3832-8. https://doi.org/10.12659/msm.913761
    PMid:31120043
24. Wang Y, Zhao L, Chen F, Guo Y, Ma H, Han B, *et al*. Diagnostic value of fractional exhaled nitric oxide and small Airway function in differentiating cough-variant asthma from typical asthma. Can Respir J. 2021;2021:9954411. https://doi.org/10.1155/2021/9954411
    PMid:34457097