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REVIEW ARTICLE

UTILITY OF EXHALED NITRIC OXIDE IN PEDIATRIC PRACTICE REVIEW OF LITERATURE

Arnaudova-Danevska Ivana[1], Balkanov T[2], Boshkovska K[1], Jakjovska T[1], Popova G[1], Cakalarovska I[1]

[1]Institute for Respiratory Diseases in Children - Kozle, Skopje, Republic of North Macedonia
[2]Institute of Preclinical and Clinical Pharmacology with Toxicology- Skopje

ABSTRACT

The field of interest for measurement of exhaled nitric oxide (NO) and nasal NO is significantly evolving over the last 25 years, with over 1000 publications published in that area. Inflammation of the airways is a central process in asthma and other lung disorders, but the monitoring of the inflammation has not been included in the current recommendations. The exhaled air contains volatile media such as nitric monoxide, carbon monoxide, ethane, pentane and non-volatile substances in the liquid phase in the exhalation, as a condensate (hydrogen peroxide). It is increasingly confirmed that the measurement of exhaled mediators in general, and especially NO, is a new way to monitor certain aspects of asthma, COPD and interstitial lung disease, which cannot be estimated with other methods, like lung function. In asthma, exhaled NO is recommended to be used as a marker for diagnosis, for monitoring the response of anti-inflammatory drugs, confirming the safety of therapy and predicting asthma exacerbation. Measurements of FeNO are easily performed, they are reproducible and technically less expensive than the analysis of induced sputum. In symptomatic patients, high FeNO levels (> 50 ppb), refer to significant eosinophilia in the airways, which will most likely respond to treatment with ICS. The current data provides support for the diagnostic use of FeNO in children with symptoms of asthma. For patients with chronic and/or severe asthma, FeNO levels are useful for determining whether eosinophilic inflammation of the airways is active or not. Both high (> 50ppb) and low (<25 ppb) levels of FeNO can be used to for predicting the outcome in patients with a definitive history of asthma who are currently in remission and who have stopped treatment with ICS.

Key words: asthma, children, exhaled nitric oxide, recommendations.

The field of interest for measurement of exhaled nitric oxide (NO) and nasal NO is significantly evolving over the last 25 years, with over 1000 publications published in that area. Interest is growing because the measurement of airways inflammation can contribute in the management of lung diseases and the same measurement may be included in the clinical practice [1].

Evaluation of the airway inflammation

Inflammation of the airways is a central process in asthma and other lung disorders, but the monitoring of the inflammation has not been included in the current recommendations [2].Taking a direct sample of the airway cells and the mediators is invasive technique, as well as analysis of induced sputum or bronchoscopy with lavage and biopsy. The exhaled air contains volatile media such as nitric monoxide, carbon monoxide, ethane, pentane and non-volatile substances in the liquid phase in the exhalation, as a condensate (hydrogen peroxide). Non-invasive measurements of these exhaled mediators are appropriate for patient monitoring.

Exhaled NO

The presence of NO in the exhaled air in both animals and humans for the first time was described in 1991. It has been shown that correlates with other indicators of moderate asthma (eosinophils in induced sputum) and bronchial reactivity in non-steroid-treated patients. Generally, the exhaled NO does not correlate with the lung function parameters in asthma. It is increasingly confirmed that the measurement of exhaled mediators in general, and especially NO, is a new way to monitor certain aspects of asthma, COPD and interstitial lung disease, which cannot be estimated with other methods, like lung function. In asthma, exhaled NO is recommended to be used as a marker for diagnosis [3], monitoring the response of anti-inflammatory drugs, to confirm the safety of therapy [4] and to predict asthma exacerbation [5-7]. It is believed that the adaptation of anti-inflammatory drugs led by the monitoring of non-invasive markers as eosinophils in the sputum and the exhaled NO can provide good control of the asthma.

Nasal NO

Concentrations of nasal NO are relatively higher according to the lower respiratory tract in humans, with the highest level in the paranasal sinuses. Patients with ciliary dyskinesia and with cystic fibrosis have extremely low NO values and accordingly, the nasal NO may be a useful clinical test for early diagnosis.

