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Exhaled nitric oxide in asthma: progress since the introduction of standardized methodology

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Abstract

The measurement of nitric oxide (NO) in exhaled breath has given us the ability to learn about and monitor the inflammatory status of the airway through a non-invasive method that is easy to perform and repeat. This has been most useful in the diagnosis and management of asthma and has promised a seemingly unlimited potential for evaluating the airways and how clinical decisions are made (Grob N M and Dweik R A 2008 *Chest* **133** 837–9). The exhaled NO field was initially limited, however, due to the absence of standardized methodology. The ATS and ERS jointly released recommendations for standardized methods of measuring and reporting exhaled NO in 1999 that were revised in 2005 (1999 *Am. J. Respir. Crit. Care. Med.* **160** 2104–17; 2005 *Am. J. Respir. Crit. Care. Med.* **171** 912–30). In this paper, we summarize the literature that followed this standardization. We searched the literature for all papers that included the term ‘exhaled nitric oxide’ and selected those that followed ATS guidelines for online measurement for further review. We also reviewed cut-off values suggested by groups studying exhaled nitric oxide. We found a wide range of NO values reported for normal and asthma populations. The geometric mean for FE_{NO} ranged from 10 ppb to 33 ppb in healthy adult control populations. For asthma, the FE_{NO} geometric mean ranged from 6 ppb to 98 ppb. This considerable variation likely reflects the different clinical settings and purposes of measurement. Exhaled NO has been used for a multitude of reasons that range from screening, to diagnosis, to monitoring the effect of therapy. The field of exhaled NO has made undeniable progress since the standardization of the measurement methods. Our challenge now is to have guidelines to interpret exhaled NO levels in the appropriate context. As the utility of exhaled NO continues to evolve, it can serve as a good example of the crucial role of the standardization of collection and measurement methods to propel any new test in the right direction as it makes its way from a research tool to a clinically useful test.

Introduction

The nitric oxide (NO) field has come a long way since the identification of NO as what been known as ‘endothelial-derived relaxing factor’ in the late 1980s [4,5]. Soon after that in the early 1990s, the ability to measure NO in exhaled breath by chemiluminescence opened a new window in the ability to study lung physiology and disease [6]. While the field of exhaled breath research had already made significant strides by then, rapid development and standardization of exhaled NO measurements brought this method to the forefront of breath analysis as a non-invasive tool in diagnosing and monitoring disease. Nearly every year

brings more discoveries in exhaled NO than the year before (figure 1). Asthma is one of the diseases most studied by this technique and will be the primary focus of this paper.

NO is endogenously synthesized by a group of enzymes, nitric oxide synthases (NOS), that help regulate the tone of blood vessels and airways, provide signals for neurotransmission in the bronchial wall, and control ciliated beating of epithelial cells [7–11]. One form of NOS, inducible NOS (iNOS), is thought to be the source of increases in NO during inflammation: its expression is induced by inflammatory mediators (TNF- α , IL-1 β , IFN- α) and inhibited by corticosteroids [12,13]. In asthma, a disease characterized by airway inflammation, exhaled NO levels have been found to correlate with eosinophilic airway inflammation [14–17]. The ability to learn the inflammatory status of an airway through a non-invasive measurement has promised a seemingly unlimited potential for evaluating the airways and how clinical decisions are made [1].

Exhaled NO can be measured by two main techniques: online and offline measurements. The online measurement refers to the fractional concentrations of orally exhaled nitric oxide (FENO) testing with a real-time display of NO breath profiles; the offline testing refers to a collection of exhalate for later analysis [18]. FENO levels measured may vary depending on the technique [19]. Similarly, the flow rate at which FENO is measured is another important component of variability. At higher flow rates, the transit time for NO to diffuse into the airway is limited: as the flow rate increases, FENO decreases [20–22].

Early work: before method standardization

Many promising trends were discovered early in the investigations of FENO. Smoking was found to reduce FENO [19,23–25], while asthmatic smokers still had elevated FENO [26]. Age also had an effect on FENO: FENO increased with age in children [27–29]. The ingestion of caffeine and nitrates influenced the FENO measurement [30,31]. Respiratory track infections also elevated FENO in asthmatics [32,33]. This milieu of trends (table 1) suggested the need for a task force to outline guidelines to standardize this new measurement technique to reduce the confounding effects of many of these newly discovered variables on future NO measurements.

