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New products

Series editors: Donald Y. M. Leung, MD, PhD, Harold S. Nelson, MD, Stanley J. Szeffler, MD, Philip S. Norman, MD, and Andrea Apter, MD, MSc

The Aerocrine exhaled nitric oxide monitoring system NIOX is cleared by the US Food and Drug Administration for monitoring therapy in asthma

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The Aerocrine exhaled nitric oxide monitoring system NIOX is cleared by the US Food and Drug Administration for monitoring therapy in asthma

Philip E. Silkoff, MD, (guest editor),^a Mats Carlson, MSc,^b Trevor Bourke, BSc,^c Rohit Katial, MD,^a Elisabeth Ögren, MPharm,^{b,†} and Stanley J. Szeffler, MD^a Denver, Colo, Stockholm, Sweden, and New York, NY

The Aerocrine exhaled nitric oxide (NO) monitoring system NIOX was cleared by the US Food and Drug Administration for clinical application in patients with asthma in May 2003. The fractional concentration of exhaled NO has been extensively researched as a marker of airway inflammation in asthma and other diseases and is now poised to enter clinical application. The American Thoracic and European Respiratory Societies' current guidelines recommend measurement at constant expiratory flow, which is difficult for some adults and children. The NIOX NO monitoring system was designed to facilitate standardized measurement according to guidelines. A clinical study was performed together with *in vitro* testing to obtain clearance. Exhaled NO levels were measured in unstable steroid-naïve adult and pediatric asthmatic subjects and again after a 2-week treatment with inhaled corticosteroids. Exhaled NO levels decreased highly significantly, with 95% confidence limits for the decrease of –40% to –60% accompanied by clinical improvement. This trial, together with extensive *in vitro* testing, led to the clearance of NIOX by the US Food and Drug Administration. This article in the journal's "New products" feature section will describe background material regarding exhaled NO, special features of the NIOX NO monitoring system, and how this tool can be incorporated into clinical asthma management. (J Allergy Clin Immunol 2004;114:1241-56.)

Key words: Exhaled, nitric oxide, asthma, inflammation, monitoring

THE HISTORY OF EXHALED NITRIC OXIDE

It was only in the late 1980s that the small molecule nitric oxide (NO) was found to be an endogenous mediator of profound importance in both health and disease synthesized by complex heme-containing NO synthases.¹ It is hard to imagine biologic science without this mediator today, which is reflected in NO being crowned "Molecule of the Year" by *Science* in 1992.² The profound achievements of Furchgott, Ignarro, and Murad in unraveling the NO story brought them the Nobel Prize for Medicine in 1998,³ although other scientists were also deserving.⁴

In 1991, a pivotal discovery was made by Gustafsson et al⁵ that exhaled breath in animals and human subjects contains NO, and this was independently confirmed by means of gas chromatography mass spectroscopy.⁶ In the early 1990s, several investigators reported that fractional concentration of exhaled NO (F_{ENO}) levels are increased in asthmatic patients compared with those in healthy individuals.⁷⁻¹⁰ Shortly after that, F_{ENO} levels were found to be decreased after corticosteroid use^{7,11-13} and inhaled NO synthase inhibitor use,¹³ confirming the endogenous origin and synthesis. NO and exhaled NO have been the topics of recent clinical forums in this journal.^{14,15} Landmarks in the history of exhaled NO appear in Table I.

Exhaled NO measurement issues

Initial concern about contamination of exhaled samples by NO produced in high concentrations by the nose and paranasal sinuses was allayed by the discovery that exhaling against resistance with a positive mouth pressure excluded nasal NO by means of velum closure.¹⁶ In 1997, F_{ENO} levels were found to be inversely proportional to expiratory flow rate, a characteristic termed flow dependence, and this was explained by the fact that exhaled NO is in part derived from a diffusion-based process in the

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[†]We regret to announce that Elisabeth Ögren from Aerocrine passed away tragically during the preparation of this article.

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Abbreviations used

ATS:	American Thoracic Society
C_{alv} :	Steady-state alveolar NO concentration
C_w :	Airway wall NO concentration
D_{NO} :	Airway NO transfer factor
FDA:	US Food and Drug Administration
F_{ENO} :	Fractional concentration of exhaled NO
ICS:	Inhaled corticosteroid
NO:	Nitric oxide
ppb:	Parts per billion
TSS:	Total symptom score

airways.^{16,17} This flow dependence led to a need for standardization of the expiratory flow rate and also led to the development of physiologic models for NO excretion from the human lung.¹⁸

A major step forward was the involvement of the European Respiratory Society and the American Thoracic Society (ATS) in the standardization of exhaled NO measurement, which was encouraged by enthusiastic investigators in the field who wished to unify methods as a basis for comparison and for future clinical application. The European Respiratory Society's task force document appeared in 1997,¹⁹ and the ATS published recommendations in 1999²⁰; the latter is due to be updated in 2004 and ratified by both societies. There is also a separate pediatric exhaled NO measurement document available.²¹ NO in human breath can be measured accurately and reproducibly according to guidelines published by these societies.^{19,20}

Recommended technique for exhaled NO measurement

The recommended method for exhaled NO measurement has taken into consideration the flow dependency of exhaled NO.^{16,22} The standardized single-breath technique requires inhalation to total lung capacity followed by exhalation at a constant flow rate of 50 mL/s against resistance to exclude nasal NO by means of velum closure.^{19,20} F_{ENO} is defined as a 3-second NO plateau at the end of the exhalation. Nasal NO, which might contaminate exhaled NO,²³ is excluded by means of velum closure facilitated by exhaling against resistance with positive mouth pressure, with subjects targeting a flow or pressure displayed on a monitor. Simple systems for controlling the very low flows required by the recommendations include the use of biofeedback of exhalation pressure,¹⁶ exhalation flow,²⁰ or both; mass flow controllers²⁴; starving resistors²⁵; and servo systems that adjust resistance in relation to airway pressure and operator-controlled resistance.^{26,27} Despite diverse technologies, exhalation at a constant flow rate remains difficult or impossible for some adults and for the younger child.²⁸ Readers wanting greater details are recommended to access the documents on the societies' Web sites (<http://www.thoracic.org> and <http://www.ersnet.org>).