Recommendations for a standardized procedure for the measurement of exhaled NO in adults

Basic principles for clinical use of NO measurement

In Europe, clinical testing began in the late 1990s, while American food and drug administration recommended the first NO analyzer for clinical monitoring of anti-inflammatory therapy in asthma in 2003, which was produced by Aerokrin AB from Stockholm, Sweden. Online measurements include measurement of the fraction of exhaled nitric oxide (FeNO) in real time, whereas offline measurements refer the collection of the exhale air in an appropriate time for a delayed analysis [8]. Measurement of FeNO, as a clinical agent, requires standardized measurement techniques that are obtained from previously processed data from different adult groups.

Standardization of terminology and measuring units for exhaled NO

Online measurement. FeNO is expressed in parts per billion (ppb), which is equivalent to nanoliters per liter. The output of NO is the level of the exhaled NO, which is designated as volume of NO. It is obtained when the concentration of NO in nanoliters per liter is multiplied with the level of expiratory flow per minute corrected by BTPS (body temperature, pressure, saturated).

V_{NO} (nl/min) = NO (nl/l) x expiratory flow (l/min)

Offline measurements. FeNO refers to NO concentration in the exhaled air from the vital capacity. If the exhalation is carried out under a constant flow (in liters per second) and it should be written as a subscript.

Basic principles for the measurement of exhaled NO

Source of exhalated NO. It is currently thought that NO is produced in the upper and lower respiratory tract and diffuses into the lumen according to the concentration gradient. Alveolar NO is probably very low because of the large hemoglobin uptake in the pulmonary capillary blood.

Although the level of gastric NO is high, it is considered that there is no effect on the level of exhaled NO due to the closure of the upper and lower esophageal sphincter.
Contamination with nasal NO. The nasal NO can be accumulated in relatively high concentrations in the low respiratory tract. But techniques that provide a lower respiratory tract NO should prevent the contamination of the sample with nasal NO.
Ambient NO Because environmental NO can reach high levels relative to those in exhaled breath, standardized techniques must prevent the contamination of biological samples with ambient NO.
Dependence on the level of expiratory flow. This flow dependence is a feature of the diffusion-based processes and relates to the transfer of NO from the wall to the lumen of the airways and can be easily explained. The fast flow minimizes the time of transit of the alveolar gas in the airways and consequently reduces the amount of transferred NO. The output level of NO as well is higher in the flow rate, analogous to the loss of respiratory heat.
Air retention. The air retention results in the accumulation of NO in the nose, the lower airways and probably in the oropharyngs, which leads to a NO peak in the NO exhalation profiles relative to the time. Accordingly, air retention is not used in standard techniques [1].

Other patient-related factors that affect the values of the exhaled NO
Age/Sex: Exhaled NO levels increase with the age. As there are contradictory data for the effects of the sex, menstrual cycle and pregnancy, these characteristics should be taken in account at the time of the measurement [9-12].
Respiratory maneuvers: Spirometry reduces the level of exhaled NO and accordingly, NO analysis should be performed prior to spirometry [13, 14].
Airway caliber: It turned out that the level of FeNO may vary in relation to the degree of the airway obstruction or after bronchodilator, probably due to the mechanical effect of the NO output. Because of this, it is recommended to note the time of administration of the bronchodilator and some measurements of the caliber of the airways, like FEV1 [13].
Food and liquids: Patients should be restrained from food and drink before analyzing NO. There is an increase in FeNO after ingestion of nitrates or food containing nitrates. Drinking water and caffeine intake may lead to transient change of the levels of FeNO. It is likely that the mouthwash can also reduce the effect of foods containing nitrates. Patient should avoid to eat or drink one hour before the measurement and should be asked about the food recently consumed. The alcohol intake reduces FeNO [15].
Daily rhythm: It is not yet certain how FeNO values vary during the day and therefore it is recommended that serial measurements of NO should be done in the same time of day and the time should be recorded [16].
Smoking reduces FeNO levels. However, smokers with asthma have elevated levels of FeNO. Patients should not smoke one hour before the measurement and the history of short or long active or passive smoking should be recorded [17].
Viral infections of the upper and lower respiratory tract lead to elevated levels of FeNO in asthma. HIV infection is associated with reduction of the levels.
Other factors: The change in the blood flow in the lungs has no effect in humans, but hypoxia reduces the exhaled NO. Positive end-expiratory pressure application increases the level of FeNO in animals, while the pressure of the airways in humans does not influence the level of the exhaled NO. There are studies about the impact of exercise, with different conclusions and it is therefore considered to avoid physical effort one hour before the measurement.

