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ERS TASK FORCE

Measurement of exhaled nitric oxide in children, 2001

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This statement, prepared by a joint European Respiratory Society/American Thoracic Society (ERS/ATS) Task Force, provides practical recommendations and suggestions for the measurement of fractional exhaled nitric oxide concentration (FENO) and nasal nitric oxide (NO) in paediatrics, particularly in young children who cannot actively cooperate. In the past, great efforts have been made, by the ERS and the ATS, to standardize NO measurement procedures [1, 2], but the experience in young children was limited at that time. This document is based on available literature and on the consensus of participants obtained during two meetings held in the year 2000. The aims of the Task Force were three-fold: to describe the available methods for FENO and nasal NO measurement in young children in order to improve their clinical use; to promote uniformity of measurement techniques; and to define areas of clinical application for future research of FENO in paediatrics.

In the last decade there has been an explosion of interest in the measurement of FENO and other volatile substances in exhaled air. Although understanding about the link between NO and airway inflammation remains incomplete, increasing evidence supports the contention that FENO may be considered as being a readout of certain aspects of airway inflammation, particularly in atopic asthma. FENO measurement has characteristics (instantaneous, non-invasive, repeatable, safe) that make it ideally suited for children. The need for developing practical, non-invasive markers to reflect asthmatic airway inflammation is universally recognised [3]. It is especially important in young children who wheeze, in whom

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Table 1. – Methods for the measurement fractional exhaled nitric oxide in children

Age yrs	Single-breath on-line[#]	Single-breath flow controlled off-line[#]	Flow controlled during spontaneous breathing on-line	Uncontrolled flow (single-breath or tidal breathing) on- or off-line	Single-breath forced exhalation on-line[¶]
5–16	+	+			
2–5			+	+	
<2				+	+

+: method used in the age group indicated; [#]: Dynamic flow restrictors are recommended in children with poor cooperation (see under single-breath on-line measurement); [¶]: Rapid thoraco-abdominal compression technique, sedation required; +: method indicated was used.

other objective diagnostic tools, such as spirometry or induced sputum, cannot be easily applied in clinical practice. Several methods can be used to measure FENO and selection depends on the age and cooperation of the child (table 1). For children who cannot perform the standardized single-breath on- or off-line exhalations [2], alternative methods are now available that do not require as much active cooperation. Their potential roles and limitations will be discussed in this document. The techniques for nasal NO measurement, a new diagnostic utility in children with primary ciliary dyskinesia and other airway diseases, are also discussed. The present document can be considered a paediatric update of recommendations by the ERS and ATS Task Forces published previously [1, 2], and the reader is invited to refer to these previous reports for more extensive details on NO measurements in cooperative subjects.

The biology of nitric oxide in paediatric airways

FENO is a marker of childhood asthma that may reflect airway inflammation. Abnormalities in expired NO concentration are also increasingly recognised in a variety of other paediatric diseases. However, the biological explanation for variations in FENO concentrations has proven to be surprisingly complex. A brief overview of biochemical, molecular, cellular, anatomical and genetic determinants of expired NO that are relevant to the interpretation of measurements made in children are presented here.

Nitric oxide synthases

Endogenous NO is derived from L-arginine by the enzyme NO synthase (NOS), of which at least three distinct isoforms exist [4]. Two of these enzymes are constitutively expressed and are activated by small rises in intracellular calcium concentration. Neuronal NOS (nNOS, NOS1) is predominantly expressed in neurons and endothelial NOS (eNOS, NOS3) in endothelial cells. A third enzyme that is inducible (iNOS, NOS2), may have a much greater level of activity, is independent of calcium concentration and may be induced by inflammatory cytokines.

Cellular sources of exhaled nitric oxide

Airway epithelial cells may express all NOS isoforms and therefore contribute to NO in the lower respiratory tract [5–8]. In inflammatory diseases, such as asthma, the increase in exhaled NO may reflect, at least in part, induction of NOS2. In adult asthmatic patients there is evidence of increased expression of NOS2 in airway epithelial cells [9]. Pro-inflammatory cytokines (T-helper cell (Th)1), which may at times be involved in asthmatic inflammation, induce the expression of NOS2 in cultured human airway epithelial cells [6, 10]. NOS2 may be expressed in other cell types, such as alveolar macrophages, eosinophils and other inflammatory cells [11]. Further evidence that the increase in exhaled NO is derived from increased NOS2 expression is the observation that corticosteroids inhibit induction of NOS2 in epithelial cells [10] and in bronchial biopsies of adult asthmatic patients [11], and that they also reduce exhaled NO concentrations in asthmatic patients [12]. Epithelial cell and nonadrenergic, noncholinergic NOS1 may also play an important role in determining both the asthmatic phenotype and the expired NO concentration.