The Joint American Thoracic Society (ATS) European Respiratory Society (ERS) guidelines

The ATS and ERS jointly released recommendations for standardized methods of measuring FENO in 1999 that were revised in 2005 [2,3]. One of the main reasons that stimulated the development of the guidelines was the seminal observation that the exhaled NO levels were dependent on the expiratory flow rate [20]. The recommendations provided standards for online and offline procedures including background information regarding the choice between the two techniques. The documents included descriptions of NO terminology and units, factors influencing FENO values, techniques for exhaled NO measurement, recommendations for expiratory flow rate and interpretations of NO single breath values. The recommendations also included information for optimizing NO measurement in special populations, including children.

Recent work: since the guidelines

Since the guidelines have been released, FENO has emerged as a highly reproducible technique for non-invasively examining the airways. Before the guidelines were released, research in this field focused on studying the different methods and determining whether NO changes could be found in specific conditions. Since the guidelines were published, however, the field has moved much further: NO levels have been determined in several conditions such as asthma, chronic obstructive pulmonary disease, bronchiectasis, cystic

fibrosis, sarcoidosis, upper respiratory infections, primary ciliary dyskinesia, pulmonary hypertension, lung transplantation rejection and others. In asthma, one of the diseases most examined by this technique, FE_{NO} values have been used to predict exacerbations [34–36], predict the outcome of inhaled corticosteroid (ICS) withdrawal [37], monitor adherence to treatment [38] and to aid in the management of asthma [39]. There has also been significant work in establishing normal FE_{NO} values [40–45].

We have searched the literature for all papers that included the term ‘exhaled nitric oxide’ and selected those that followed ATS guidelines for online measurement for further review (table 2). Despite the new guidelines, we found a wide range of NO values reported for normal (table 3) and asthma (table 4). The geometric mean for FE_{NO} ranged from 10 ppb to 33 ppb in healthy adult control populations; in healthy children FE_{NO} ranged from 5 ppb to 14 ppb. For asthma, the FE_{NO} geometric mean ranged from 6 ppb to 98 ppb; in asthmatic children, this range was from 11 ppb to 44 ppb. Groups such as Travers *et al* used multivariate modeling to suggest that reference NO values should be grouped by gender, atopy status and smoking status; this study was one of the first to publish the expected reference values for exhaled nitric oxide based on the presence of these variables [46]. Sex, atopy and smoking status significantly impacted normal values for exhaled nitric oxide; the presence of multiple factors had a multiplicative effect on reference ranges determined by multivariate modeling [46].

We also reviewed cut-off values suggested by groups studying exhaled nitric oxide. They include the following suggestions for adults:

- FE_{NO} must be greater than 10 ppb to diagnose asthma [47].
- FE_{NO} greater than 45 ppb suggests poorly controlled asthma [48].
- Treatment should be initiated if FE_{NO} is greater than 35 ppb [49].
- FE_{NO} greater than 47 ppb predicts steroid responsiveness [50].
- FE_{NO} of 35 ppb is the threshold value for the prediction of inhaled corticosteroid responsiveness [48].
- The inhaled corticosteroid treatment dose increased if FE_{NO} is greater than 26 ppb [48].
- The inhaled corticosteroid treatment dose decreased if FE_{NO} is less than 16 ppb or less than 26 ppb on two consecutive occasions [48].
- FE_{NO} less than 30 ppb suggests that asthma exacerbation is unlikely to occur [48].
- If FE_{NO} increases by more than 4 ppb, the increase is most likely due to inflammation rather than normal variability [51].

Similar recommendations were made for children and nitric oxide:

- For children, FE_{NO} should be less than 15 to 25 ppb, depending on age and atopy [52,53].
- For children, FE_{NO} of 49 ppb predicts relapse of symptoms [36].
- For children, a FE_{NO} of 22.9 ppb predicts an asthma exacerbation [52].
- For children, FE_{NO} greater than 22 ppb during the reduction of an inhaled corticosteroid dose suggests that the reduction has not been successful [54].
- For children with asthma, using FE_{NO} for ICS dose titration every 3 months for 1 year is superior to the conventional treatment guided by symptoms alone; FE_{NO}

leads to a similar clinical asthma control and less airway inflammation with a similar ICS dose [54].

