REVIEW ARTICLE

Exhaled Nitric Oxide in Children: A Noninvasive Marker of Airway Inflammation

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This article is an academic review of the application in children of the measurement of fractional exhaled nitric oxide (FE_{NO}). We outline the joint American Thoracic Society/European Respiratory Society recommendations for online measurement of FE_{NO} in both cooperating children and children unable to cooperate, offline measurement with uncontrolled exhalation flow rate, offline measurement with controlled exhalation flow rate using a dynamic flow restrictor, and offline measurement during tidal breathing in children unable to cooperate.

This is followed by a review of the normal range of values for single-breath online measurements obtained with a chemiluminescence FE_{NO} analyzer (geometric mean, 9.7 parts per billion [ppb]; upper limit of the 95% confidence interval, 25.2 ppb). FE_{NO} values above 17 ppb have a sensitivity of 81% and a specificity of 80% for predicting asthma of an eosinophilic phenotype. We discuss the response of FE_{NO} values to anti-inflammatory treatment and the use of this marker in the management of asthma.

Results obtained with chemiluminescence and portable electrochemical analyzers are compared. The portable devices offer the possibility—in children over 5 years of age—of accurate and universal monitoring of exhaled nitric oxide concentrations, an emerging marker of eosinophilic inflammation in asthma that facilitates diagnosis, monitoring of disease progression, and assessment of response to therapy.

Key words: *Exhaled nitric oxide. Methodology. Portable analyzers. Children. Asthma.*

Óxido nítrico exhalado en niños: un indicador no invasivo de la inflamación de las vías aéreas

En este artículo se presenta una revisión académica sobre la aplicabilidad de la medida de la fracción exhalada de óxido nítrico (FE_{NO}) en niños. De acuerdo con las normas conjuntas de la American Thoracic Society/European Respiratory Society, se describen los métodos de medida *on-line* en niños colaboradores y no colaboradores, los registros *off-line* sin control de flujo de exhalación y con control de flujo de exhalación mediante restrictor de flujo dinámico, y el registro *off-line* a respiración corriente en niños no colaboradores.

Se revisan los valores de normalidad, fundamentalmente con los analizadores de la FE_{NO} por quimioluminiscencia, mediante registro *on-line* de una única respiración (media geométrica: 9,7 ppb –partes por mil millones–; límite superior del intervalo de confianza del 95%: 25,2 ppb). Los valores de la FE_{NO} superiores a 17 ppb aportan un 81% de sensibilidad y un 80% de especificidad para predecir asma de fenotipo eosinofílico. Se analiza la respuesta de la FE_{NO} al tratamiento antiinflamatorio y al seguimiento del asma.

Por último, se comparan los resultados entre los analizadores por quimioluminiscencia y los electroquímicos, portátiles. Estos últimos ofrecen la posibilidad, en niños mayores de 5 años, de un seguimiento adecuado y universal del óxido nítrico exhalado como indicador emergente de la inflamación eosinofílica en la enfermedad asmática, de modo que facilitan el diagnóstico, el control evolutivo y el seguimiento terapéutico.

Palabras clave: *Óxido nítrico exhalado. Metodología. Medidores portátiles. Niños. Asma.*

Introduction

In 1987, a group of researchers at the University of California at Los Angeles recognized that endothelium-derived relaxing factor from veins and arteries possessed biological and chemical properties identical to those of nitric oxide (NO).[1] Five years later, in 1992, NO was named "Molecule of the Year" by the journal *Science*.[2] In 1998, Furchgott, Ignarro and Murad were awarded the Nobel Prize for Medicine for their work on this molecule,[3] a decision that gave rise to controversy among other NO researchers.[4] The NO molecule has a very simple atomic structure, acts like a free radical, has a very short half life, and functions as a messenger in a variety of processes including the regulation of peripheral circulation, immune responses, platelet function, and neurotransmission.

During the 1990s, numerous authors reported that fractional exhaled nitric oxide concentration (FE_{NO}) was

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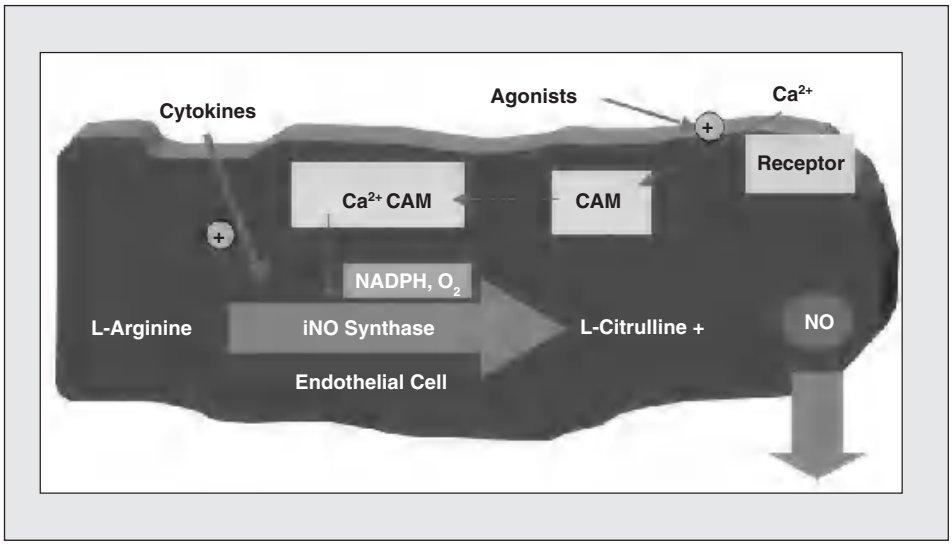

Figure. 1. Cellular synthesis of nitric oxide (NO). CaM indicates calmodulin, a calcium-binding protein; iNO synthase, inducible NO synthase; NADPH, reduced nicotinamide adenine dinucleotide phosphate-oxidase

higher in asthmatic patients than in healthy controls,[5,6] that these levels decreased in asthmatic patients treated with inhaled corticosteroids,[7] and that this parameter was closely linked to eosinophilic inflammation.[8]

NO Synthesis

NO is an endogenous and diffusible simple free radical that generates cyclic guanosine monophosphate (cGMP). Mammals synthesize NO by way of the NO synthase (NOS) enzyme, which converts the amino acid L-arginine into L-citrulline and NO (Figure 1). The 3 known isoforms of the NOS enzyme are classified according to their activity, location, and molecular cloning; 2 are constitutive and the third is inducible.

