

# Nitrogen oxide content in the expired air of patients with asthma and chronic obstructive pulmonary disease, as related to disease progression

## Abstract

The nitrogen oxide content (Feno, ppb) was assessed in 113 people, with 26 healthy individuals (the control group), 64 patients with asthma, and 23 patients with COPD. Among the patients with asthma, 10 had mild disease severity, 50 had moderate disease severity and 4 had severe asthma. Twenty patients had both asthma and COPD. The results of the study demonstrated that Feno depends on a number of factors in asthma sufferers:

- i. Disease severity, with patients with moderate and severe asthma having much higher Feno values than patients with mild asthma
- ii. Disease phase, with Feno values being significantly higher during asthma exacerbation than during asthma remission
- iii. Responsiveness to treatment, with patients with difficult-to-treat asthma demonstrating significantly higher Feno values.
- iv. With COPD displayed low Feno levels, which were significantly lower than in asthma patients. Patients with COPD had low Feno levels even with a high cellular sputum content, which indicates significant airway inflammation.

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**Abbreviations:** A, bronchial asthma; A<sub>1</sub>, bronchial asthma without cough and sputum; A<sub>2</sub>, bronchial asthma with cough and sputum; COPD, chronic obstructive pulmonary disease; NO, nitric oxide; FENO, nitric oxide in the expiratory air; PPB, content of nitric oxide

## Introduction

Nitrogen oxide (NO) is well known as an atmospheric pollutant, which is contained in automobile exhaust fumes and cigarette smoke. Over the past 20 years, since the numerous biological functions of NO have become known, this gas has been studied in healthy individuals and in the presence of pathology. Nitrogen oxide was discovered during an experiment to activate guanylate cyclase to form cyclic guanosine monophosphate in 1977.<sup>1</sup> The year 1980 can be considered as the beginning of NO studies for clinical purposes, when it was discovered that some vasodilators do not have an effect when the vascular epithelium is damaged.<sup>2</sup> It was found that nitrogen oxide is the factor synthesised by the coronary artery epithelium, which causes vessel dilation.<sup>3</sup> The first publication about NO content in human and animal expired air was published in 1991.<sup>4</sup> In 1993, the Journal Science called NO "molecule of the year". It was later discovered that NO is formed not only in the endothelium, but also in other cells, such as the epithelium, neurons, monocytes and lymphocytes.<sup>5</sup> It was determined that NO is one of the most important mediators in the cardiovascular, respiratory, nervous, immune, gastrointestinal and genitourinary systems.<sup>6</sup>

Nitric oxide is formed from the guanidine atom of the L-arginine oxide by the nitric oxide synthase. The nitric oxide synthases are grouped according to their physiological properties into a constitutive, including neuronal (type I) group, and endothelial group (type III), and an inducible group. Endogenous nitric oxide, after a complex

conversion cascade, forms stable compounds such as nitrates, nitrites, S-nitrosothiols, and nitrotyrosines.<sup>7</sup> Of particular interest is the information about which internal and external factors can lead to changes in the nitric oxide content in expired air (Feno). These factors may include genetic variations, age, gender, atopy, weight, height, smoking, and type of diet. The effect of smoking and non-infective allergens on Feno levels has been confirmed by all researchers.<sup>8</sup> Studies in children and adults have shown that sensitization to household allergens is accompanied by an increase in Feno levels.<sup>9</sup> Children with bronchial asthma display increased Feno after sensitization to cat and Dermatophagoides allergens.<sup>10</sup> Another study showed the effect of sensitization to cat and dog allergens on an increase in Feno.<sup>11</sup> Feno levels were shown to drop a week after moving from the city to the countryside.<sup>12</sup> Children with asthma, living in ecologically clean environments, demonstrate lower levels of Feno than children living in polluted areas.<sup>13</sup> Inhaling formaldehyde and acetaldehyde vapour has been shown to increase Feno levels in both healthy schoolchildren and those with asthma.<sup>14</sup> Berhane K et al.,<sup>15</sup> believe that increased air pollution may be a cause of increased Feno levels, which are associated with airway inflammation. The hereditary predisposition to respiratory NO formation dysfunction can be explained by the well-studied genetic link in allergic conditions. Increased Feno levels were noted in practically healthy children, whose parents suffered from allergic conditions.<sup>16</sup> The data gathered so far indicates that NO is an essential component of bodily functions. Barnes P<sup>17</sup> believes that NO, when produced in physiological quantities by the constitutive NO synthase, participates in maintaining cellular equilibrium. NO regulates the proliferation of endothelial cells and angiogenesis, and is overall responsible for normal vessel function.<sup>18</sup> NO plays a key role in almost all aspects of lung biology, as well as in the pathophysiology of numerous lung conditions, such as bronchial asthma.<sup>19</sup> NO also