Drugs: FeNO levels decrease after treatment with topical or oral corticosteroids in patients with asthma or after inhalation of NO synthetase inhibitors. Leukotriene antagonists also reduce FeNO. The drugs containing NO as well as oral, inhallatory and intravenous L-arginine increase the level of FeNO. Even if a medicine does not influence the production of NO can change the levels through other mechanisms, like changing the airways caliber.

Recommendations for online and offline measurement of exhaled NO in children

Measurements of exhaled NO in children aged 4-5 years

Single breath online measurement: This method is preferred in all children who can cooperate. The child should comfortably sit down and be breath normally for about 5 minutes to acclimatize. The child inhales close to vital capacity and immediately expires under a constant flow of 50 ml/s while plateau of NO is obtained for at least 2 sec. which may be registered in an outbreak of at least 4 sec. The vaporized gas should contain low NO concentrations (<5 ppb). The expiratory pressure should be from 5 to 20 cm water column to close the velum. Repeated exhalations (2-3 which correspond to 10% or 2 to 5%) are performed at intervals of at least 30 seconds and the mean value is recorded. Audiovisual devices can ease the inhaling to the vital capacity and control the expiratory flow of 50 ml/s. The use of dynamic flow restrictors that allow the child to exhale with various pressure in the mouth, allows a constant level of expiratory flow. Dynamic flow restrictors are simple manual or mechanical devices that change their resistance depending on the blow pressure and their use is recommended in children [18, 19].

Offline method with constant flow level: The child blows air through a tube in the mouth that is made of a material that does not interfere with NO, whereas nasal contamination is protected by the closing of the velum in the air extraction under oral pressure of at least 5 cm water column. The gas collection can be in the Milar or Tedlar balloons. The size of these balloons is recommended to be similar or slightly higher than the vital capacity of the patient. No nasal clips or retention of air is recommended, since they may potentially lead to nasal contamination. Concentrations of NO in balloons may be stable for a few hours and the measurements can be carried out elsewhere (school or home) [20]. Great progress in offline measurements can be expected by incorporating dynamic flow restrictors into a collection system that will provide easy feasibility for children under 4 years of age. This led to a consensus and recommendations that the flow rate should be 50 ml/s for both online and offline [21].

Alternative methods for pre-school children and infants

Online measurements of FeNO during spontaneous breathing. This method can be applied to children from 2 to 5 years of age. FeNO can be measured in spontaneous breathing while exhaled flow can be adjusted by changing the exhaled resistance [22].This ensures a stable and reproducible model of breath to breath. The child breathes slowly and regularly through a tube connected to a two-way valve. It is recommended to calmly breathe with normal frequency.

The level of exhaled flow is adjusted to 50 ml/s (from 40-60) with continuous adaptation of exhaled resistance manually or with automatic flow controllers.

The method still requires passive co-operation in order that the child breathes calmly and regularly through a connector, which is a limiting factor. The use of a biofeedback allows the child to visualize a model of normal respiration. Measurements in spontaneous breathing lead to variability, as there is no control over the lung volume, but the flow is measured. According to this, the measurements of NO levels during spontaneous breathing do not equate with single

breathe online measurements and it is necessary to specify this method by including a description of the normal values in healthy children [22].

Techniques of normal breathing with uncontrolled flow rates: There is no standardized method to be recommend for clinical use in infants and young children, and further investigations should resolve some methodological problems [23].

Offline measurement: Exhaled air should be collected through a tube connector or face mask connected to a valve that allows inhalation of air without NO, from a NO inert tank to avoid contamination from the ambient NO. Examples of NO are collected in NO inertial bags that are attached on the expiration side. The expiratory side provides expiratory resistance [24]. This resistance allows avoiding nasal contamination if the face mask does not cover the nose. With NO analyzers with quick response, small samples of exhaled air may be sufficient for analysis.

Online normal breathing techniques for measuring NO and the model of normal breathing in neonates and infants are described with good reproducibility [25, 26].Infants breathe through a mask covering both the nose and mouth and the NO concentrations should be recorded during the normal-breathing stages. Reproducibility is a significant problem of the normal breathing methods and is described by many researchers [23, 27]. Since it is resistance dependent, the data for the flow rate will vary. The inconvenient side of the mixed exhaled air is that it can be contaminated with ambient NO and NO from the upper airways. As long as the contribution of NO from the upper airways is not known, it's better to collect a sample of orally exhaled air. This is facilitated by the use of a mask covering only the mouth, while the nostrils are occluded or by using a double-chamber mask.