- In children with asthma, a therapy regimen aimed at lowering FE_{NO} improved parameters of a small airway function, but was not able to improve the clinical markers of asthma control [52].

Combined with the fact that there is considerable overlap in FE_{NO} between healthy individuals and asthmatics, defining different cut points for different clinical settings may be more clinically useful than normative values. Once the clinical setting is taken into consideration, certain patterns begin to emerge. The FE_{NO} levels above 45–50 ppb may predict steroid responsiveness while the levels below 35 ppb can suggest optimal asthma control in an asthmatic on therapy. The FE_{NO} levels above 20–25 ppb suggest the presence of asthma in a steroid naive individual with symptoms while the lower levels are not likely to be associated with airway inflammation [1]. What needed now are interpretation guidelines to make the FE_{NO} levels more clinically useful to practitioners [1]. These are currently under development by the American Thoracic Society (ATS).

Looking ahead

Although the field has made undeniable progress, many important questions remain before FE_{NO} and its clinical implications can be fully understood. The pathobiologic role of NO, as fundamental as it is, remains incompletely understood. Some groups believe NO is involved in pro-inflammatory conditions that trigger immunomodulatory reactions [55], while others consider its effect on smooth muscle relaxation a protector against inflammatory reactions such as airway hyperresponsiveness [56,57]. A fundamental understanding of the role of NO in physiology and how that function is altered in pathology is key to being able to interpret FE_{NO} values.

In addition to learning more about NO, several steps must be taken in order to improve the integration of FE_{NO} into the clinical setting. First, more work needs to be done in order to better define a normal healthy reference population. Many studies thus far with normal healthy controls report 'normal' FE_{NO} without addressing the effect of potential confounders in this control population (i.e., atopy, smoking status, gender, etc). Other studies that focus on establishing reference values for the healthy population have been too small and the ranges of accepted values have been too great to draw conclusions from. There is a need to establish a large normal healthy reference population, taking note of the presence of these known confounders, in order to obtain a better understanding of what a normal FE_{NO} really is. The recent inclusion of FE_{NO} measurement in the National Health and Nutrition Examination Survey NHANES is expected to provide more useful population-based normative values.

Next, once normal values are better understood, relationships between FE_{NO} and confounders need to be determined. For example, a clinician should be able to adjust the expected FE_{NO} for a healthy patient after a change in the smoking status. The ultimate goal of being able to predict a FE_{NO} measurement in a patient based on knowledge of their smoking status, age, gender, height and other factors is known to impact the FE_{NO} measurements. Until such predicted values are available for FE_{NO} , certain cut-off points may be helpful in deciding how to use a particular FE_{NO} value in a particular individual. However, in addition to the possible confounding variables, the clinical context, the reason for the test and the pretest probability of the outcome may all need to be taken into consideration. This requires the development of guidelines for the interpretation of FE_{NO} , a need that is currently being addressed by the ATS which has already taken on this task of developing interpretation guidelines for FE_{NO} .

As the utility of exhaled NO continues to evolve, it can serve as a good example of the crucial role of the standardization of collection and measurement methods to propel any new test in the right direction as it makes its way from a research tool to a clinically useful test.

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Figure 1.

The number of novel publications on exhaled nitric oxide, by year. Data were collected from an internet search for 'exhaled nitric oxide' and 'year.' The circled dates are the years of ATS guidelines.

Table 1

Factors affecting the exhaled nitric oxide measurement. Adapted from Kharitonov *et al* [58].