plays the role of a neurotransmitter,<sup>20</sup> and nitric oxide is a mediator in the non-adrenergic and non-cholinergic systems.<sup>21</sup> NO is well known as an inflammatory mediator.<sup>19</sup> In this way, excessive NO production as part of the inflammatory and immune processes can be seen as a protective mechanism, despite the fact that the 'price' of this defence is damage to the respiratory tract with high concentrations of NO and its metabolites.

Lilly C et al.,<sup>22</sup> determined that bronchial dilation can occur due to NO accumulation. Experiments on models of isolated trachea and bronchi showed that NO can be seen as a mediator preventing bronchoconstriction.<sup>23</sup> Nitric oxide, when secreted by normal vascular endothelium, has important vasodilator activity.<sup>24</sup> In 1998, the American scientists Robert Furchgott, Louis J. Ignarro and Ferid Murad received the Nobel Prize for studying the endothelium-derived relaxing factor, which is directly related to nitric oxide. In normal physiological conditions, NO acts as a weak mediator of smooth muscle relaxation and prevents bronchial hyperactivity.<sup>19</sup> Nitric oxide also has notable antimicrobial activity<sup>25</sup> and impedes replication of the AIDS virus.<sup>26</sup> While vital for normal function, nitric oxide can have a damaging effect on various cells and tissues in the presence of pathology, and can cause lipid peroxidation and damage to cellular membranes, as well as having a cytotoxic effect. This is caused less by NO itself and more by its metabolites, such as superoxide.<sup>27</sup> Active forms of nitrogen (NO, peroxynitrite and others) participate in the formation of a lipid peroxidation cascade, damaging the bronchial mucosa, causing epithelial desquamation and leading to inflammation in the presence of obstructive pulmonary disease.<sup>28</sup>

In the area of inflammation, a high NO concentration alters the secretory and metabolic activity of alveolar macrophages.<sup>29</sup> The synthesis of inflammatory mediators increases, cyclooxygenase activity is stimulated, and leukotriene production increases.<sup>30</sup> Excessive NO production retards lymphocyte proliferation.<sup>31</sup> As an intercellular mediator, NO assists eosinophilic and neutrophilic infiltration of the airways.<sup>32</sup> The excessive accumulation of NO, caused by the expression of NO synthase, can lead to direct airway constriction via increasing vascular permeability, as well as inflammatory oedema, as a result of reactive oxygen species accumulation and an increased production of proinflammatory cytokines.<sup>33</sup> NO plays a key role in all aspects of lung biology and in the pathophysiology of numerous respiratory diseases, including asthma.<sup>19</sup> NO passes into the expired air from the respiratory epithelium, where it is formed as a result of inflammatory NOS2 activation.<sup>34,35</sup> A link has been established between Feno and eosinophilia in the airways of people with asthma, COPD,<sup>36</sup> and eosinophilic bronchitis.<sup>37</sup> A correlation has been established between Feno and blood eosinophilia,<sup>38</sup> eosinophilia in bronchoalveolar lavage,<sup>39</sup> and bronchial biopsy samples.<sup>40</sup> A low value can be seen as evidence of the absence of eosinophilic inflammation and the likely lack of effect from IGCS.<sup>41</sup> Independent from the features of airway inflammation, Feno demonstrates dynamic relationships between a response to an allergen or other triggers, and the activation of eosinophilic inflammation in the airways, otherwise known as bronchial hyperactivity.<sup>19,42</sup>