Single breath technique during forced exhalation. Modification with rapid increase in volume, with the technique of thoraco-abdominal compression. FeNO is measured online and the NO plateau is obtained at a steady rate of expiratory flow that can vary from 10 to 50 ml/s and uses a two-chamber mask [28]. The negative side of this technique is that sedation is necessary and nowadays still it is not clear how this technique can be compared with other techniques such as single breath online or the technique of normal respiration.

Rational use and interpretation of NO

There are two key issues: 1) high significant interaction of NO and eosinophilic inflammation of the airways; 2) important relationship between eosinophilic inflammation of the airways and steroid response [29].This can be summarized as:

a) FeNO measurements are highly correlated with eosinophilic inflammation on the airways. Many studies confirm that FeNO measurements correlate with eosinophilia in induced sputum, (30) bioptic material [31-33] and bronchoalveolar lavage as well [34].

In one study, a significant relationship of FeNO and blood eosinophilia is explained [35]. In addition, there is also a correlation between FeNO and the eosinophilic cation protein.

b) Eosinophilic inflammation of the airways is associated with a positive response to steroid treatment.

Treatment with inhaled corticosteroids (ICS) results in reduction of eosinophilia in asthmatic airways and improvement in clinical parameters. In contrast, in asthma that is not characterized by eosinophilia, the response to steroids is scarce [36].These findings are common in patients with constant obstruction of the airways and enable distinction between asthma and COPD [37].

According to this, determination of the character of inflammation of the airways (eosinophilia) is important in the initial management of patients with chronic respiratory symptoms in order to identify those who would benefit from the steroid treatment.
c) Increased levels of FeNO predict positive steroid response in patients with non-specific respiratory symptoms. The clinical benefit of increasing steroid treatment in patients with asthma is higher in patients with increased levels of NO [38]. Some studies have shown that in determining the outcome of treatment with inhaled Fluticasone, the level of FeNO as predictor is superior than spirometry, bronchodilator test and bronchial hyperactivity. It is important that this study identifies the optimal value for the steroid response to 47 ppb. This has been proved in studies in children and adults [39, 40].
d) The use of ICS in asthma results in decreased levels of NO and according to that, the link between ICS and FeNO is dose-dependent and has a significant correlation between changes in FeNO and changes in eosinophilia in induced sputum and ICS therapy [41].
These data provide a fundamental confirmation that FeNO measurements play an important role in the evaluation and treatment of patients with airway diseases. FeNO can be used as a surrogate marker for diseases of the airways that are characterized by eosinophilia, such as atopic asthma, asthma like coughing and eosinophilic bronchitis. Because of the close relationship between the steroid response and eosinophilia in the airways, FeNO measurements play important role in predicting and monitoring the response of the treatment with ICS [29].

Diagnosing airway diseases

Asthma: FeNO measurements are useful in distinguishing asthma from non-asthma [42]. The test should be used as diagnostic tool when chronic symptoms with a duration of 6 weeks and more are present, as the virus infection can lead to a false positive result. The predictive value is almost identical with the cell count in induced sputum. The combination of the FeNO > 33 ppb and abnormal spirometry (FEV1 <80% PV) provides great sensitivity (94%) and a specificity of 93% for diagnosing asthma. Normal values do not exclude diagnosis of asthma. This confirms the heterogeneity of asthma phenotype and FeNO measurements provide only one aspect of asthma syndrome [43, 44].

Non-specific respiratory symptoms: FeNO measurements have a widespread role in the examination of patients with undiagnosed chronic respiratory symptoms. Children with recurrent wheezing bronchitis, cystic fibrosis, congenital abnormalities of the airways and primary ciliary dyskinesia should also be considered. For the eosinophilic bronchitis and Cough variant asthma, which are characterized by eosinophilic inflammation of the airways and elevated FeNO levels, the positive effect of corticosteroid treatment is more likely. For other diagnoses, like vocal cord dysfunction which is presented as asthma, many clinicians often give empirical steroid treatment with very small benefit.

Pre-school children: Taking into account that spirometry and sputum induction cannot be easily performed in pre-school children, non-invasive measurements of the airway inflammation are potentially very useful.