Anatomical sites of nitric oxide formation

The levels of NO in the nose and nasopharynx are much higher than those expired from the mouth, suggesting that upper airways are a major contributor to exhaled NO, at least in normal individuals [13]. However, the lower respiratory tract contributes substantially to exhaled NO. Direct sampling via fibreoptic bronchoscopy in asthmatic patients shows a similar elevation of NO in trachea and main bronchi to that recorded at the mouth, thus indicating that the elevated levels in asthma are derived from the lower airways [14, 15].

Biological relevance of exhaled nitric oxide

Concentrations of NO present in expired air are considered to be too low to be of physiological relevance [16, 17]. That is to say that nM concentrations of NO are unlikely to have substantial bioactivities in the lung (as NO) where there is continuous exposure to a high flow rate of mM haemoglobin concentrations which avidly bind NO [18, 19]. However, NOS activation does not result in the formation of NO alone [20]. It may form a variety of nitrogen oxides with a broad range of bioactivities [20, 21], such as nitrate, nitrite (NO_2^-) and peroxynitrite. For example, the reaction of NO and superoxide forms

peroxynitrite. Depending on concentrations of the reactants and on the redox environment this reaction may be either cytoprotective or cytotoxic [22]. Nitrate and NO_2^- are additional oxidation products that have been thought of as inert byproducts, although it is now apparent that NO_2^- may be critical as a capacitor and storage pool for NO bioactivities in the lung [23, 24]. S-nitrosothiols (SNOs) may be formed during NOS activation [20] and/or from the reactions of NO with protein or low-mass thiols in the presence of electron acceptors [25–27]. SNOs store and execute NO bioactivities, such as bronchodilatation, ciliary motility, antimicrobial effects and airway-hydration effects [25, 28].

The chemistry of each of these nitrogen oxides may substantially affect expired NO concentrations. This has been most clearly demonstrated in paediatric lung disease. For example, both prokaryotic organisms and superoxide formed in the oxidative environment of the cystic fibrosis (CF) airway consume NO [29, 30] that seems to be already decreased in these patients because of a lower iNOS expression [31]. Conversely, expired NO concentrations may increase as a result of acid-induced NO evolution from NO_2^- during acute asthma exacerbations [24]. Paradoxically, breath condensate NO_2^- concentrations fall with a decrease in airway pH during an asthma exacerbation [24]. Similarly, breakdown of SNOs, particularly S-nitrosoglutathione, may be accelerated in childhood asthma [32, 33] and may contribute to elevated FENO. All of these issues must be considered when interpreting the clinical implications of a given measurement of expired NO or of other nitrogen oxides.

Genetics of the neuronal nitric oxide synthase pathway in asthma

There is evidence that NOS1 is involved in the genetics of asthma. The gene that encodes NOS1 in humans is localised on the long arm of chromosome 12 in region 12q13–q24.2. This chromosomal region is of special interest since multiple genome-wide screening studies in different ethnic populations have shown evidence of linkage of this region to the development of asthma [34–38]. Studies using polymorphic markers within the NOS1 gene have established a genetic association between NOS1 and the diagnosis of asthma. Allelic associations have been found for both a $(CA)_n$ repeat polymorphism and a bi-allelic marker in exon 29 of NOS1 in Caucasian Americans [39, 40]. In a British adult asthma population a significant association was found for a polymorphism in intron 2 of NOS1, but not for markers in the NOS2 and NOS3 genes [41], and data from mouse models indicate that NOS1 contributes substantially to FENO [42, 43]. In humans, a study involving mild asthma patients revealed that the size of an $(AAT)_n$ repeat polymorphism in intron 20 of NOS1 was significantly associated with FENO [44]. The relationship of the size of an intronic $(AAT)_n$ repeat and the level of FENO was recently confirmed in patients with CF [45]. These data suggest that variability of FENO may, at least in part, be explained by genetic predisposition, i.e. variants within the NOS1 gene.

Nitric oxide and lung development

Although distribution of murine NOS isoforms varies little with ontogeny [46], rat NOS3 messenger ribonucleic acid (mRNA) and protein expression peaks as the foetus approaches term and falls after birth [47]. Human foetal NOS2 immunoreactivity in large cartilaginous airways during the second trimester is not significantly different from that seen postnatally [8]. Near term, however, changes in NOS3 activation may have an additional role in mediating the transition from foetal to newborn circulation patterns [48, 49]. There is little direct evidence for postnatal changes in NOS distribution in humans.

Nasal nitric oxide production

The nasal cavity and sinuses produce relatively large amounts of NO compared to the lower respiratory tract [50]. A substantial amount of NO production in the nose is present early in the development, and even in preterm infants [51, 52]. NO is produced both in the nasal cavity and in the paranasal sinuses [53, 54]. In newborns, paranasal sinuses are not yet developed. However, the nose is also the primary NO source in newborns, with low levels of NO coming from the lower respiratory tract [55].