TABLE I. Landmarks in exhaled NO and asthma

Discovery of NO in biology	Late 1980s
Discovery that NO is present in breath	1991
Discovery that exhaled NO levels are increased in patients with asthma	1993
Discovery that exhaled NO levels decrease after steroid therapy in patients with asthma	1994-5
Relationship of exhaled NO to reactivity and airway inflammation	2001
Expiratory flow dependence of exhaled NO	1997
Modeling of the secretion of NO in the airway with flow-independent parameters	2001
Exhaled NO found to be useful in predicting asthma exacerbations	2001
Approval of NIOX in Europe	April 2000
Clearance of NIOX by the FDA	May 1, 2003

Factors influencing F_{ENO} levels

There are many determinants of exhaled NO levels, which might vary considerably in health and disease. In healthy subjects exhaled NO levels appear to increase in children with age²⁹ and to be higher in men than in women.³⁰ These characteristics might be related to measurement at a fixed absolute flow rate, which constitutes a different relative flow when related to airway size. There are also genetic determinants, including described polymorphisms of NO synthases.³¹ Cigarette smoking also decreases exhaled NO levels,³² and food and drink might acutely alter levels.³³ Disease states that have been associated with an increase of exhaled NO levels include asthma,⁷ atopy without asthma,³⁴ viral infection,³⁵ mycobacterial infection,³⁶ liver disease,³⁷ lung cancer,³⁸ bronchiectasis,³⁹ lung transplant rejection,^{40,41} and chronic obstructive pulmonary disease.⁴²⁻⁴⁴ Decreases of exhaled NO levels are reported in cystic fibrosis⁴⁵ and ciliary dysmotility syndromes.⁴⁶

Reference values of F_{ENO}

In healthy adult individuals exhaled NO levels fall in the 10- to 20-ppb range, but as with other biologic parameters, there are some individuals with unexplained higher levels. As stated above, viral infection increases exhaled NO levels, even in healthy individuals, and the airways are continuously exposed to pollution and allergens. There are reports that exhaled NO levels increase with ambient air pollution.^{47,48} F_{ENO} levels in asthmatic patients usually fall in the 25- to 80-ppb range but vary greatly depending on the extent of the airway inflammation and the treatment used.⁴⁹ Levels increase in individuals experiencing exacerbation⁵⁰ or in cases of uncontrolled disease⁵¹ and are lower in patients with stable asthma receiving anti-inflammatory treatment.^{52,53} Monitoring F_{ENO} levels before and during anti-inflammatory therapy can serve as a tool for monitoring asthma and the effects of treatment.⁵⁴

Evidence that FE_{NO} is a marker of airway inflammation

Evidence that exhaled NO is a marker of asthmatic airway inflammation can be classified as indirect and direct. The indirect evidence includes the fact that exhaled NO levels are increased in asthma, an inflammatory airway syndrome, and decrease after anti-inflammatory treatments, such as corticosteroids⁵² and leukotriene axis modifiers,^{55,56} but not bronchodilators, such as salmeterol.^{57,58} Direct evidence includes correlations between exhaled NO and sputum eosinophils^{59,60} and eosinophilic markers in bronchial biopsy specimens.⁶¹⁻⁶³ Recently, Mahut et al⁶⁴ have reported significant correlations between exhaled NO levels and not only indices of inflammation but also airway remodeling (eg, subbasement membrane thickening). Undoubtedly, FE_{NO} will not reflect all the components of asthmatic inflammation, the phenotype of which might vary considerably from patient to patient and over time. Thus this marker might not be of value in all patients.

Exhaled NO has been found to correlate with other standard outcomes in asthma, including airways reactivity to inhaled histamine^{65,66} and methacholine^{67,68} and bronchodilator responsiveness,⁶⁹ but in general not with lung function⁷⁰ or indices of asthma severity.⁷¹ This is probably because this marker is assessing the inflammatory asthma component, and the latter has complex relationships to airway obstruction and asthma severity. Indeed, if exhaled NO correlated well with lung function, there would be little value in its assessment. Exhaled NO has also proved useful in predicting a good FEV_1 response to inhaled corticosteroid (ICS) therapy⁷² and oral steroids.⁷³

Targeting asthma control

In the past, therapies in asthma were assessed mainly for their ability to improve asthma outcomes, such as symptom scores, peak flow, lung function, and reactivity, compared with placebo. More recently, therapies and their means of administration are being evaluated for their ability to improve asthma control. In the broadest sense asthma control might include the traditional outcomes mentioned above but, more importantly, will also include reduction in asthma exacerbations, urgent care visits, use of steroids, and health care use, together with improvement in quality of life. The ideal therapy would also reduce asthma morbidity and mortality and carry a favorable benefit/risk ratio. Long-term improvement of asthma control could prevent tissue remodeling, although there is no evidence to support this.

Can monitoring other parameters in addition to symptoms and spirometry contribute to asthma control? Sont et al⁷⁴ reported that monitoring reactivity to methacholine, as a basis for adjusting ICS dose, helped reduce asthma exacerbations. Jones et al⁷⁵ studied exhaled NO weekly in subjects with asthma who abruptly stopped their ICSs. In subjects who eventually experienced loss of control, exhaled NO levels had increased significantly higher and

more rapidly than in those remaining clinically stable. Importantly, exhaled NO levels at the visit before loss of control were predictive of the upcoming exacerbation at a time when symptoms remained stable. Green et al,⁷⁶ in a pivotal study performed in an outpatient setting, used eosinophils in sputum at each visit as a parameter to adjust steroid therapy and compared this with British Thoracic Society standard care. There was a highly significant reduction in the exacerbation rate in the sputum arm, and moreover, this did not carry an increased steroid burden. Exhaled NO levels were significantly lower in the sputum arm, most probably because of reduced eosinophilic inflammation. Studies are ongoing to see whether exhaled NO measurement, which is simpler and less invasive than sputum testing, could perform as well as sputum testing in this scenario. The main message from the studies of Sont et al,⁷⁴ Jones et al,⁷⁵ and Green et al⁷⁶ is that monitoring inflammatory outcomes might allow real-time adjustment of therapy in proportion to airway inflammation, thus improving control and, most importantly, reducing exacerbations.

Modeling NO secretion in the airway

Recently, several investigators have proposed physiologic models that explain the flow dependency of exhaled NO and also allow the derivation of additional parameters that describe the lung excretion of NO.^{17,25,77,78} The most common proposal is a 2-compartment model consisting of alveolar and airway compartments. After inspiration, any NO that is carried to or produced in the alveolar region (derived from inspired air, the nasal cavity, and the conducting airways) diffuses rapidly across the alveolar-capillary membrane to bind with hemoglobin, and a steady-state low alveolar concentration (C_{alv}) is established. As exhalation occurs, alveolar gas with an NO concentration equal to C_{alv} ascends the airway and is conditioned with NO diffusing from the airway wall under a concentration gradient, which is equivalent to the airway wall concentration of NO (C_w) minus the luminal concentration of NO. The diffusivity of NO in the airways is described by a transfer factor for NO between the airway wall and the lumen (D_{NO}). The 3 measures, C_{alv} , C_w , and D_{NO} , have been termed flow-independent parameters by Shin et al.⁷⁹ A review of this growing area and how these parameters are perturbed in airway and parenchymal lung diseases appeared recently.¹⁸ It remains to be seen whether the measurement of the flow-independent parameters will contribute additional utility to measurement at a single flow rate, although an increase of alveolar NO levels has been described in chronic obstructive pulmonary disease, nocturnal asthma, and interstitial lung disease.⁸⁰⁻⁸² It might, however, be essential to measure the alveolar-capillary diffusion factor for NO to distinguish between increased alveolar NO production, decreased alveolar-capillary NO diffusion, or both, per the study of Hsia et al.⁸³