The 2 constitutive isoforms are the neuronal isoform (nNOS, or NOS_1) and the endothelial isoform (eNOS, or NOS_3). As these constitutive isoforms are both calcium-dependent, they produce small quantities of NO in response to agonists that increase intracellular calcium. nNOS is present in nerve cells and skeletal muscle and eNOS in blood vessels and platelets. Both the constitutive forms are also found in the airway epithelium, where they produce picomolar concentrations of NO.

The inducible isoform (iNOS, or NOS_2 is calcium-independent. iNOS is expressed in vivo in the bronchial epithelial cells in both healthy and asthmatic individuals and its activity increases during certain inflammatory processes. iNOS is also expressed in vitro following stimulation with cytokines, endotoxins, and lipopolysaccharides. iNOS produces nanomolar concentrations of NO and is inhibited by corticosteroid treatment, which does not affect the constitutive isoforms.

Another NOS, which is expressed constitutively but is calcium-independent and not inhibited by corticosteroids, has been found in human paranasal sinuses.

The upper airway produces large quantities of NO. The highest concentration—3000 parts per billion (ppb)—is found in the mucosa of the paranasal sinuses, while measurement in the airway in tracheostomized patients or performed by way of bronchoscopy reveals NO concentrations of the order of 5 ppb.

Lane et al[9] studied the expression of the different NOS isoforms in macrophages and the epithelial cells of the respiratory tree in an unselected population of 41 children aged between 6 and 16 years. They found a median FE_{NO} of 15.5 ppb (95% confidence interval [CI], 10 to 18.1 ppb) and significantly higher concentrations in atopic patients than in healthy children or children with controlled asthma ($P<.05$). They also reported a positive correlation between FE_{NO} and NOS_2 expression ($R=0.672$, $P<.001$), which was stronger in asthmatic children ($R=0.828$, $P=.006$) than in asymptomatic atopic ($R=0.752$, $P=.02$) or healthy children ($R=0.525$, $P=.008$). The bronchial epithelial cells express NOS_2 and contribute to the total NO produced in the distal airway. In another study of children, FE_{NO} was found to be associated mainly with atopy and bronchial hyperresponsiveness rather than with a diagnosis of asthma.[10] However, there does not appear to be any correlation between FE_{NO} and NOS_1 or NOS_3 expression.[9]

In a recent study of 230 families with asthma (842 individuals), Batra et al[11] studied the association of the polymorphisms of the *NOS_2* gene located in the C-C chemokine gene cluster on chromosome 17q11.2-q12 with atopy, peripheral blood eosinophilia, and asthma severity. They found the following statistically significant associations: asthma severity ($P=.04$) and eosinophilia ($P=.001$) with allele 3 of *M3* (an intron 4 GT repeat); and serum total immunoglobulin E concentrations ($P<.001$) and FE_{NO} ($P=.03$) with the *M1* allele (a CCTTT repeat in allele 4).

In healthy children, age has been found to be a significant predictor of FE_{NO} ($P=.014$) but not of NOS_2 ($P=.402$),[12] perhaps because of the correlation between FE_{NO} and the volume of anatomic dead space, a parameter that increases with age in healthy children ($r=0.68$, $P<.01$).[11,12] It has therefore been suggested that normal values for FE_{NO} in school-age children should be related to age and weight.[13]

Malmberg et al,[14] who studied 114 healthy school children, found FE_{NO} to be significantly associated with

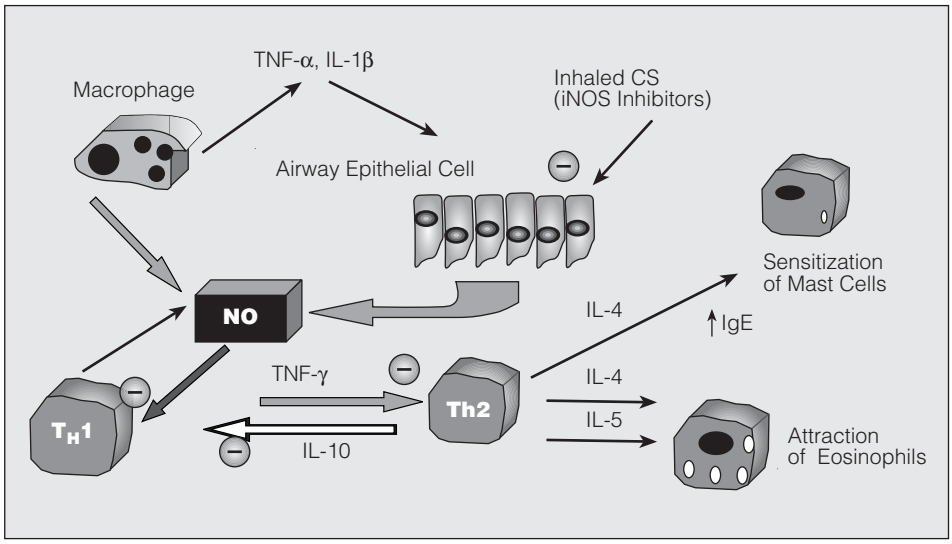

Figure 2. Nitric oxide (NO) and asthmatic inflammation. CS indicates corticosteroids; IFN-γ, interferon-γ; IgE, immunoglobulin E; IL, interleukin; iNOS, inducible NO synthase; Th, T helper cell; TNF-α, tumor necrosis factor-α.

age, height, weight, and body mass index (P<.0001), with height as the strongest independent predictor. No significant differences were found with respect to the sex of the patient (P=.42).