A lot of data has been gathered about the clinical assessment of Feno levels. Feno can be used to assess the risk of asthma development. Children with high levels of Feno develop asthma twice as often as children with low levels of Feno.<sup>43</sup> Feno is higher in children with a hereditary predisposition to allergic conditions and episodes of wheezing.<sup>44</sup> High levels of Feno indicate the presence of

subclinical respiratory inflammation even in the absence of symptoms and with normal lung function.<sup>45</sup> Some researchers suggest that Feno should be used to diagnose asthma<sup>46,47</sup> and it has been shown that measuring Feno levels can differentiate asthma sufferers from healthy patients.<sup>48,49</sup> There is a noted correlation with Feno levels and asthma severity, with more severe asthma corresponding to higher Feno levels.<sup>50,51</sup> Smith et al.,<sup>47</sup> believe that Feno has greater diagnostic potential than spirometry and is comparable to studying sputum eosinophilia. Another study determined that Feno values are well correlated with the results of functional lung studies and the presence of eosinophils in patient sputum.<sup>52</sup> It has been established that Feno can be used in the differential diagnosis in patients with symptoms of cough but no definite diagnosis.<sup>53</sup> Perer-de-Liano et al.,<sup>54</sup> recommend the use of Feno as a diagnostic test in patients with undiagnosed respiratory symptoms. It has also been recommended that Feno is used to evaluate disease progression in patients with asthma. When patients with difficult-to-treat asthma were treated with oral glucocorticoids, clinical improvement was accompanied by a normalisation in the spirometry readings and a reduction in Feno levels.<sup>55</sup> There is also data to show that this indicator can be used to monitor the status of patients with atopic asthma.<sup>56</sup> Alongside the positive evaluations of using Feno for diagnosis, treatment selection and monitoring, there are other studies that contradict the absolute value of this indicator. Taylor et al.,<sup>57</sup> believe that normal Feno values do not exclude the presence of asthma in a patient. A patient may have typical asthma symptoms and yet have normal Feno levels. Kwok et al.,<sup>58</sup> are of the opinion that Feno concentrations do not correlate with other indicators, which characterise condition severity in children with acute asthma. Petsky HL et al.,<sup>59</sup> compared the effectiveness of treating children and adults with asthma, with and without considering Feno levels, and found that treatment based on Feno levels was no more effective.

According to a group of authors Dweik et al.,<sup>60</sup> further study of Feno is important.

- i. The most frequent reasons for determining Feno levels:
- ii. To determine the eosinophilic phenotype of the asthma;
- iii. To evaluate the effectiveness of anti-inflammatory therapy, especially IGCS;
- iv. To determine the initial Feno levels in patients with chronic, stable asthma, for later comparisons;
- v. As a criterion for changing anti-inflammatory medication doses (step up, step down, cessation)
- vi. As a way to monitor patient compliance with medical recommendations regarding anti-inflammatory therapy;
- vii. To assess how much respiratory inflammation is responsible for poor asthma control, especially in the presence of complicating factors (rhinosinusitis, anxiety, gastroesophageal reflux, obesity, ongoing allergen presence).

The authors of the same article give an answer to the question of whether Feno can be used for asthma diagnosis. As we find the answer particularly relevant and corresponding to our ideas, we quote it here verbatim: "The diagnosis of asthma is done clinically and cannot be based on just one criterion. Asthma pathophysiology is often, but not always, based on eosinophilic inflammation in the airways. The right interpretation of Feno results is therefore vital. According to many experts, Feno is a diagnostic test for asthma, but if the disease is not

associated with eosinophilic inflammation, the level of Feno may stay low. We recommend that Feno is used to predict the effectiveness of treatment with GCS in patients with chronic symptoms of respiratory pathology, which is likely caused by airway inflammation.” In order to interpret the data obtained during Feno testing, it is important to know normal values for a particular person. Feno levels can be affected by a number of factors: evaluation methods, the flow of expired air, the addition of nasal NO, the type of analyser, age, height, smoking, medications, etc.<sup>61</sup> There are contradictory findings regarding the effect of gender, age and many other indicators on Feno values. The presence of multiple factors that affect changes in Feno levels preclude this indicator from being used in everyday clinical practice. The expert committee believes that clinically significant threshold levels are more important than specific values, when interpreting Feno.<sup>41</sup>