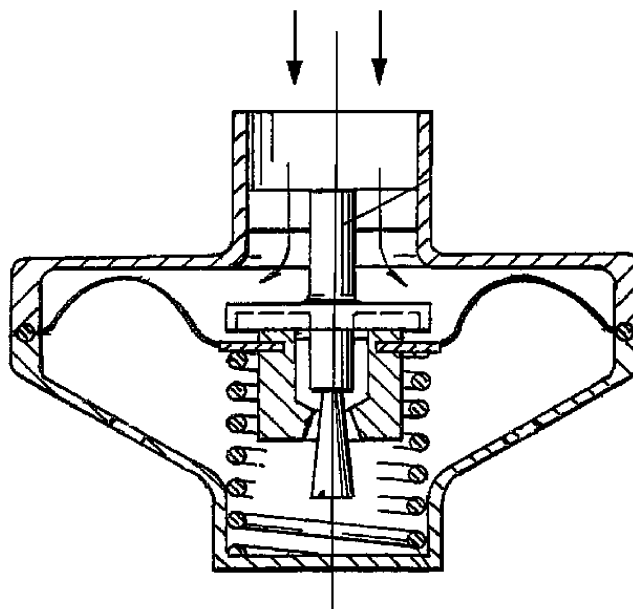


FIG 1. Schematic of NIOX flow control. The dynamic flow controller principle is based on exhalation force controlling forward (increased force) or backward (decreased force) position of a longitudinal piston that has a gradually increasing diameter. The movement of the elongated element varies the aperture and thus the exhalation flow through the control. This design facilitates the maintenance of a constant exhalation flow at low rates and is validated to maintain an exhalation flow of 50 ± 5 mL/s, conforming to ATS standards.¹²

Measurement scenarios

Online F_{ENO} measurement. Although adult online single-breath measurement has been described above, some adults and very young children have difficulty in maintaining a constant expiratory flow rate.²⁸ The 1999 ATS statement dedicated a section to exhaled NO measurement in adults and children, but the pediatric section has now been updated in a separate document that appeared in the *European Respiratory Journal* in 2000²¹ and also can be accessed on the ATS Web site (<http://www.thoracic.org>). The adult single-breath method described above is usually applicable to children of school age but unsuitable for the preschool child, who usually cannot maintain a steady exhalation flow rate. Online tidal breathing methods have been applied to children and infants with some success.⁸⁴ Ways to facilitate a steady flow include the dynamic flow resistor, which is incorporated into the NIOX System (Fig 1). This spring-mounted channel advances irrespective of whether mouth pressure increases, thereby occluding orifices and reducing flow, or retracts if mouth pressure decreases, opening apertures and increasing flow. The single-breath method has been applied in neonates by using a chest compression technique with exhalation flow control.²⁴

NO measurement in ventilated patients. NO measurement in ventilated patients presents a challenge for single-breath measurement, but a recent study described a practical methodology in the intubated subject.⁸⁵ Others have collected exhaled gas from tidal breathing and evaluated NO levels in patients with adult respiratory distress syndrome.⁸⁶

Offline F_{ENO} measurement. Offline NO measurement involves exhaling in a standardized manner and collecting exhalate for delayed measurement, and a section in the ATS statement deals extensively with this topic.²⁰ Such techniques are particularly useful for the bedside or emergency department or for large-scale studies in the workplace or school or in remote locations. Offline and online exhaled NO values show good correlations.⁸⁷ NIOX can be supplied with an adaptor for offline sampling. The development of more portable NO monitors, such as that described later in the section dealing with future developments, might obviate the need for offline techniques.

Nasal NO. Nasal NO, discussed above as a potential contaminant of exhaled NO, has been measured in its own right in nasal research. There are diverse ways of measuring nasal NO that are described in the ATS statement from 1999.²⁰ A nasal sampling kit is available for NIOX for those wishing to do nasal sampling. Nasal NO shows great promise as a tool for screening and noninvasive diagnosis for ciliary dysmotility syndromes, in which levels are extremely low.⁴⁶

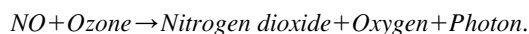
THE NIOX NO MONITORING SYSTEM

Aerocrine AB was founded by researchers at the Karolinska Institute in Stockholm, Sweden, in 1997, and a US subsidiary, Aerocrine Inc, Chicago, Illinois, was established in 2001. The company is entirely dedicated to the field of exhaled NO instrumentation and its clinical applications.



FIG 2. The Aerocrine NIOX NO monitoring system.

The Aerocrine NO Monitoring System, NIOX, was designed to facilitate constant-flow online measurement of F_{ENO} according to European Respiratory Society and ATS guidelines (Fig 2). The NO sensor is based on chemiluminescence, in which NO in the exhaled sample is drawn to a reaction chamber and reacts with ozone according to the following reaction:



The photons released by the reaction are detected with a photomultiplier tube, which generates a voltage linearly proportional to the amount of NO in the sample. The NIOX analyzer has a lower detection limit of 2 ppb, a suitable range for human breath analysis. To facilitate exhalation at a constant flow, which is essential for reproducible F_{ENO} values,¹⁶ the system incorporates a dynamic flow controller (Fig 1) that adjusts flow despite varying airway-opening pressure and monitors exhalation parameters to ensure conformity with guidelines.

Exhalation flow is monitored with a pneumotachograph to ensure that this remains within the ATS-recommended range of 45 to 55 mL/s.²⁰ In the design of the system, user friendliness was enhanced by inbuilt quality controls to ensure functionality with minimal need for human intervention. The repeatability of F_{ENO} measurements with the NIOX NO monitoring system is excellent in healthy children and adults and in children and adults with stable asthma.⁴⁹

THE PROCESS TO OBTAIN US FOOD AND DRUG ADMINISTRATION CLEARANCE FOR NIOX

The NIOX NO monitoring system was approved for clinical use in the European Union in April 2000. In the United States market authorization for medical devices is the responsibility of the US Food and Drug Administration (FDA) Center of Devices and Radiological Health. The NIOX NO monitoring system

was deemed an *in vitro* diagnostic and nonsignificant risk device by the FDA, which required validation of the intended clinical application and safety assurances. The first 510(k) application was rejected in March 2000; NIOX was deemed nonsubstantially equivalent because there was no accepted predicate device for comparison and was recommended to follow the *de novo* pathway.

Documented *in vitro* testing was required for validation of technical performance and safety, but a clinical trial was also necessary in the United States to confirm whether NIOX was able to detect a decrease in F_{ENO} levels in patients with unstable asthma after corticosteroid therapy, the initial intended clinical use. It was also necessary to provide a point-of-care analysis. The latter examines the interface between the system and the personnel required to perform the testing. The review process followed the *de novo* (risk-based classification) process, which in short means that NIOX was evaluated as a new device without comparison to a previously approved equivalent product but reclassified as low risk and thus cleared with similar documentation requirements as for a 510(k) instead of requirements for a full premarket approval.