Avital et al[15] and Kissoon et al[16] also observed a significant age-related increase in FE_{NO} in asthmatic patients (P<.0001), although this effect was less marked in preschool children (between 2 and 5 years of age).

Functions of Endogenous NO

NO has the following properties and functions that affect the respiratory system: *a)* a weak bronchodilator effect; *b)* a strong vasodilator effect; *c)* noncholinergic and nonadrenergic neurotransmitter activity; *d)* antibiotic activity; *d)* a role as a modulator of cell differentiation; and *f)* a role as an amplifier of airway inflammation.

1. NO relaxes bronchial smooth muscle via cGMP. The bronchodilator effect is more marked in the tracheobronchial region than in the small airways. Studies in humans show that NO functions as a weak bronchodilator. The reversal of methacholine-induced bronchoconstriction brought about by the administration of inhaled NO produces only a slight increase in forced expiratory volume in 1 second (FEV_1).

2. Since the concentration of NO in the bronchial region depends on the fraction of inspired oxygen, NO production decreases in hypoxia and remains stable under normal and hyperoxic conditions. NO produced in the bronchoalveolar epithelium diffuses freely to the smooth muscle cells of the pulmonary vessels, where it activates the enzyme guanylate cyclase and stimulates the production of cGMP. This nucleotide activates Ca^{2+} channels, and the resulting relaxation produces vasodilation and a consequent improvement in the ventilation-perfusion ratio.

3. NO functions as a neurotransmitter of the nonadrenergic noncholinergic (NANC) system, the only neural bronchodilator mechanism. NO induces an inhibitory response (i-NANC) and, consequently, bronchodilatation. Activation of the excitatory nonadrenergic noncholinergic (e-NANC) system (excitation of the C-fibers) induces bronchoconstriction, vasodilation, edema, and mucus secretion. This activation is mediated by neurokinin A and P substance, 2 tachykinins that trigger the release of cytokines and an increase in acetylcholine

4. Increased NO production is associated with an increase in the host's resistance, while inhibition of NO synthesis increases the replication of the infectious agent in the infected macrophages. NO seems to play a particularly important role in the defense against intracellular pathogens, which it can damage by way of deamination of DNA, enzyme inhibition, protein oxidation, and the peroxidation of lipids in the membranes of these microorganisms.

5. High local concentrations of NO favor both the inhibition of the differentiation of $CD4^+$ T-helper (T_H) cells into T_H1 cells (producers of interleukin (IL) 2 and interferon-γ) and the increase of T_H2 cells (secretors of IL-4, IL-5, and IL-10 that promote the production of immunoglobulin E and the adherence and accumulation of eosinophils). In other words, from the immunologic point of view, an increase in local NO production favors and perpetuates a cellular and inflammatory mediatory pattern similar to that found in both atopic and nonatopic asthmatic patients, such that inhibiting the activity of iNOS may lead to the inhibition of eosinophilic inflammation in asthma. It is known that corticosteroids effectively inhibit the activity of iNOS and also control asthma and eliminate airway inflammation.

Under conditions of pathological oxidative stress, NO inhibits T_H1 cells and interferon-γ production, decreases eosinophil apoptosis, and acts as a modulator in diseases such as asthma, amplifying the inflammatory response (Figure 2). In asthmatic children there is an increase in the expression of iNOS in epithelial cells, eosinophils, neutrophils, and mast cells, giving rise to large quantities of NO and metabolites (such as peroxynitrite) that provoke bronchial hyperresponsiveness, damage the epithelium, and inhibit surfactant function.[17]

6. NO tends to intensify edema and plasma exudation and cause denudation and desquamation of the epithelial lining. Moreover, a correlation has been observed between increases in FE_{NO} and increases in 8-epi-isoprostane $F_{2\alpha}$, a marker of lipid peroxidation and a powerful pulmonary vasoconstrictor. It has been suggested that this substance could be a marker of oxidative stress in asthma.

Measurement of NO

NO can be measured precisely using chemiluminescence. NO and ozone react in a cooled chamber to form NO_2 and this photochemical reaction emits infrared light that can be detected by a photomultiplier tube with a linear response.

Procedure

The procedures used to measure FE_{NO} differ depending on the patient's condition. Measurement is performed online in patients able to cooperate and offline in those who cannot. The general principles, methods, and technical characteristics of FE_{NO} measurement were published in the 1990s by the European Respiratory Society (ERS) and the American Thoracic Society (ATS).[18,19] The main difference between the recommendations of the 2 societies was the expiratory flow rate at which measurement was made.

1. Online measurement in children able to cooperate. Baraldi et al,[20] in association with the ERS and the ATS, published recommendations for FE_{NO} measurement in children. The ATS recently published joint ATS/ERS recommendations.[21]

Single-breath online measurement (SBOL) is the technique of choice in children able to cooperate. The child inhales NO-free air (NO <5 ppb) for 2 to 3 seconds through a mouthpiece. A nose clip may be used if the patient is unable to avoid nasal inhalation or exhalation. The patient should inhale deeply to total lung capacity and then exhale completely at a constant flow rate until a plateau of at least 3 seconds is reached during an exhalation of at least 4 seconds in children under 12 years of age and over 6 seconds in patients over 12 years of age. In general, a 10-second exhalation time—the maneuver recommended in adults—produces a more stable plateau. FE_{NO} values are then captured and recorded in real time. Exhalation against resistance generates increased mouth pressures that close off the soft velum (pressure 5-20 cm H_2O) and reduce NO contamination from nasal air. A variability of no more than 10% over 3 measurements or no more than 5% over 2 measurements is recommended.