Recommendations were developed for the clinical application of Feno levels.<sup>41</sup>

- i. Low Feno levels (<25ppb in adults and <20ppb in children) indicate that eosinophilic inflammation and successful treatment with GCS is unlikely.
- ii. Feno levels of >50ppb in adults and >35ppb in children make eosinophilic inflammation likely, and GCS may be effective in treating clinical symptoms.
- iii. Values between 25 and 50ppb in adults, and between 20 and 30ppb in children need to be considered together with the clinical situation. High Feno values have a particular interest. The following explanations are given for this fact.
- iv. Non-compliance with the IGCS treatment regimen.<sup>62</sup>
- v. Continuing or increasing contact with aerial allergens.<sup>63</sup>
- vi. A highly reactive disease phenotype.<sup>60</sup>
- vii. High levels of Feno associated with factors other than eosinophilic inflammation in the airways.

When analysing the results of repeat Feno testing, it is important to consider the variability of the obtained results with and without pathology, the so-called variability coefficient, which equals to 10% in healthy people and can increase to 20% in patients with asthma.<sup>64</sup> We assessed Feno levels in patients with asthma and COPD. The study sought to determine the following:

Feno levels in practically healthy people, for the purpose of determining normative values;

- i. Feno levels in patients with asthma, depending on disease severity, in patients with COPD, and in patients with both conditions;
- ii. Changes in Feno levels during treatment and disease remission;
- iii. Feno levels in patients with asthma and COPD, depending on the degree of bronchial inflammation (as shown in cytology sputum studies)
- iv. Feno levels in relation to the content of neutrophils and eosinophils in sputum.

The study included 113 patients, of which 26 were healthy (control group), 64 had asthma, and 23 had COPD. Among the asthma sufferers, 10 had mild asthma, 50 had moderate asthma, and 4 had severe asthma. Fifteen patients had non-productive cough (A<sub>1</sub>),

twenty-nine patients had productive cough (A<sub>2</sub>), and twenty patients had concomitant asthma and COPD (A+COPD). Testing was repeated in 62 patients with asthma and 22 patients with COPD, before and after treatment, during the exacerbation and remission phases.

Statistica 5.1 Stat Soft, Inc. was used for statistical analysis. The critical significance level was considered 0.05 when interpreting the results. Descriptive statistics for quantitative data used mean and standard deviation (M ± s). Student's t-test and Pearson's correlation analysis was also used. Patients with A<sub>1</sub> and A<sub>2</sub> were predominantly women, while patients with COPD and A+COPD were predominantly men, with the average age between 17 and 61 years. Patients with A<sub>1</sub> and A<sub>2</sub> tended to be younger than patients with COPD and A+COPD. We conducted a general clinical examination and assessed Fenopbb using the NO breath device (Bedfont Scientific Lts, Great Britain).

Patients with spontaneous sputum had cytology testing to determine the degree of airway inflammation. The degree of inflammation was based on the number of leukocytes in the visual field count of the sputum sample:

- i. First degree inflammation: 3-5-8 leukocytes in the visual field count—mild inflammation, 10-12-15—mild-to-moderate inflammation;
- ii. Second degree inflammation: >15-18-20 leukocytes in the visual field count—moderate inflammation, >20-25-30 leukocytes in the visual field count—moderate-to-severe inflammation;
- iii. Third degree inflammation: >30-40-50 leukocytes in the visual field count—significant inflammation, 60-70-80 leukocytes in the visual field count as a solid layer—severe inflammation.