Increased nitric oxide	Decreased nitric oxide
Drugs: papaverin, sodium nitroprusside L-arginine, enalapril	Drugs: oxymetazoline, NOS inhibitors
Air pollution exposure	Repeated spirometry, sputum induction
Ingestion of arginine or nitrite/nitrate rich foods	Exercise, menses, reduction in body temperature, moderate altitude
Chemical exposure: flouride, ozone, chlorine dioxide, latex, formaldehyde	Chemical exposure: water vapor, nitrous oxide, heptane, carbon dioxide, 100% oxygen
Upper respiratory tract infections	Smoking, caffeine, alcohol ingestio

Table 2

Demographic information from published papers that studied the role of nitric oxide in the diagnosis (A) and treatment (B) of asthma; healthy reference population values are listed in (C). All papers followed ATS guidelines for online measurement.

First author	Date	n	Flow rate (ml s ⁻¹)	Device	Country	Population	Confounders studied
(A)							
Warke [59]	2002	71	50	Sievers NOA280	UK	Asthma, children	Atopy, asthma
Khaitonov [51]	2003	59	50	Aerocrine NIOX	UK	Asthma, adults & children	Gender
Malmberg [60]	2003	96	50	EcoPhysics CLD 77AM	Finland	Asthma, children	Treated and probable asthmatics, chronic cough
Olin [61]	2004	228	50	Eco Physics CLD700AL	Sweden	Asthma, adults	Exposure to ozone, atopy, asthma and ICS
Smith [62]	2004	47	50 (& 200)	Unknown	New Zealand	Asthma, adults	EBC higher oxides of NO
Nguyen [63]	2005	44	50	Aerocrine NIOX	USA	Asthma, adults	Smokers not excluded, age, sex
Smith [64]	2005	52	50	Aerocrine NIOX	New Zealand	Asthma, adults	Gender, atopy, smoking status
Travers [46]	2007	528	50	Aerocrine NIOX	New Zealand	Healthy, adults	Atopy, smoking
Kostikas [47]	2008	961	50	MINO	Greece	Asthma, adults	
(B)							
Beck-Ripp [38]	2002	46	50	Logan LR 2000	Germany	Asthma, children	ICS administration
Berry [15]	2005	566	250	Logan LR 2000	UK	Asthma, adults	Sputum eosinophil, smoking
Pijnenburg [36]	2005	40	50	Sievers NOA280	Netherlands	Asthma, children	Relapse, time after decrease in ICS
Pijnenburg [65]	2005	85	50	Aerocrine NIOX	Netherlands	Asthma, children	ICS doses
Smith [50]	2005	97	250 (50 calculated)	Unknown	New Zealand	Asthma, adults	
Spergel [49]	2005	30	50	Sievers NOA280	USA	Asthma, children	ICS dose
Zacharasiewicz [54]	2005	40	50	Aerocrine NIOX	UK	Asthma, children	Asthma control, age
Fritsch [66]	2006	47	50	Aerocrine NIOX	Austria	Asthma, children	Bariatric surgery, BMI, ACT
Gruffydd-Jones [67]	2007	37	50	Aerocrine NIOX	UK	Asthma, adults & children	Treatment, withdrawal, atopy
Maniscalco [68]	2007	22	50	Sievers 280 NOA	Italy	Asthma, adults	Asthma severity
Montuschi [69]	2007	14	50	Aerocrine NIOX	Italy	Asthma, children	Corticosteroid dose
Robroeks [70]	2007	114	50	Aerocrine NIOX	Netherlands	Asthma, children	Severity of asthma
Shaw [71]	2007	118	50	Not stated	UK	Asthma, adults	