Since asthma is an inflammatory syndrome characterized by varying degrees of airway obstruction, lower expiratory flow rates will discriminate better and have greater sensitivity in the determination of FE_{NO} concentrations despite variation over time in the degree of inflammation. As FE_{NO} is dependent on the expiratory flow rate at which measurement is made, values obtained at higher flow rates will be lower. Excessively high expiratory flow rates will lead to a rapid decline in lung volume making it extremely difficult for the patient to sustain exhalation for long enough to achieve the recommended plateau, particularly in the case of small children. However, if the expiratory flow rate is too low, exhalation will be unsustainable in patients with severe asthma.[22]

In a study of 32 healthy adolescents between 15 and 18 years of age, Kissoon et al[23] measured FE_{NO} at the following flow rates: 46, 31, 23, 15, 10, 7, 5, and 4 mL/s. They found that FE_{NO} increased as flow rates decreased, a correlation that was particularly strong at the 4 highest flow rates (0.85-0.93, $P<.001$). Moreover, they reported that expiratory flow, body surface, age, and forced midexpiratory flow rate were significant predictors of FE_{NO} in healthy adolescents and proposed that the ideal flow rate for healthy adolescents was between 30 and 50 mL/s. The ATS recommendations published in 2005 proposed measurement at a constant expiratory flow rate of 50 mL/s.[21]

In a study of 40 children aged between 7 and 13 years, Kharitonov et al[24] measured FE_{NO} twice a day for 5 consecutive days. They found a correlation coefficient of 0.99 and reported that FE_{NO} was significantly higher in the asthmatic children (32.9 ppb) than in the healthy controls (16.3 ppb). They observed no significant day-to-day variations or learning effect and concluded that measurement of FE_{NO} was a simple and reproducible technique free from diurnal or day-to-day variations, making it a suitable parameter for use in the diagnosis and monitoring of childhood asthma in routine clinical practice. The online measurement method also allows the operator to assess the pressure and flow of each measurement and select only the best exhalations, discarding those that are substandard.

It has also been recommended that FE_{NO} measurement should be performed before the other methods used to assess airway inflammation, such as induced sputum, spirometry, or a bronchial challenge test, because several authors have found concentrations to be significantly lower ($P<.05$) when FE_{NO} was measured after such tests.[25,26]

2. Online measurement in children unable to cooperate. It is extremely difficult to obtain valid FE_{NO} measurements during tidal breathing in preschool children (between 2 and 5 years of age) without sedation, even when visual cues and animation are used to motivate these small children.

Buchvald and Bisgaard,[27] who studied 51 preschool children aged between 2 and 5 years (14 healthy and 37 with asthma), described a method for obtaining valid online FE_{NO} measurements during tidal breathing at a constant flow rate (40-60 mL/s) with an exhalation time close to 1 second and a flow of 50 mL/s. The flow rate was controlled by continuous adjustment of expiratory resistance during exhalation. As the results they obtained agreed with those reported for FE_{NO} measurements performed in school-age children ($P<.05$) using the SBOL method, the authors of this study proposed FE_{NO} measurement as a noninvasive tool for monitoring the severity and activity of asthma disease in preschool children

in whom accurate results are hard to obtain with forced spirometry.[28] They also stressed the need for the definition and publication of normal values for healthy preschool children.

Another recently published article described a method for online and offline measurement of FE_{NO} during tidal breathing using a face mask in children between 2 and 7 years of age. The results obtained were excellent.[29]

3. Offline measurement with uncontrolled exhalation flow rate. Jöbsis et al[30] measured FE_{NO} offline using a Tedlar or Mylar balloon in school-age children between 8 and 13 years of age without controlling either the flow or exact pressure of the exhalation. The samples obtained were analyzed within 12 hours of collection. Those authors reported that offline FE_{NO} measurement was a simple and reliable method that discriminated between various lung diseases in school-age children (asthma, respiratory allergy, viral infections) in the context of ambient NO levels of less than 10 ppb. Nasal contamination can be avoided by closure of the soft velum through expiratory pressure in the mouth of 5 cm H_2O and the use of a nose clip is not, in principle, recommended.

Offline measurement has advantages over the online method primarily in children unable to sustain a constant and uniform exhalation flow. It can be used to obtain better results in children who are less well able to cooperate.[31,32] While the correlation between online and offline FE_{NO} values is good, significant differences preclude direct comparison between the results obtained with the different methods, particularly when exhalation flow is uncontrolled in the offline technique.

4. Offline measurement with exhalation flow rate controlled by a dynamic flow restrictor. This is considered to be the best offline measurement technique because the standardization of flow with a dynamic flow restrictor or biofeedback improves reproducibility, making it possible to compare the values obtained with the results of online measurement at fixed flow in school-age children.[33]

In a study of 101 children with a mean age of 14 years, Jöbsis et al[34] found that when an offline procedure using controlled flow and excluding the dead space volume (by discarding the first 220 mL of exhaled air) was used, the values obtained offline (mean [SD] FE_{NO}, 17.7 [1] ppb) and online (16 [1.2] ppb) were similar if a constant flow of 50 mL/s was used. A high degree of agreement was found between the 2 measurements at a flow of 50 mL/s (0.95 ppb; 95% CI, 0.63-1.27 ppb) and, furthermore, the offline measurement method discriminated significantly (P<.0001) between healthy individuals (13.6 [1] ppb) and those affected by asthma or allergic rhinitis (33.3 [1.1] ppb).