The single breathing technique for measuring FeNO at this age is not suitable, so other alternatives that vary from modification of standard online techniques to offline spontaneous breathing method with flow control are developed [45-51]. Generally, these techniques are less sensitive to discriminating between asthmatic and non-asthma patients [53, 53].

In acute wheezing episode FeNO level is significantly higher in those with recurrent wheezing versus healthy controls, whereas in children with first wheezing episode FeNO levels do not differ from normal children. Also, montelucast reduces the FeNO values of small children with early onset of asthma [54, 55].

Atopy: There is a strong correlation between FeNO levels with total and specific IgE [56, 57].This suggests that asymptomatic atopic children may have moderate inflammation of the airways leading to a raised FeNO level [58].

Chronic obstructive pulmonary disease (COPD):FeNO levels are non-consistent in patients with COPD. This may be the result of smoking or the heterogeneity of the airways inflammation [59, 60].

Cystic fibrosis (CF): In patients with CF, it has not been found that FeNO measurements can be clinically useful. First, there is a reduced expression of nitric oxide synthetase in patients with CF and second, elevated levels of nitrites are found in the breathing condensate of patients with cystic fibrosis [61].

Primary Ciliary Dyskinesia (PCD): FeNO levels are significantly lower in these patients than in healthy individuals. The nasal NO is extremely low in patients with PCD of all ages and completely separates affected from unaffected individuals. The measurement of nasal NO can probably become a screening test. The diagnostic sensitivity and specificity of nasal NO for PCD ranges from 89 to 100%, and the specificity is from 97 to 100% [62, 63].This is due to the reduced activity of NO synthetase and the mucus that affects the diffusion of NO from sinuses in the nasal cavity or from epithelial cells to the lumen of the airways and makes the NO elimination more difficult. Even young infants with PCD, have low nasal NO [64, 65]. Probably NO is also involved in the stimulation of the ciliary motility. The nasal NO may play a role in non-specific immunity, which includes a direct toxic effect on the microorganisms [66].

Lung transplantation: FeNO levels are increased in post-transplant patients with an unstable lung function [67].

FeNO measurement for chronic asthma management

Prediction of exacerbations: The prognostic value of FeNO measurements for asthma exacerbation seems to be limited. In a small study with steroidal reduction protocol, changes in sputum eosinophils were superior comparing with FeNO for prediction of exacerbation. In the second study, measurements of bronchial hyperactivity, sputum eosinophilia and FeNO measurements ranged from a sensitivity of 21% (sputum eosinophils> 4%) to 65% in FeNO greater than 10 ppb at a flow rate of 250 ml/s. Although positive predictive values ranged from 80 to 88%. Therefore, it is preferable to examine more parameters than just one. The increase in FeNO of 60% is considered to be optimal, but this has a 50% sensitivity with a positive predictive value of 83%. These studies used a protocol to exclude steroids in order to imitate clinical exacerbations and so they are not suitable [68, 69].

Predicting the outcome of ICS reduction in stable asthma: The main issue is when airway inflammatory markers can be used to predict a successful reduction or exclusion of ICS treatment. Studies showed that number of eosinophils in sputum was significantly more predictive than FeNO measurements in asthma control over a period of 6 months and 16 weeks.

From the results of the many studies it can be concluded that the number of eosinophils in the sputum (more than 1%) probably provides superior prognostic safety when it is necessary to determine whether a patient needs to continue treatment with ICS.

In situations where induced sputum can not be obtained (some centers and small children), the high FeNO level (> 50 ppb) probably predicts asthma relapse and a low FeNO level <20 ppb in children (<25 in adults) predicts asthma stability if the measurement is obtained at least 4 weeks after interruption or reduction in ICS in asymptomatic patient [70-73].

Increasing ICS doses: Correlation between airway inflammation and/or symptoms and/or pulmonary function is very poor. So, the use of these treatment should be considered as the second best [29].

Measurement and interpretation

Normal Values and Clinically Important Changes

In the past, studies showed that the upper limit is age dependent and ranges from 15,7 ppb for the age of 4 years to 22,5 ppb for adolescents. The reason for this age-dependence is still unknown, but it can be result of an increase of the surface area in the airways which is age-related, age-depended induction of NO synthetase as a result of recurrent immunological stimulation or progressive reduction of the constant exhalation level that is relatively high in children. So, that's why the upper limit for healthy adults is 33 ppb, while for school children is 25 ppb [74]. Measurement of FeNO level when asthma is stable may be the basic refrence point for a single patient. It is considered that the mean value of FeNO change that occurs between stability and "loss of control" after stopping ICS treatment is 16,9 ppb (or mean 24,9) [41].

