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

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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) or 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 asthmatics [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.

![Flow diagram of selection process](image.png)

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 oxide (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 NOS1 and NOS3 are both corticosteroid resistant, steroids have no effect on NO release. Under normal settings, the inducible form (iNOS or NOS2) is presumably produced continuously by human airway epithelial cells and is induced by pro-inflammatory cytokines. In contrast to constitutive nitric oxide synthase (cNOS), NOS2 is glucocorticoid sensitive. Once NOS2 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 NOS2 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>
<thead>
<tr>
<th>Characteristic pathological features of asthma</th>
<th>Etiology</th>
<th>Sputum cytology</th>
<th>Transcriptional profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic eosinophilic inflammation</td>
<td>Allergens</td>
<td>Eosinophil++Neutrophil –</td>
<td>Production of IL-4, IL-5, IL-13, prostaglandin D2 (PGD2), upregulation of Muc2 expression, synthesis IgE</td>
</tr>
<tr>
<td>Non-Allergic eosinophilic inflammation</td>
<td>Pollutants, microbes</td>
<td>Eosinophil++Neutrophil –</td>
<td>Production of IL-5, IL-23, IL-22, prostaglandin D2 (PGD2), synthesis IgE</td>
</tr>
<tr>
<td>Non-eosinophilic asthma/ paucigranulocytic</td>
<td>Pollutants, oxidative stress</td>
<td>Eosinophil –, Neutrophil –</td>
<td>IL-10, IL-6, IL-8, IL-17 and interferon γ (IFN-γ)</td>
</tr>
<tr>
<td>Mixed granulocytic asthma</td>
<td>Pollutants, oxidative stress, microbes</td>
<td>Eosinophil++Neutrophil++</td>
<td>Production of MCP4, IFN-γ, IL-6, PGD2</td>
</tr>
<tr>
<td>Type 1 and type 17 neutrophil inflammation</td>
<td>Pollutants, oxidative stress, microbes</td>
<td>Eosinophil++Neutrophil++</td>
<td>Activation of Th1 and Th7, production of ILC3, IL-17, IL-23, CXCL-8</td>
</tr>
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</table>

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]](image)

**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 (0.849-0.938), 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) use 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**

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