5. Offline measurement during tidal breathing in children unable to cooperate. Tidal breathing (inspired air with a NO level <10 ppb) can be performed through a mouthpiece or face mask connected to a 2-valve system that prevents rebreathing of exhaled air and stores air in a collection bag for later FE_{NO} analysis. This technique requires no active cooperation and is a reliable method for obtaining valid measurements in preschool children, newborn infants, and patients with neuromuscular diseases.[35,36]

In 2004, Silkoff et al[37] described a system for monitoring FE_{NO} using the SBOL method and the Aerocrine NIOX analyzer. This study was undertaken to facilitate the standardization of the measurement procedures recommended by the different ATS/ERS guidelines[20,21] and a method approved in 2003 by the United States Food and Drug Administration for application in asthmatic children in routine clinical practice. Exhalation time should be at least 6 sec, expiratory flow rate varies between 45 and 55 mL/s, and expiratory pressure must be between 10 and 20 cm H_2O. The degree of precision ranges between ±2.5 ppb for values below 50 ppb and ±5% for values of 50 ppb and higher. The NIOX device, which is a stationary chemiluminescence analyzer requiring calibration every 14 days, is currently the FE_{NO} measurement system of choice in children.

Napier and Turner,[38] who used the NIOX analyzer to study 91 children (mean age 5.3 years), found that the FE_{NO} values obtained were valid and reproducible in children of at least 5 years of age but not in 4-year-olds. In a study of 522 healthy children aged between 4 and 17 years, Buchvald et al[39] found a mean FE_{NO} value of 9.7 ppb and reported an age-related increase, particularly in children aged 10 years or older. Repeatability was good, with a variability of 1.6 ppb (95% CI, 1.49-1.64 ppb) over 3 measurements. Most of the healthy subjects had values under 15-25 ppb, depending on age and reported symptoms of atopy on the days leading up to the test.

However, some medical facilities have limited access to these chemiluminescence analyzers and this has led to the development of a new portable FE_{NO} measurement device (the NIOX-MINO) based on electrochemical analysis. This device was designed to fulfill ATS criteria[19,21] and to greatly simplify the measurement of FE_{NO} in routine clinical practice in both specialized respiratory clinics and primary care centers. The exhalation time required varies from 3 to 6 seconds and the required expiratory pressure, which is between 10 and 20 cm H_2O, facilitates a flow rate between 45 and 55 mL/s. These parameters are controlled by a flow-pressure regulator guided by interactive light images and sounds that encourage the child to achieve the desired optimum flow rate. The total time required to perform the measurement is under 2 minutes.

In a study of 55 children aged between 4 and 15 years (mean age 9 years), McGill et al[40] measured FE_{NO} with both stationary NIOX (gold standard) and handheld NIOX-MINO analyzers to validate the latter (Table 1 and Figure 3). Altogether, 61% of the children were able to perform both maneuvers reliably and (in line with earlier studies in adults[41]) the investigators found a good correlation between the results obtained with the 2 devices (correlation coefficient = 0.98). Vahlkvist et al[42] found the FE_{NO} values obtained with the NIOX-MINO analyzer to be slightly higher than those obtained with the NIOX system (P<.01), and this difference was more evident at higher FE_{NO} values,

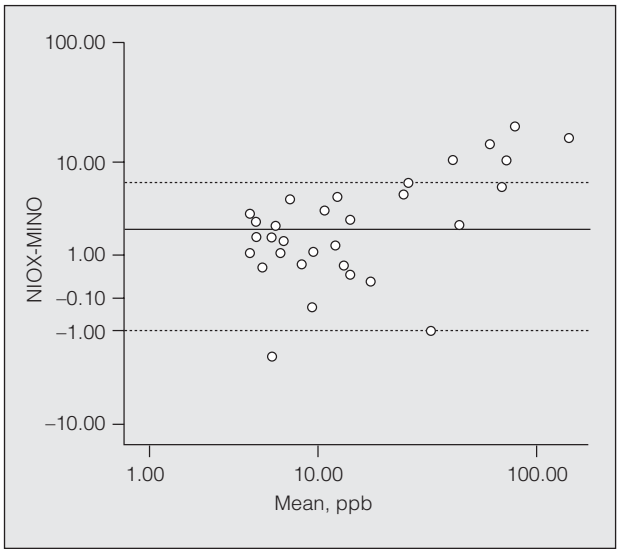

Figure 3. A Bland-Altman plot comparing the differences between the measurements obtained with the 2 devices (the NIOX and the portable MINO) used to measure fractional exhaled nitric oxide concentration (FE_{NO}) and the mean of measurements from the 2 devices. ppb indicates parts per billion. (Modified from McGill et al.[4])

although this was not clinically significant for either the diagnosis or management of asthma in these children. McGill et al[40] found the NIOX analyzers are somewhat more precise (±2.5 ppb for values under 50 ppb in the NIOX as compared to ±5 ppb for values under 50 ppb or ±10% for values of 50 ppb or higher in the NIOX-MINO). In that study, the range of measurements was from 0 to 200 ppb in the NIOX compared to 5 to 300 ppb in the NIOX-MINO (Table 1). The chief disadvantage of the NIOX-MINO is that it does not produce a graphic representation of the FE_{NO} plateau corresponding to the exhaled bronchial fraction: the handheld system provides only a digital readout of the final FE_{NO} value.

In the study by McGill et al,[40] children of 5 years or older proved capable of performing the maneuver required for the measurement of FE_{NO} with the handheld NIOX-MINO system. Furthermore, the intersubject coefficient of variation was lower for the NIOX-MINO (11%) than for the NIOX analyzers. This means that there were no differences between the first and subsequent measurements and that a single measurement, as recommended by the manufacturer, was sufficient. However, current ATS/ERS guidelines recommend a minimum of 2 successive measurements.[21]

Children have a smaller total lung capacity than adults and this difference could, in some cases, make it difficult for them to exhale correctly for longer than 6 sec. Taking this into account, McGill et al[40] validated the use of a 6-second exhalation time with the handheld NIOX-MINO analyzer as the ATS/ERS guidelines indicated that a 6-second maneuver might be valid for children aged between 6 and 12 years.[21]