First author	Date	n	Flow rate (ml s ⁻¹)	Device	Country	Population	Confounders studied
Michhils [48]	2008	341	50	Logan LR200	Belgium	Asthma, adults	Asthma severity, eosinophils, neutrophils, tissue markers
Shannon [72]	2008	50	50	Not stated	Canada	Asthma, adults	
(C)							
Van der Lee [73]	2002	117	250	Eco Physics CLD 77AM	Netherlands	Healthy, adults	Gender
Buchvald [53]	2005	405	50	Aerocrine NIOX	Denmark	Healthy, children	Age, atopy
Olin [74]	2006	2200	50	Aerocrine NIOX	Sweden	Healthy, adults	Height, age, atopy, smoking status
Olivieri [75]	2006	204	50 (&100, 200)	Ecomedics CLD88	Italy	Healthy, adults	Gender
Olin [40]	2007	3376	50	Aerocrine NIOX	Sweden	Healthy, adults	Age, height, atopy
Stark [76]	2007	21	50	Sievers NOA280	Finland	Healthy, adults	Diurnal, seasonal, day-to-day
Sundy [77]	2007	994	50	Sievers 280i	USA	Healthy, adults	Smoking, age
Zietkowski [78]	2007	26	50	Sievers 280i	Poland	Asthma, adults	
Bohadana [42]	2008	39	50	NIOX Aerocrine	France	Healthy, adults	Smoking, gender
Kerckx [79]	2008	21	50	Logan LR200	Belgium	Asthma, adults	
Kovesi [45]	2008	657	50	Eco Medics CLD88sp	Canada	Healthy, children	Race, age, height, gestation, exercise, gender, BMI, age, height, FEV1
Levesque [44]	2008	895	50	Sievers 208i	USA	Healthy, adults	Race, Gender, URI, height, weight, BP, IgE, ECP, nitrate

Abbreviations: asthma control test (ACT); blood pressure (BP); body mass index (BMI); eosinophil cationic protein (ECP); inhaled corticosteroids (ICS); upper respiratory infection (URI).

Table 3

Summary of reviewed exhaled nitric oxide values (ppb) for the normal healthy population: adults (A), children (B).

First author	n	Mean	Standard deviation	Standard error	Median	Interquartile range
(A)						
Kharionov [51]	2003	59	16.3	8.4		
Olin [61]	2004	228			15.7	
Smith [62]	2004	47	15.7	12.9		
Nguyen [63]	2005	44			13.3	8.8–19.2
Olin [74]	2006	2200			17	12.7–23.5
Olivieri [75]	2006	204	10.8	4.7		
Olin [40]	2007	3376	17.9	5.47		
Stark [76]	2007	21	14.8	5.8		
Travers [46]	2007	528	14.1			
Sundy [77]	2007	994	20.5	21.3		
Zietkowski [78]	2007	26	18	5.59		
Bohadana [42]	2008	39	16.2–33.1	1.5–2.4		
Levesque [44]	2008	895	19.7			
(B)						
Warke [59]	2002	71			9.5	4.9–13.6
Malmberg [60]	2003	96	5.3			
Buchvald [53]	2005	405	9.7	7.75		
Robroeks [70]	2007	114	14.1			8.2–27.3
Kovesi [45]	2008	657	14.1			

Table 4

Summary of reviewed exhaled nitric oxide values (ppb) for the asthma population: adults (A), children (B).

First author	Date	n	Mean	Standard deviation	Standard error	Median	Interquartile range
(A)							
Kharionov [51]	2003	59	32.3		25.9		
Olin [61]	2004	228	24.7				
Smith [62]	2004	47	52	34			
Berry [15]	2005	566	6.7	log 0.65			
Nguyen [63]	2005	44				17	9.5–41.4
Olin [74]	2006	2200				19.9	14.6–31.4
Gruffydd-Jones [67]	2007	37				31.2	11.5–61.9
Maniscalco [80]	2007	14	32.9	6.4			
Shaw [71]	2007	118				29.2	14.0 – 61.0
Travers [46]	2007	528	25	15.2			
Zietkowski [78]	2007	26	98.9	55.37			
Kerckx [79]	2008	21	46.5	22.1			
Kovesi [45]	2008	657				20	14–31
Michils [48]	2008	341	32.9				
Shannon [72]	2008	50	13.3	8.5			
(B)							
Warke [59]	2002	71				24.3	10.5–66.5
Beck-Ripp [38]	2002	46	14.8	1.9			
Malmberg [60]	2003	96	22.1	2.6			
Pijnenburg [36]	2005	40	11.2	2.05			
Pijnenburg [65]	2005	85	26.4				
Spergel [49]	2005	30	44	49			
Zacharasiewicz [54]	2005	40				21.5	8.5–33
Fritsch [66]	2006	47				34.6	17.5–58.6
Gruffydd-Jones [67]	2007	37				55.3	11.6–102.1
Montuschi [69]	2007	14				42.6	28–66.4
Robroeks [70]	2007	114	15.4		1.37		