On May 1, 2003, the FDA cleared NIOX for clinical use on the basis of the clinical trial results and *in vitro* testing. The FDA then published a guidance document on the documentation requirements of breath NO test systems.

THE CLINICAL TRIAL REQUIRED FOR FDA CLEARANCE

An open-label and prospective study was performed at 6 centers in the United States. Children (5-17 years old) and adults (>18 years old) with asthma, as defined by the National Asthma Expert Panel Report II,⁸⁸ were recruited. The protocol was approved by institutional review boards, and informed consent was obtained from all subjects, legal guardians, or both. Subjects were recruited from outpatient clinics by physicians who also served as study investigators.

TABLE II. Clinical trial showing testing by visit

Assessments	Visit 1	Visit 2
Medical history and physical examination	X	
FE _{NO} measurement	X	X
Spirometry before and after inhaled β_2 -agonist	X	X
ICS treatment started	X	
Documentation of medications	X	X
Adverse event assessment		X

Rescue medication and symptoms recorded daily by subjects between visits 1 and 2.

Inclusion and exclusion criteria

Subjects had uncontrolled asthma defined as 3 or more of the following symptoms for 2 or more days in the week before visit 1: cough, wheezing, dyspnea, chest tightness, expectoration, nocturnal asthma, and limited activity. Asthma was confirmed by previously documented reversibility or at visit 1. Because the purpose was to ascertain whether NIOX can detect a decrease in FE_{NO} levels after steroids and some asthmatic subjects do not exhibit an increased FE_{NO} level, subjects with a FE_{NO} level of less than 30 ppb at visit 1 were excluded. Other exclusion criteria were smoking in the 6 months before the study or a greater than 5 pack-year history; use of oral corticosteroids within 1 month; use of inhaled or nasal corticosteroids, leukotriene-modifying drugs, sodium cromoglycate, nedocromil sodium, or theophylline in the 2 weeks before the beginning of the study; and significant medical or other respiratory conditions.

At visit 1, eligibility was verified, and pretreatment testing was performed. Subjects then began taking open-label high-dose ICSs defined by the National Asthma Expert Panel Report on asthma.⁸⁸ Some subjects received oral prednisone in addition to ICSs at the treating physician's discretion. Visit 2 was at 14 ± 2 days, and this was when posttreatment assessments were made. Daily symptoms and medications were recorded by all subjects. A summary of procedures appears in Table II.

Measurement of FE_{NO} levels

FE_{NO} levels were measured according to the ATS guidelines²⁰ by using the NIOX NO monitoring system. Exhalation parameters were monitored so that parameters were within the limits specified by the ATS guidelines. Repeated exhalations were performed up to a maximum of 8 to obtain 3 reproducible exhalations that agreed within 10%.

Point-of-care analysis

The level of personnel education and training was recorded for each of the sites in the trial.

Statistical methods

Data from all centers were combined for intent-to-treat and protocol-correct analysis on separate adult and pediatric data sets, as well as for the groups combined with

TABLE III. Demographic characteristics

Variable	Age group		
	Adult	Pediatric	Combined
No. of subjects	30	32	62
Age (y)	34.1 ± 15.4	11.9 ± 3.34	22.6 ± 15.60
Sex, M/F	15/15	18/14	33/29
Ethnic background			
Asian-Pacific Islander	1	2	3
Black	8	5	13
White	21	21	42
Other (Hispanic)	0	4	4

SAS Version 6.12 (SAS Institute, Cary, NC). Kruskal-Wallis tests were performed to test for homogeneity of data across the sites. The changes in FE_{NO} levels, spirometric results, and total symptom scores (TSSs) from visits 1 and 2 were examined by using a 2-tailed paired *t* test. The changes in FE_{NO} levels were related to the changes in spirometric results and symptom scores with the Pearson correlation coefficient. All significance testing was at an α level of .05.

Results

Sixty-five subjects (33 children and 32 adults) were recruited at 6 centers, of which 62 were included in the statistical analysis. Demographic data are presented in Table III.

Change in FE_{NO}. The results are summarized in Table IV and Fig 3. For both age groups, separately and combined, there was a highly significant decrease in FE_{NO} levels from visit 1 to visit 2. The mean $\pm$ SD percentage change in FE_{NO} levels was $-50.5\% \pm 31.41\%$ ($P < .001$). The mean $\pm$ SD percentage change was $-57.1\% \pm 24.1\%$ in the pediatric group ($P < .001$) and $-43.3\% \pm 36.80\%$ in the adult group ($P < .001$). The FE_{NO} response was heterogeneous, with 9 adults and 2 children experiencing a decrease of less than 20% or an increase in FE_{NO} levels. The 11 patients who did not show a decrease of greater than 20% in FE_{NO} levels after anti-inflammatory therapy did not experience any improvement in symptoms.

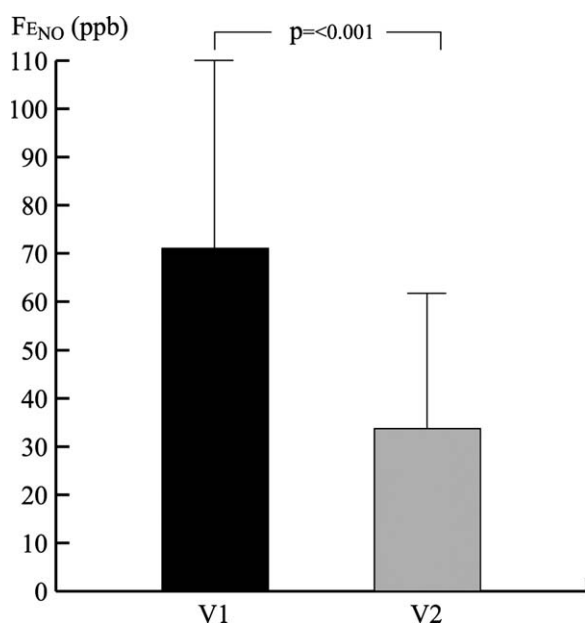
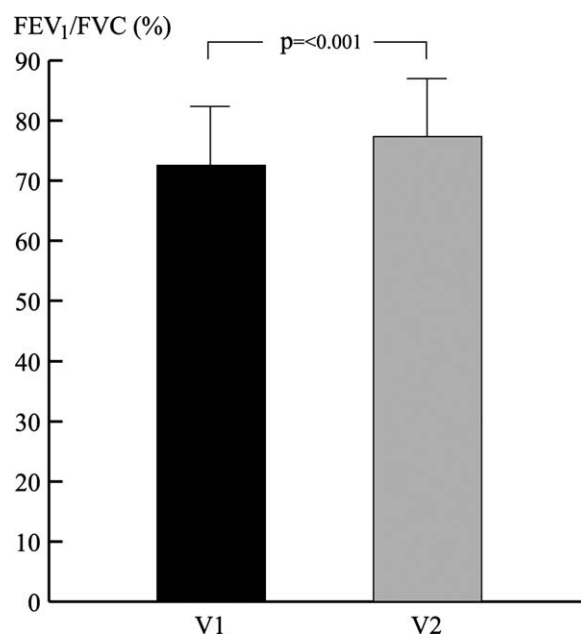
Spirometric parameters. In all subjects combined, a statistically significant percentage change from visit 1 to visit 2 was detected for the following parameters: FEV₁/forced vital capacity ($P = .010$), prebronchodilator FEV₁ ($P < .001$), prebronchodilator forced vital capacity ($P = .003$), and prebronchodilator forced expiratory flow at 50% of vital capacity ($P < .001$). Data are presented in Figs 4 and 5.