Feno levels in healthy individuals are shown in Figure 1. Healthy participants displayed a significant variability in Feno values: 23% had <4ppb, while 12% had >16ppb. From these findings, we considered >16ppb as raised Feno levels. Feno levels in patients with asthma, as compared to Feno levels in patients with COPD, depending on disease severity and the combination of asthma with COPD during an episode of asthma exacerbation, are presented in Table 1. The information in Table 1 demonstrates that patients with mild asthma had significantly lower levels of Feno than patients with moderate and severe asthma. Patients with A<sub>1</sub> had higher levels of Feno than patients with COPD. Patients with asthma and a productive cough (A<sub>2</sub>) had significantly lower levels of Feno than A<sub>1</sub> patients. Feno levels in A<sub>1</sub> patients were higher than in patients with COPD, but this difference was not statistically significant. Five patients with uncontrolled asthma, who were then prescribed oral prednisolone, had Feno levels of 56.87±28.01, while the other 56 patients with controlled asthma had Feno levels of 20.819±18.01, which is less than half (p=0.000204). Changes in Feno levels throughout treatment and upon disease remission are shown in Table 2. The raised Feno level in patients during disease exacerbation and before treatment is significantly reduced after treatment, during the remission phase. Feno levels in patients with COPD before treatment also reduce during treatment and as disease remission occurs, but this decrease is not statistically significant. The significant reduction in Feno levels becomes obvious when the changes are evaluated in the group as a whole (84 patients). Table 3 demonstrates the comparison between Feno levels and bronchial inflammatory activity, as measured by sputum cytology. The data in Table 3 demonstrates the cellular sputum content in patients with asthma did not significantly influence Feno levels, while patients with COPD have a convincing reduction in the

ability to produce NO, and their Feno levels were not increased even with significant levels of cellular infiltration of the sputum (3<sup>rd</sup> degree of inflammation).

In 16 patients with asthma and productive cough, a correlation was made between the percentage content of neutrophils and eosinophils in the sputum, and Feno levels. It appears that there is a difference in the correlation between Feno levels and neutrophils as compared to eosinophils. With increased neutrophil content, there is a tendency to lower Feno levels, while increased eosinophil content leads to a statistically significant increase in Feno levels (Figure 2). The reduction in Feno levels associated with an increased neutrophil content in sputum correlates with our view that neutrophils have a protective function in patients with mild atopic asthma, thus retarding the development of inflammation.<sup>65</sup> The increase in Feno levels associated with higher eosinophil content in sputum supports the widespread view that Feno can identify the eosinophilic asthma phenotype.<sup>41</sup>

**Table 1** Feno values in patients with asthma, depending on disease severity, in patients with COPD, and in patients with both asthma and COPD, during an episode of asthma exacerbation.

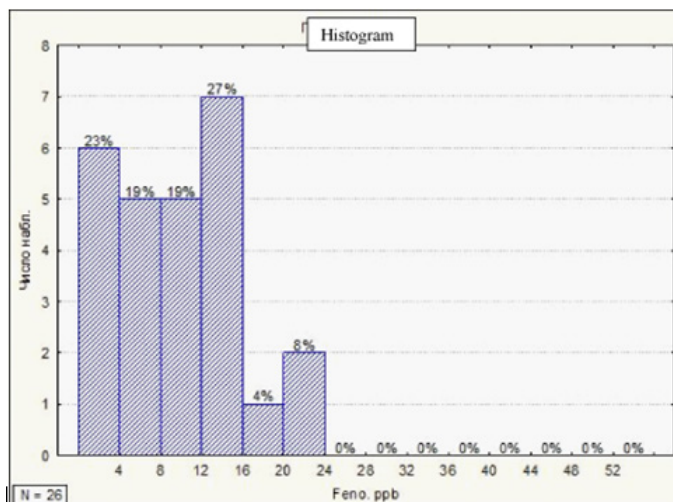
Disease	Number of patients	M	Standard deviation	P
Mild asthma	9	9.222	7.981	
Moderate +Severe asthma	35	28.457	20.241	0.008
A <sub>1</sub>	15	34.099	23.927	
COPD	23	16.391	13.763	0.006343
A <sub>1</sub>	15	34.099	23.927	
A <sub>2</sub>	29	19.57	15.8	0.02
A <sub>1</sub>	15	34.099	23.927	0.15587
A+COPD	20	21.684	25.778	