In a recent study, Menzies et al[43] validated results obtained with the portable MINO analyzer against those obtained with a NIOX in 101 asthmatic children and 50 healthy controls. They observed an excellent correlation between the 2 systems in both the asthmatic children and the healthy controls (r=0.94 and 0.96, respectively). In a study of 28 healthy nonsmoking adults, Fortuna et al[44] compared the results obtained with a conventional chemiluminescence analyzer (N-6008, SIR, Madrid, Spain) with those obtained using the portable NIOX-MINO analyzer, following current international recommendations in both cases.[21] They found a direct and statistically significant correlation between measurements from the 2 analyzers (R=0.92; P=.001). However, in line with the results of earlier studies,[40,45] those authors found that FE_{NO} values obtained with the portable NIOX-MINO analyzer were significantly higher and proposed a correction of ×1.5+10 for each FE_{NO} measurement obtained with the N-6008. They also reported normal reference values that agree with those previously published in the literature—geometric mean FE_{NO} for the N-6008 system of 7 (5) ppb (range, 1-19 ppb) and of 20 (7) ppb (range, 8-41 ppb) for the NIOX-MINO—and proposed a cutoff of 20 ppb for the chemiluminescence analyzer and 35 ppb for the portable system.

In conclusion, the results obtained with the 2 measuring devices are comparable, valid, and reproducible. However, while school-age children in the cited studies were capable of performing both techniques reliably, preschool children obtained better results with the NIOX.

Reference Values

For SBOL measurement with chemiluminescence analyzers using an exhalation flow rate of 45-55 mL/s, normal FE_{NO} values in healthy adults are in the range of

TABLE 1
Comparison of the NIOX and NIOX-MINO Analyzers Used to Measure Exhaled Nitric Oxide Concentration[a]

	NIOX	NIOX-MINO
Dimensions: height, width, depth, cm	50 × 30 × 40	24 × 13 × 10
Weight of the analyzer, kg	40	0.8
Accuracy of measured value		
In measurements <50 ppb	±2.5	±5
In measurements >50 ppb	±5%	±10%
Range of measurements, ppb	0-200	5-300
Lower detection limit, ppb	1.5	5

Abbreviations: ppb, parts per billion

TABLE 2.
Diseases and Other Factors That Modify Fractional Exhaled Nitric Oxide Concentration (FE_{NO})

Increased FE_{NO}	Decreased FE_{NO}	Variable FE_{NO}
Asthma	Cystic fibrosis	COPD
Exposure to pulmonary allergens	Primary ciliary dyskinesia	Bronchiectasis
Pollution	Pulmonary hypertension	Fibrosing alveolitis
Apnea	Pneumonia	Sarcoidosis
Bronchodilators (transient)	Gastroesophageal reflux	Systemic sclerosis
Viral respiratory infection	Laryngeal tracheomalacia	
Pulmonary tuberculosis	Sputum induction	
Allergic rhinoconjunctivitis. Nasal polyposis	Bronchoconstriction	
Posttransplant bronchiolitis	Forced spirometry (transient)	
Chronic inflammatory intestinal disease	HIV infection	
Hepatopulmonary syndrome	Smoking	
Liver cirrhosis	Alcohol	
	Caffeine	
	Menstruation	
	Nebulizer therapy with distilled water	

Abbreviations: COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus.

5-15 ppb.[44] The same reference values are found in children over 5 years of age, as confirmed by the following studies:

- Jöbsis et al[34] in 73 children, FE_{NO} of 10.5 (1.1) ppb
- Kharitonov et al[24] in 20 children, FE_{NO} of 15.6 (9.2) ppb
- Pedroletti et al[46] in 15 children, FE_{NO} of 12.5 (3.2) ppb
- Scollo et al[47] in 23 children, FE_{NO} of 10.1 (4.1) ppb
- Malmberg et al[48] in 62 children, FE_{NO} of 5.3 (0.4) ppb.

Buchwald et al[39] defined normal values for FE_{NO} in a multicenter study of 405 healthy children between 4 and 7 years of age. In that study, geometric mean FE_{NO} was 9.7 ppb (increasing with age) and the upper 95% confidence limit was 25.2 ppb.

In 2007, Daniel et al[49] published reference values for children aged between 2 and 7 years for measurement of FE_{NO} during tidal breathing with a face mask both online (geometric mean concentration, 3.9 ppb; 95% CI, 3.5-4.2 ppb) and offline (geometric mean concentration, 3.0 ppb; 95% CI, 2.7-3.3 ppb).

If NO is measured with an electrochemical analyzer following the ATS/ERS recommendations,[12,14] FE_{NO} values are slightly higher (range, 20-30 ppb) than those obtained with chemiluminescence analyzers.

Clinical Application of FE_{NO} Measurement in Childhood Respiratory Diseases

Asthma

Diagnosis. Elevated FE_{NO} values have been reported in children with asthma and other inflammatory lung diseases (Table 2), and this variable is now considered to be a valid and reproducible noninvasive marker for monitoring and managing airway inflammation and a parameter with a high discriminatory capacity for diagnosing asthma. In 1998, Cobos Barroso et al[50] reported the results of the first European pediatric study of FE_{NO} and nasal NO in 73 school-age children (36 with asthma and 37 healthy controls) with an expiratory flow rate of 250 mL/s. They observed differences between asthmatic children and controls (P<.0001) in both the subgroup receiving no treatment (for mild intermittent asthma) and the subgroup receiving inhaled corticosteroid therapy. Four years later, Balboa de Paz et al[51] reported similar results (study of 79 asthmatic children and 105 controls using an expiratory flow of 250 mL/s) and concluded that FE_{NO} could be useful for monitoring airway inflammation and evaluating patient response to anti-inflammatory treatment.