Symptom scores. In all subjects combined, TSSs decreased significantly ($P < .001$, Fig 5) from visit 1 to visit 2.

Correlations. FE_{NO} levels were not correlated at a single time point to spirometric parameters. There was, however, a significant correlation between the percentage change in FE_{NO} levels and the percentage change in prebronchodilator FEV₁ in the pediatric group ($P = .015$) and between the percentage change in FE_{NO} levels and the percentage

TABLE IV. Change in F_{ENO} levels from visits 1 to 2 in the adult and pediatric groups and combined data

Variable	Age group		
	Adult (n = 30)	Pediatric (n = 32)	Combined (n = 62)
F _{ENO} (ppb) at visit 1			
Mean ± SD	71.26 ± 38.290	70.86 ± 42.337	71.05 ± 40.099
Median	56.70	57.63	56.70
Min, max	33.9, 185.1	30.8, 193.8	30.8, 193.8
F _{ENO} (ppb) at visit 2			
Mean ± SD	36.51 ± 30.736	31.13 ± 26.419	33.74 ± 28.481
Median	26.73	22.52	24.75
Min, max	10.9, 151.2	3.5, 116.4	3.5, 151.2
Change in F _{ENO} (ppb) from visit 1 to visit 2			
Mean ± SD	−34.74 ± 38.159	−39.73 ± 26.928	−37.32 ± 32.666
Median	−30.65	−32.23	−31.83
Min, max	−161.0, 32.1	−129.8, 7.8	−161.0, 32.1
Percentage change in F _{ENO} from visit 1 to visit 2			
Mean ± SD	−43.35 ± 36.805	−57.13 ± 24.063	−50.47 ± 31.408
Median	−56.50	−62.98	−61.65
Min, max	−87.0, 64.4	−91.6, 23.2	−91.6, 64.4
95% CI	−57.10 to −29.61	−65.81 to −48.46	−58.44 to −42.49
P value	<.001	<.001	<.001

Test method: 2-tailed paired *t* test.**FIG 3.** The change in F_{ENO} levels (mean ± SEM) from visit 1 (V1) to visit 2 (V2) for the intent-to-treat analysis in the combined adult and pediatric populations.**FIG 4.** The change in prebronchodilator FEV₁/FVC ratio (mean ± SEM) from visit 1 (V1) to visit 2 (V2) for the intent-to-treat analysis in the combined adult and pediatric populations.

change in TSSs in both the entire population ($P = .017$) and the pediatric group ($P = .007$).

Subject acceptability of exhaled NO measurement. The maximum allowable number of exhalations per subject by protocol was 8. A mean ± SD of 3.8 ± 1.39 (range, 3–8) and 3.8 ± 1.45 (range, 3–8) exhalations per subject at visit 1 and 3.3 ± 0.45 (range, 3–4) and 3.3 ± 0.83 (range, 3–6) exhalations per subject at visit 2 in adults and children,

respectively, were needed to obtain 3 acceptable exhalations, indicating that the procedure was practical to perform. There were no reported device-related adverse events.

Point-of-care analysis. The education and experience of the research personnel that supervised the F_{ENO} measurements varied (Table V). All personnel encountered

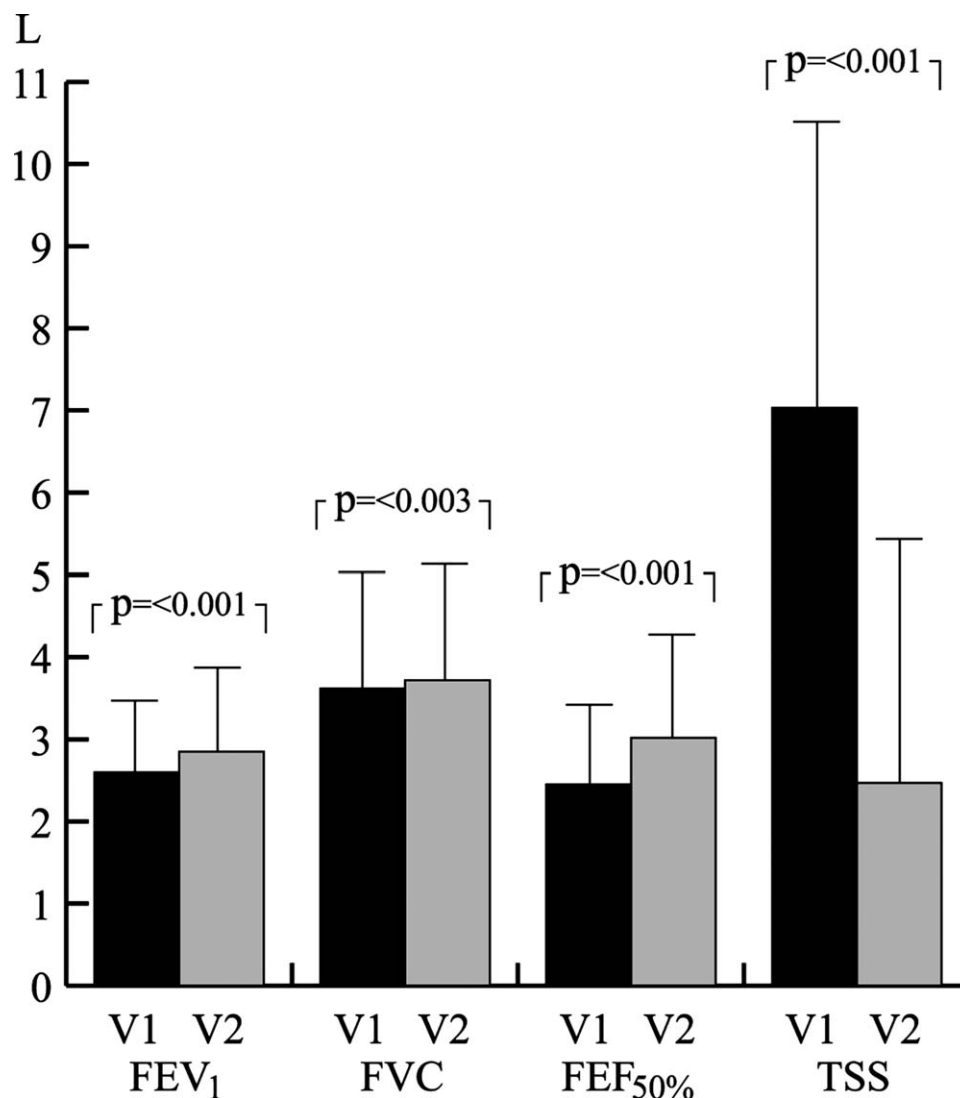


FIG 5. The change in prebronchodilator, FEV₁, FVC, forced expiratory flow at 50% vital capacity (FEV_{50%}), and TSS (mean ± SEM) from visit 1 (V1) to visit 2 (V2) for the intent-to-treat analysis in the combined adult and pediatric populations.