**Table 2** Feno levels in patients with asthma and COPD, before and after treatment (from the exacerbation phase to the remission phase, M ±standard deviation)

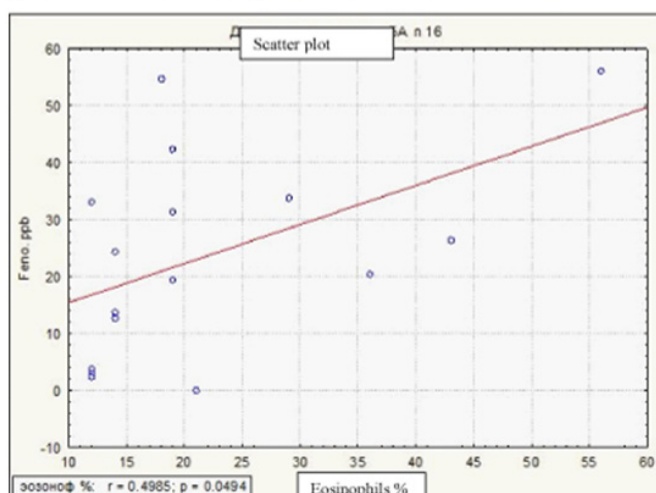
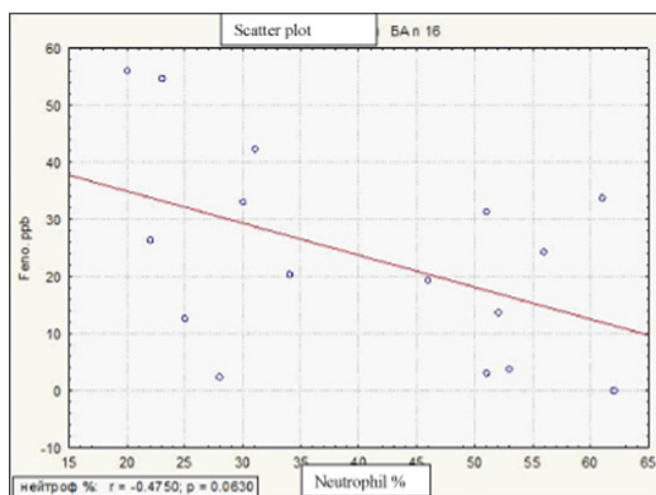
Disease	Number of patients	Before treatment	After treatment	P
Asthma	42	23.643±17.923	14.454±12.001	0.001
COPD	22	16.848±13.907	11.621±10.274	0.085
All patients	84	21.397±19.178	13.321±11.954	0.000035

**Table 3** Feno levels in patients with bronchial asthma and COPD, depending on bronchial inflammatory activity, as measured by sputum cytology.

Disease	1 <sup>st</sup> and 2 <sup>nd</sup> degree of inflammation		3 <sup>rd</sup> degree of inflammation		P
	Number of patients	Feno M ±standard deviation	Number of patients	Feno M ±standard deviation	
Asthma	11	22.061±17.293	5	26.8±20.208	0.636
COPD	13	21.487±15.945	7	7.714±4.828	0.040



**Figure 1** Feno levels in practically healthy individuals (control group, n=26). Feno – neutrophils



**Figure 2** Feno Diagrams demonstrating the correlation between neutrophil and eosinophil content in sputum (in %) and Feno (ppb) in patients with asthma.

### Conclusion

- i. Feno in healthy individuals ranged from 1 to 24ppb, in 65% from 4 to 16ppb.

- ii. Feno is higher in patients with moderate and severe. A than in patients with mild A. Also Feno is higher in the acute phase of the disease than in the remission phase. Feno is higher in patients with hard curable A than in other patients.
- iii. Patients with COPD (even with marked inflammation of respiratory system) have low level of Feno, significantly lower than in patients with A.

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## Conflict of interest

The author declares no conflict of interest.

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