In another study of FE_{NO} and childhood asthma, Warke et al[52] compared NO values in bronchoalveolar lavage fluid in healthy children and asthmatics. They found that FE_{NO} correlated in asthmatic children with percentage eosinophils (r=0.78; P<.001) and eosinophilic cationic protein (r=0.53; P<.01) but not with other inflammatory cells in the bronchoalveolar lavage fluid. In that study, a FE_{NO} value higher than 17 ppb had a sensitivity and specificity of 81% and 80%, respectively, for predicting asthmatic inflammation. FE_{NO} would therefore be a valid and reproducible noninvasive marker for measuring eosinophilic airway inflammation.[53]

Malmberg et al[54] also observed that high FE_{NO} values (≥3 SD) correlated with clinical asthma (odds ratio, 16.3; 95% CI, 5.4-49.7; P<.0001) and sputum eosinophilia (odds ratio, 12; 95% CI, 4.1-35; P<.0001), with clinical asthma and sputum eosinophilia being the strongest predictors of high FE_{NO} values followed by atopy and bronchial hyperresponsiveness. Those authors concluded that FE_{NO} was primarily a marker of airway eosinophilia and that significantly elevated FE_{NO} values could be a useful indicator for identifying patients with atopic or nonatopic asthma.

When those authors compared various lung function tests (spirometry, forced oscillometry, and bronchodilation test) and induced sputum with FE_{NO} in children with suspected asthma, they found that the diagnostic

sensitivity of forced spirometry was lower (47%) than that of either FE_{NO} (88%) or induced sputum (86%).[54] The specificity of FE_{NO} was 92%, reflecting the greater discriminatory capacity of this parameter in preschool children with and without asthma and its capacity to detect the presence of inflammation in the early stages of the disease. The combination of an increase in FE_{NO} of at least 33 ppb and abnormal spirometry (FEV_1<80% of the theoretical value) resulted in a sensitivity and specificity for the diagnosis of asthma of 94% and 93%, respectively.[48,55]

As there are different asthma phenotypes, however, a normal FE_{NO} value does not rule out a diagnosis of asthma, particularly in the case of a noneosinophilic asthma phenotype.[56] Moreover, in children, the different asthma phenotypes are not as clearly defined as they are in adults, especially those relating to severe asthma in which symptoms and lung function decline persist despite treatment. Although severe childhood asthma is a heterogeneous entity, the predominant pattern is thought to be that of T_H2 cell-mediated chronic airway inflammation. However, the question remains of whether evolution from one phenotype to another over the course of a lifetime is possible in a single individual.[57,58]

Monitoring treatment. FE_{NO} has been used satisfactorily to monitor anti-inflammatory treatment with inhaled corticosteroids in asthmatic children. This success is due to the great sensitivity of this parameter and its ability to rapidly detect deterioration in lung function and changes in response to doses of inhaled corticosteroids. Jones et al[59] studied 78 patients with moderate-to-severe asthma receiving inhaled corticosteroid therapy and compared FE_{NO}, induced sputum analysis, and bronchial hyperresponsiveness as methods for diagnosing and predicting deterioration of lung function and loss of control over asthma. They found that FE_{NO} correlated more closely with the respiratory symptoms indicative of loss of control of the disease (P<.0001) than either FEV_1 obtained by forced spirometry (P<.002) or induced sputum analysis (P< .0002). An increase in FE_{NO} of more than 60% over baseline had a positive predictive value between 80% and 90% for loss of control over the disease. Moreover, FE_{NO} monitoring facilitates the adjustment of steroid treatment to the minimum effective dose while maintaining control over the inflammatory process.[60] It can also be used even before symptoms develop to identify patients who will respond well to inhaled corticosteroids since a FE_{NO} value of 47 ppb or higher has been shown to be a better positive predictor of response to corticosteroids in asymptomatic patients than spirometry, the bronchodilator test, or bronchial hyperresponsiveness. This makes it a reliable method for ensuring appropriate management of asthma in routine clinical practice.[61,62]

Lung function and FE_{NO} has also been studied in children aged between 2 and 5 years with asthma being treated with montelukast (4mg/d)[63]; treatment reduced mean FE_{NO} from 33.1 to 11.6 ppb and improved airway resistance measured using the interrupter technique with a single occluding valve. No changes were observed in bronchodilator response.

Similarly, Pijnenburg et al[64] studied FE_{NO} as a marker of eosinophilic airway inflammation in a group of 49 children (mean age 12 years) receiving treatment with inhaled budesonide or an equivalent corticosteroid (mean dose, 400 µg; range, 100-400 µg). They measured FE_{NO} levels after withdrawal of treatment with inhaled corticosteroids and found them to be an effective indicator of relapse or stability at both 2 weeks (35.3 ppb as compared to 15.7 ppb; 95% CI, 1.2-4.1 ppb; P=.01) and 4 weeks (40.8 ppb as compared to 15.9 ppb; 95% CI, 1.3-5.1 ppb). These results demonstrate that a FE_{NO} value of 49 ppb 4 weeks after withdrawal of inhaled corticosteroid treatment had a sensitivity of 71% and a specificity of 93% for determining whether the asthma is in remission.

Infant With Recurrent Wheezing

FE_{NO} measurement is also useful in the differential diagnosis of recurrent wheezing in preschool children, in whom spirometry and induced sputum analysis are more difficult to perform. In a study of 31 children with recurrent wheezing and a family history of atopy, Moeller et al[65] performed spirometry and measured FE_{NO} before and after the children received treatment with inhaled corticosteroids or placebo for 4 weeks. They found that treatment with moderate doses of inhaled corticosteroids significantly reduced FE_{NO} values (from 35 ppb to 16.5 ppb for the active treatment, compared to from 35.2 ppb to 30.2 ppb for placebo; P<0.05), a result that reflects the potential of FE_{NO} as a marker of airway inflammation requiring treatment even in the absence of respiratory symptoms and significant changes in lung function. Consequently, in the case of a FE_{NO} level under 20 ppb in a patient with symptoms indicative of asthma, various possible diagnoses should be considered, such as asthma of a neutrophilic phenotype, gastroesophageal reflux disease, rhinosinusitis with postnasal drip, left ventricular dysfunction, primary ciliary dyskinesia, vocal chord dysfunction, and so on. This use was demonstrated by Gabriele et al,[66] who studied 118 infants aged between 4 and 25 months. They found a different geometric mean FE_{NO} in healthy infants (10.4 ppb; 95% CI, 9.1-12.0 ppb) compared to those with recurrent wheezing (18.6 ppb; 95% CI, 15.6-22.2 ppb), bronchopulmonary dysplasia (11.7 ppb; 95% CI, 8.2-16.8 ppb), or cystic fibrosis (6.9 ppb; 95% CI, 3.4-10.1 ppb). Infants with recurrent wheezing had higher FE_{NO} values than the controls (P=.009), those with bronchopulmonary dysplasia (P=.038), and those with cystic fibrosis (P<.001). Moreover, the atopic infants with recurring episodes of wheezing had higher FE_{NO} than nonatopic infants with wheeze (P=.04).