NIOX for the first time in this study, and all received a 2- to 4-hour training session. When comparing the number of exhalations needed to obtain 3 approved FeNO measurements between the centers, the mean number of exhalations per subject varied between 3.4 and 4.4 at visit 1 and 3.0 and 3.6 at visit 2 (Table V) for adults and children combined. There were no statistically significant differences between centers or research personnel with different training concerning success rates.

Discussion

The clinical trial confirmed that the NIOX NO monitoring system detected a highly significant decrease in FeNO levels ($P < .001$) after 14 days of anti-inflammatory treatment (inhaled ± oral corticosteroids), which is in keeping with the results of many previous reports.^{12,17,89}

Additionally, on the basis of the number of exhalations required for a reproducible and acceptable test, the NIOX NO monitoring system is a practical and acceptable method to facilitate measurement of exhaled NO at a constant exhalation flow rate, even in adult and pediatric patients with uncontrolled asthma. There were no device-related adverse events. This study, together with *in vitro* validation, was submitted to the US FDA and was the basis for medical device clearance in May 2003. The 95% confidence limits of the magnitude of the change in FeNO levels were -40% to -60%, which is in close agreement with previous reports,^{10,12,53,90,91} confirming that NIOX can reproduce the published findings. The magnitude of the change in FeNO levels in this study is greater than that observed in the spirometric parameters (95% confidence limits, 7%-25%) and greater than the variability in a serial study in asthma with NIOX.⁴⁹ The profound decrease in

TABLE V. Success rate at each center defined as the number of exhalations needed to obtain 3 valid F_{ENO} measurements related to educational level and research experience of the research coordinator

Center	Education	Research experience	No. of subjects	Success rate, visit 1 (mean $\pm$ SD)	Success rate, visit 2 (mean $\pm$ SD)
1	BSc respiratory therapy	12 y	11	4.4 $\pm$ 1.57 (range, 3-7)	3.6 $\pm$ 0.69 (range, 3-5)
	Registered nurse	2.5 y			
3	Registered nurse	13 y	13	4.2 $\pm$ 1.86 (range, 3-8)	3.1 $\pm$ 0.49 (range, 2-4)
	Registered nurse	2.5 y			
	Registered nurse	1.5 y			
	Registered nurse	<1 y			
4	Registered nurse	3 y	20	3.6 $\pm$ 1.00 (range, 3-6)	3.5 $\pm$ 0.89 (range, 3-6)
5	Registered nurse	2.5 y	14	3.4 $\pm$ 1.34 (range, 3-8)	3.1 $\pm$ 0.36 (range, 3-4)
	Registered nurse	8 y			
	BA Ph	3.5 y			
	BSc	6 mo			
9	Certified clinical research coordinator	3 y	4	3.5 $\pm$ 1.00 (range, 3-5)	3.0 $\pm$ 0.00 (range, 3-3)

There were no statistically significant differences between centers concerning success rates.

Note: Centers 2 and 6 through 8 did not enroll subjects.

F_{ENO} levels in most subjects supports the high adherence to therapy reported by the majority of the subjects in their study diaries. There were, however, 11 subjects (9 adults and 2 children) who experienced a decrease of less than 20% or even an increase in F_{ENO} levels. This might be explained either by poor compliance or steroid resistance; however, these were not assessed in this study.

Supporting the relevance of F_{ENO} as a marker of the therapeutic response, there were significant improvements in symptoms and many spirometric parameters from visit 1 to visit 2, confirming both subjective and objective responses to therapy. Furthermore, there was a highly significant correlation between the percentage change in F_{ENO} level and the total symptom score ($P = .017$), with borderline correlations between the percentage change in F_{ENO} levels and prebronchodilator FEV_1 ($P = .051$) and postbronchodilator FEV_1 ($P = .060$) in all subjects combined. This further supports the validity of F_{ENO} as a relevant marker for the monitoring of asthma because the degree of decrease in F_{ENO} levels is not only accompanied by changes in standard outcomes (spirometry and symptoms) in the expected direction (ie, improvement) but also proportionate to this improvement. Similar to other reports, F_{ENO} levels were not correlated at a single time point to spirometry or symptoms. The lack of a correlation with spirometry can be explained by the concept that airway inflammation, reflected by F_{ENO} , is considered the central pathogenic feature in asthma, leading to several components. These components include symptoms, airflow obstruction, bronchial hyperresponsiveness to inhaled agents (eg, methacholine and allergens), and airway remodeling leading to fixed obstruction. In any individual the components can be present at different degrees, and this might also vary from time to time.

By way of illustration, an individual subject might have normal spirometric results but marked airway inflammation, whereas another might have marked chronic airflow obstruction but little inflammation. The time course of

change in the different components is also different. After treatment, F_{ENO} changes over days, symptoms change within a week, airflow obstruction improves over weeks, and reactivity takes several months to fully respond.

The point-of-care analysis provided recommendations for the level of training and personnel necessary to ensure reliable operation of the instrument.

In conclusion, the clinical study established that the NIOX NO monitoring system was able to detect a significant decrease in F_{ENO} after corticosteroid therapy for unstable asthma. The dynamic flow control incorporated in NIOX facilitated constant flow measurement of F_{ENO} in a clinical setting in adult and pediatric patients with uncontrolled asthma. The method was acceptable to patients and technicians alike. The decrease in F_{ENO} levels after corticosteroid therapy was accompanied by significant amelioration of symptoms and improvement in spirometric parameters. The study, together with the *in vitro* validation described below, served as the basis for the recent FDA clearance of NIOX for clinical use in patients with asthma.