Interpretation

Based on the currently available data, two algorithms for interpreting the FeNO results in everyday practice had been developed. First, as a diagnostic tool and second for further management of asthma (Shown on the tables):

1) Diagnostic use is pretty clear (Table 1).

Table 1. FeNO as a diagnostic tool

FeNO (ppb)	Level	Eosinophil inflammation	Children
5-25	Low (< 20 in less than 12 years, <25 in more than 12 years)	Probably not	Obstructive bronchitis, GER, ENT disturbances, CF, PCD (FeNO<5 ppb check nasal NO), congenital airway anomalies, other immune deficiencies
30-45	Intermediate	Present but moderate	Interpretation based on the clinic presentation
50-65	High	Significant	Atopic asthma, if FEV1 <80% PV, diagnosis for asthma is highly probable. Eosinophilic bronchitis, a positive response to steroid treatment.

2) In order to monitor patients correctly, increased levels of FeNO in symptomatic patients reffer to uncontrolled eosinophilic inflammation. This is very often result of a weak compliance with anti-inflammatory therapy or a poor inhalation technique than inadequate dosing of ICS. Low levels of FeNO implicate the absence of eosinophilic inflammation of the airways. When we assume that the result is not credible, as long as the patient has symptoms or has atopic asthma, this may be due to the use of cigarets which reduces the level of FeNO up to 60%.

It is commonly referred to nonatopic (probably neutrophilic) asthma, gastroesophageal reflux (GER), rhinosinusitis with postnasal drip or left heart failure (Table 2).

Table 2. FeNO results for management of asthma

FeNO (ppb)	Level	Eosinophil inflammation	Children
5-25	Low	Probably not	If there are symptoms revision of the diagnosis. Considering Obstructive bronchitis, congenital airway anomalies, CF, PCD. If there are no symptoms and uses ICS: good compliance with treatment, reduction of the dose of ICS or, in case of a low dose, exclusion of ICS
30-45	Intermediate	Present but moderate	If there are symptoms: infection as a cause of worsening, high exposure to allergens, adding therapy other than ICS (for example long-acting β agonist). Consider increasing ICS doses, inadequate treatment with ICS, check compliance, check the inhalation technique and consider a metered dose inhaler or spacer if the patient uses a dry powder inhaler If there are no symptoms: no changes in the ICS dose
50-65	High	Significant	If there are symptoms of inadequate treatment with ICS: check the compliance, check the inhaler technique, inadequate ICS dose, high exposure to allergens, exacerbation or relapse, consider the metered dose inhaler or spacer if the patient uses an inhaler with a dry powder If there are no symptoms: no changes in the ICS dose.

The data shown in the tables is for guidance only. Further studies may lead to certain changes. Patients with various clinical phenotypes may have different referent values of FeNO. Since asthma is stable, FeNO levels may remain high. Prove that "normalization" of FeNO leads to a clinical benefit is not confirmed. It may be preferable to have individual "FeNO typing" on the refrent values [29].

CONCLUSION

FeNO measurement offers a step ahead in the assessment of the airway diseases. As the "inflamometer," FeNO provides information about the nature of airway inflammation. Measurements of FeNO are easily performed, they are reproducible and technically less expensive than the analysis of induced sputum. FeNO results require careful consideration with the clinical aspect. In symptomatic patients, high FeNO levels (> 50 ppb), refer to significant eosinophilia in the airways, which will most likely respond to treatment with ICS.

The current data provides support for the diagnostic use of FeNO in children with symptoms of asthma. Whether or not it needs to be used to predict the response of steroids or to guide the need for ICS in small children with recurrent wheezing, is still not clear. For patients with chronic and/or severe asthma, FeNO levels are useful for determining whether eosinophilic inflammation of the airways is active or not. Both high (> 50ppb) and low (<25 ppb) levels of FeNO can be used to for predicting the outcome in patients with a definitive history of asthma who are currently in remission and who have stopped treatment with ICS. Depending of symptoms, at the same time, high and low levels of FeNO offer assistance to clinicians in adjustment of the dose of ICS.

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