In a prospective double-blind placebo-controlled study of 24 infants (10-26 months old) with recurrent wheezing randomized to receive montelukast (4 mg/d) or placebo, Straub et al[67] did not find that FE_{NO} predicted those who would have recurrent wheezing episodes.

Some researchers have studied FE_{NO} in healthy newborn infants to observe whether this parameter might serve as a predictor of the onset of recurrent respiratory disease. Latzin et al,[68] after observing a cohort of 164 healthy

newborn infants, concluded that the offspring of atopic mothers with high FE_{NO} were at risk for developing respiratory symptoms (relative risk, 7.5; 95% CI, 1.7-32.4; *P*=.007). Similarly, the combination of high FE_{NO} and respiratory symptoms is associated with maternal smoking (relative risk, 6.6; 95% CI, 2.3-19.3; *P*<.001). The detection of this "pre-asthmatic state" in the newborn children of mothers who smoke or are atopic gives rise to an interesting argument in favor of the use of pharmacological therapy aimed at modifying the natural history of asthma in some of its phenotypes.[69]

Atopy

An increase in nasal NO has been reported in patients with allergic rhinitis. This declines after treatment with oral topical corticosteroids, an effect that appears to be explained by the overexpression or inhibition of epithelial iNOS in the upper airway, both effects closely related to eosinophilic inflammation. Jouaville et al[70] studied 149 children (53 untreated asthmatics and 96 healthy subjects) in whom they measured FE_{NO} and performed a prick test. Those authors found that the children with atopic asthma had higher values than the nonatopic asthmatic children (28.9 [9.1] ppb as compared to 17.1 [13.1] ppb; *P*<.0004). Atopic non-asthmatic children (2 positive allergens per prick test) with allergic rhinitis had higher FE_{NO} than nonatopic children (20.7 [13] ppb vs 11.7 [6.7] ppb; *P*<.003) and atopic children who had neither allergic rhinitis nor asthma (12.5 [6.4] ppb). Among the asthmatic children, however, FE_{NO} was higher in the atopic individuals whether or not they had allergic rhinitis. The authors concluded that, in addition to asthma and atopy, allergic rhinitis should also be taken into account when interpreting FE_{NO} in routine clinical practice.

Premature Infants

A certain number of very premature newborn infants require supplemental oxygen even after 36 weeks postmenstrual age. On discharge from hospital, these infants develop respiratory diseases, require emergency department care, and are readmitted for various respiratory diseases during the first 2 years of life. This condition, called bronchopulmonary dysplasia or prematurity-related chronic lung disease, is known to be an inflammatory process in which FE_{NO} monitoring could be of interest.

When lung function and FE_{NO} values were investigated prospectively by way of serial measurements, FE_{NO} did not predict the development of prematurity-related chronic lung disease.[71] In that study, birthweight and lung function results were more closely related to this disease.

Cystic Fibrosis

FE_{NO} values are low in cystic fibrosis despite the presence of chronic inflammation in these patients. These low values are due in part to a reduction in NOS_2 expression in the airways of children with cystic fibrosis, but the high nitrate levels found in the secretions of patients with this disease are also indicative of the metabolism of NO that occurs in these fluids.[72]

In a novel study, Mahut et al[73] used a 2-compartment model to separately analyze the production of NO in the alveolar area and the conducting airways in order to differentiate areas of inflammation and gain a better understanding of their distribution. Alveolar FE_{NO} levels were higher in alveolitis (secondary to hypersensitivity and idiopathic pulmonary fibrosis) than in asthma, and an inverse correlation was found between FE_{NO} and both lung diffusing capacity and the degree of restriction.

Primary Ciliary Dyskinesia

Low FE_{NO} levels are found in association with primary ciliary dyskinesia. Corbelli et al[74] reported that the measurement of nasal NO in accordance with ATS/ERS recommendations could help identify this disorder. Nasal NO values were very low in patients of all ages with this disease (13.7 ppb) compared to healthy controls (223.7 ppb). They also reported that the positive predictive value of a nasal NO value under 105 ppb for a diagnosis of primary ciliary dyskinesia was 89% with a specificity of 88%. It is, therefore, one of the screening techniques of choice for this diagnosis.

Other Respiratory Processes

FE_{NO} does not increase in stable chronic obstructive diseases, but may occasionally be elevated if the patient's condition becomes unstable.[75] Most authors report lower FE_{NO} in subjects with bronchiectasis than in the normal population, while alveolar FE_{NO} is similar in both groups.[76]

Conclusions

FE_{NO} is a valid, noninvasive, reproducible, and stable marker of eosinophilic airway inflammation useful in the differential diagnosis of various lung diseases in routine clinical practice[77] and as a complement to lung function testing in the diagnosis and management of childhood asthma.[78] A FE_{NO} value greater than 20 ppb measured on a chemiluminescence analyzer or 35 ppb on a portable electrochemical analyzer indicates increased inflammatory activity in the airway. Consequently, this technique facilitates better adjustment of antiinflammatory therapy and can identify possible cases of poor compliance with treatment or even indicate the need to consider withdrawal of treatment.[79]

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