***In vitro* testing**

The *in vitro* testing characterized the performance characteristics of NIOX and confirmed the safety and subject acceptability. The interference testing was essential to establish the magnitude of the possible confounding effects of humidity,^{92,93} CO_2 , alcohol, and isoprene, which are relevant to human subject testing with chemiluminescence.^{94,95}

Electrical and safety testing was performed, and the functionality of the system was validated. The following parameters were examined: accuracy, precision, linearity, and the lowest detection limit in pure laboratory gas and in simulated breath (air with added humidity and CO_2). Interference testing was performed for humidity, CO_2 , alcohol, and isoprene. The results of the *in vitro* testing are summarized in Table VI, including interferences and

TABLE VI. Summary of *in vitro* testing

Test	Results
Measurement range	2-200 ppb
Lowest detection limit	2 ppb
Offset	±3 ppb, 14 d
Sampling frequency	20 Hz
Increase time	10%-90%, <0.7 s
Lag time	<0.8 s
Response time	<1.5 s
Fall time	90%-10%, <1 s
Accuracy (deviation from mean value)	±2.5 ppb of measured value <50 ppb, ±5% of measured value >50 ppb
Precision expressed as SD for concentrations <50 ppb and coefficient variation for concentrations >50 ppb	<2.5 ppb of measured value <50 ppb (analytic and clinical precision); <5% of measured value >50 ppb (analytic precision); clinical precision was not evaluated for values >50 ppb
Linearity (integral linearity)	<2.5 ppb
Interference	
H ₂ O	<10% of measurement value at 5% volume H ₂ O
CO ₂	<10% of measurement value at 5% volume CO ₂
Ethanol	<2.5 ppb at 137 ppm ethanol
Isoprene	<2.5 ppb at 392 ppb OF isoprene
Exogenous substances	<2.5 ppb after ingestion of carbonated beverage, chewing gum, and mouth wash
Operating specifications	Permitted ambient temperature*: +15°C to +30°C; atmospheric pressure range*: 860-1060 hPa; humidity*: 30%-75% RH (noncondensing); calibration interval: recommended very 14 d

*Limits for operating temperature, pressure, and humidity to maintain accuracy are shown. The lowest detection limit of NIOX was determined by using gas mixtures of NO and N₂ to 2 ppb. Accuracy was determined by using gas mixtures of NO and N₂ in several concentrations between 2 and 200 ppb, including simulation of human breath with added CO₂ and H₂O. Analytic precision was determined by using gas mixtures of NO and N₂ in different concentrations between 2 and 200 ppb. Clinical precision was determined as the variability of consecutive measurements from the same patient measured with different instrument operators for NO concentrations of less than 50 ppb only. Linearity was determined in laboratory testing with gas mixtures of NO and N₂ in different concentrations between 2 and 200 ppb. Repeatability of exhaled NO measurements performed with NIOX was measured in a separate clinical study.⁴⁹ The coefficient of reproducibility expressed as the mean pooled SD on the basis of 229 sessions and 675 estimations was 2.11 ppb, and the intraclass correlation coefficient was 0.99 in both adults and children. The repeatability of F_{ENO} measurement within the same patient with NIOX operated by physicians, laboratory technicians, and nurses (expressed as mean intraindividual SD) was 1.40 ppb. Operator-dependent variability for F_{ENO} measurements of greater than 50 ppb has not been studied. Interference of CO₂ and H₂O with NO analysis caused by quenching was tested by flushing NO gas mixtures through CO₂ and H₂O to simulate human breath. Interference of isoprene was tested in the laboratory by generating a working curve for isoprene up to 392 ppb, detecting a chemiluminescence signal, and controlling the offset drift of the NO curve. No significant interference was detected. The interference of ethanol was studied in a clinical study with 20 healthy volunteers (10 male and 10 female patients; age, 20-65 years). F_{ENO} levels were measured within 30 minutes before ingestion of alcohol and at 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours after the start of ingestion. There was a small but statistically significant decrease of mean F_{ENO} levels after alcohol ingestion; however, the magnitude of the change of the F_{ENO} level is within the limits of variability of the NIOX System. The influence of a selection of exogenous substances (ie, chewing gum, carbonated beverage, and mouthwash) on F_{ENO} levels measured with NIOX was investigated in a clinical study. The primary end points were the differences between baseline F_{ENO} levels and F_{ENO} levels measured 1 and 2 hours after exposure to each exogenous substance. Ten healthy volunteers (5 male and 5 female subjects) were included in the study. There was a small but statistically significant increase of the mean F_{ENO} level after chewing gum and mouthwash exposure 1 hour from baseline; however, the magnitude of the change in F_{ENO} level is within the limits of variability of the NIOX System.

methods, whereas manufacturer specifications appear in Table VII.

THE FDA LABELING OF NIOX

The FDA cleared NIOX for clinical use in patients with asthma in May 2003. The labeling of this device is currently restricted to the monitoring of the response to anti-inflammatory medications, as an adjunct to established clinical and laboratory assessments, in adults up to 65 years old and children older than 4 years. The point-of-care study guided the labeling to restrict the operation of NIOX to trained physicians, respiratory therapists, nurses, and laboratory technicians. Physicians are alerted to the

possibility that an individual patient might demonstrate no change in exhaled NO after anti-inflammatory therapy because of steroid resistance, noncompliance, or other factors. Known interference with NO measurement (eg, food, beverages, smoking, infection, and physical exercise) on the basis of the literature is mentioned.²⁰

Measuring exhaled NO with NIOX is simple and noninvasive. Full details can be accessed in the instrument manuals, including calibration and maintenance requirements. Subjects should refrain from eating, drinking, and exercise for 1 hour before the test when feasible. Each center can develop a report form that will include subject details, medications, and ambient NO levels. Exhaled NO measurement should be performed before spirometry, which can perturb levels.⁹⁶

TABLE VII. Manufacturer's specifications

System	NIOX NO Monitoring System
Operating environment	
Ambient temperature	15°C-30°C
Atmospheric pressure	860-1060 hPa
Relative humidity	30%-75% (noncondensing)
Measurement range	2-200 ppb
Sampling frequency	20 Hz
Lag time	<0.8 s
Increase time	10%-90% <0.7 s
Response time	<1.5 s
Decrease time	90%-10% <1 s
Offset	±3 ppb/14 d
Accuracy	±2.5 ppb of measured value <50 ppb, ±5% of measured value >50 ppb
Precision	<2.5 ppb of measured value <50 ppb (analytic and clinical precision); <5% of measured value >50 ppb (analytic precision)*
Linearity	<±2.5 ppb integral linearity
Detection level	2 ppb
Interference	<10% of measurement value at 5% volume H ₂ O and 5% volume CO ₂

*Expressed as an SD for concentrations of less than 50 ppb or as a coefficient of variation of a measured value for concentrations of greater than 50 ppb. Clinical precision has not been evaluated for F_{ENO} values of greater than 50 ppb.

There are as yet no large studies published regarding normal values in adults or children. However, as a rough guide, exhaled NO levels are in the 10- to 20-ppb range in healthy nonatopic adults.⁴⁹ Levels in patients with steroid-treated asthma are around 30 to 40 ppb,¹⁷ whereas in patients with steroid-naïve asthma, levels are often greater than 80 ppb and can reach several hundred parts per billion.

CLINICAL APPLICATION

After FDA clearance of NIOX, a new phase of clinical application has arrived. There are several potential applications for F_{ENO} in the clinic, although for now, the FDA has only cleared the system for monitoring the effect of anti-inflammatory medication in patients with asthma.

Exhaled NO as an index of response to anti-inflammatory therapy

This application of NIOX is cleared by the FDA in adults and children. F_{ENO} levels decrease after corticosteroid therapy in patients with asthma but not after bronchodilators and is the earliest responding marker compared with symptoms, lung function, or reactivity. The use of NIOX in this context can be illustrated with a theoretic patient presenting with new-onset dyspnea, wheezing, and chest tightness. After physical examination, the F_{ENO} level is measured, followed by prebronchodilator and postbronchodilator spirometry, confirming asthma on the basis of reversible airflow limitation. If spirometric results are normal, the diagnosis might be made on the basis of reactivity to methacholine or exercise challenge. The patient is started on inhaled steroids and as-needed bronchodilators, with short-course oral steroids if indicated. Exhaled NO levels start to decrease within 1 to 2

days⁵³ and take approximately a week to reach a stable level.⁵² The patient returns after 6 weeks for follow-up. At that visit, history and physical examination are performed, and F_{ENO} measurement and spirometric testing are performed. In the ideal situation the patient reports a marked improvement in symptoms, airflow limitation is decreased, and the F_{ENO} level has decreased significantly. If, however, there has been a suboptimal response and F_{ENO} levels are still increased compared with those seen in patients with stable asthma,⁴⁹ this should lead the physician to consider increasing the dose of inhaled steroids, to ascertain adherence to therapy, and to identify ongoing exposures that might be driving airway inflammation. Alternatively, there might be an alternative diagnosis that mimics asthma (eg, hypersensitivity pneumonitis). Of note, there is some evidence that patients with asthma have increased levels of F_{ENO} after steroid therapy compared with healthy control subjects. This might be due to an increase in airway NO diffusivity, perhaps because of an increase in epithelial surface area occupied by steroid-resistant constitutive NO synthases.¹⁷ This stresses the need to establish individual stable baseline levels.

In long-term follow-up, clinical stability can be matched with the individual patient's stable exhaled NO level. If exhaled NO levels increase to greater than the stable level, this could indicate an upcoming exacerbation,⁷⁵ allowing the clinician to increase controller therapy, even at a time when the patient's symptoms remain clinically stable. Controller therapy could thus be matched to disease activity, which fluctuates in most asthmatic patients in response to factors like allergen exposure and viral infections. Use of exhaled NO in this manner holds the promise of reducing exacerbations similar to that seen in the study by Green et al,⁷⁶ who used sputum eosinophils. Exhaled NO has been used more recently in predicting response to therapy in asthmatic

individuals treated with corticosteroids.⁷² Little et al⁷³ reported a positive predictive value for an increased FE_{NO} level of 83% for an FEV_1 improvement of greater than 15% after oral steroid therapy, whereas sputum eosinophils had a positive predictive value of 68%. Regarding our patient, the clinician could perhaps use exhaled NO to decide whether the patient should be started on ICSs at all if the asthma is mild and intermittent.

Children who are able to perform spirometry can usually do exhaled NO testing. The clinical trial above included children 4 to 18 years of age, for whom NIOX is cleared for clinical use. Normal values in children for exhaled NO measured at a fixed flow increase with age,^{29,97} probably because a fixed flow is relatively larger or smaller in small and large children, respectively.

Future clinical uses for exhaled NO testing in patients with asthma

FE_{NO} in diagnosis. Recent evidence in fact suggests that FE_{NO} is an excellent way to confirm or refute a diagnosis of asthma, perhaps even superior to spirometry and reactivity.⁹⁸⁻¹⁰⁰ Similarly, FE_{NO} can differentiate chronic cough caused by asthma versus other causes.¹⁰¹ In a similar vein, if a patient returns to the clinic with increased dyspnea but spirometric results and exhaled NO levels appear stable, this should lead the clinician to suspect an alternative diagnosis (eg, heart failure or pulmonary embolism).

FE_{NO} as a compliance check. FE_{NO} levels increase within days after steroid withdrawal,⁵³ and this could be used to monitor adherence to therapy.⁵² Indeed, Beck-Ripp et al¹⁰² reported that FE_{NO} levels correlate with therapy adherence.

Summary of the application of exhaled NO for asthma in the clinic. For each patient, FE_{NO} measurement and spirometry would be performed at each clinic visit to establish a stable FE_{NO} baseline. Patients with high levels would be expected to show a significant decrease in FE_{NO} level after anti-inflammatory therapy as they improved. Failure for FE_{NO} levels to decrease would alert the clinician to increase therapy or check compliance. A FE_{NO} level that increased by more than 20% above the stable baseline should alert the physician to worsening disease or poor compliance. Worsening symptoms but a stable and low FE_{NO} level would suggest an alternative diagnosis.

FUTURE TECHNICAL DEVELOPMENTS

Sensor technologies

Chemiluminescence is the current technology used in most commercial NO analyzers both for medical and nonmedical use, but other technologies exist.¹⁰³⁻¹⁰⁵ Chemiluminescence is likely to be replaced soon by simpler, more robust, and more economic approaches to NO measurement. In the future, difficult asthmatic patients might measure exhaled NO levels at home and adjust therapy on a day-to-day basis.

Flow control

As discussed above, exhalation flow must be standardized in the single-breath maneuver, and NIOX incorporates the dynamic flow controller described earlier. Investigators have used biofeedback of exhalation pressure,¹⁶ exhalation flow,²⁰ or both; mass flow controllers²⁴; starling resistors²⁵; and servo systems that adjust resistance in relation to airway pressure and operator-controlled resistance.^{26,27} Despite diverse technologies, exhalation at a constant flow rate remains difficult or impossible for some adults and for the younger child,²⁸ and therefore new approaches to flow control are required. Moving away from constant flow maneuvers might be an option. Tsoukias et al¹⁰⁶ have recently described a single exhalation at a continuously varying flow rate, where mass flow controllers determine the flow pattern, and Condorelli et al¹⁰⁷ have described a tidal breathing method that can estimate the flow-independent NO exchange parameters. It remains to be seen whether such methods will evolve into practical scenarios for exhaled NO measurement.

Multiple flow rates

As described earlier, measurement of exhaled NO at multiple flow rates allows the derivation of flow-independent NO exchange parameters. An accessory kit of flow resistors is available for NIOX for 10, 30, 50, 100, and 200 mL/s.

OVERALL SUMMARY

Exhaled NO has stood the test of time and has now moved from research to clinical application with the FDA's clearance of the NIOX NO monitoring system. Exhaled NO serves as a prototype for the application of biomarkers to the management of the inflammatory component of asthma. This novel marker should be used in conjunction with standard asthma outcomes, and future guidelines on asthma management might include the testing of inflammation as an additional aid to improving asthma control and outcomes.

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