Single-breath on-line measurement

A recent statement of the ATS [2] recommended techniques for measuring lower respiratory tract FENO in school-aged children. Briefly, the child should be comfortably seated and breath quietly for about 5 min to acclimatise. Inspired gas should contain low NO (<5 parts per billion (ppb)). The child inhales to near total lung capacity (TLC) and immediately exhales at a constant flow of 50 $mL \cdot s^{-1}$ until an NO plateau of $\geqslant 2$ s can be identified during an exhalation of $\geqslant 4$ s. The expiratory pressure should be maintained between 5–20 cmH_2O to close the velum. Repeated exhalations (three that agree within 10% or two within 5%) should be performed with $\geqslant 30$-s intervals and mean NO should be recorded [2] (fig. 1).

However, the application of single-breath on-line (SBOL) measurements in preschool children has been reported to be difficult by several investigators [56, 57]. There are several advantages in the SBOL FENO measurement that have made this the "gold standard" technique. These include the following. 1) Maintenance of constant flow. FENO is flow dependent, low flows result in higher levels and vice versa [58]. The SBOL method keeps flow constant. 2) Exhalation from total lung capacity. SBOL exhalations are performed from (near-) TLC, the most constant and easily found lung volume. This is important as the degree of lung expansion affects FENO with values

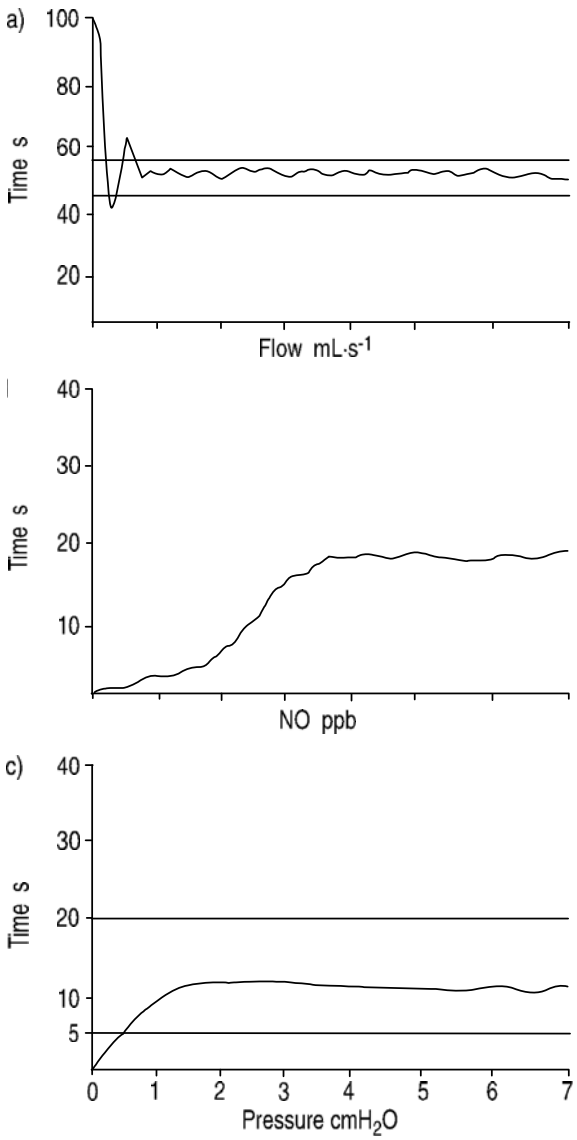

Fig. 1.–a) Flow (airflow), b) fractional exhaled nitric oxide, and c) pressure (airway pressure) tracings for a 6-yr-old female using the single-breath on-line method at a flow rate of 50 mL·s^{-1}. The exhalation lasted 7 s, a good plateau can be identified and the expiratory pressure is maintained at 11 cmH_2O during the manoeuvre. NO: nitric oxide; ppb: parts per billion.

measured at the same flow rate, from the functional residual capacity (FRC), being ~20% lower than those from TLC [58]. 3) Exclusion of nasal NO. Velum closure is simple and reliable with single-breath exhalations [58, 59]. 4) A low constant flow is easily attained. SBOL exhalation can be performed successfully at low and high flows. There are theoretical and practical reasons that have guided the selection of a flow of 50 mL·s^{-1} by the ATS [2]. Recent physiological modelling predicts that NO output from expiratory flows of 50 mL·s^{-1} is mainly derived from airway diffusion of NO [60]. As asthma is an airway disease, low flows may be more discriminative between subjects and more sensitive to temporal changes. From a practical standpoint in children, fast exhalations cause a rapid decline in lung volume and difficulty in sustaining exhalations long enough for NO values to plateau [61]. At the other end of the spectrum, expiratory flows <30 mL·s^{-1} result in an excessively long time before NO plateaus, making the test impossible for some subjects and certainly for young children [62]. Children have widely different lung volumes, and it could be argued that flow should be corrected for lung size. However, it was demonstrated that such correction does not reduce between-subject variability of FENO [63]. Hence, there seems to be no advantage to correcting flow for lung size in children. 5) Advantages of working on-line. The on-line display of flow or pressure control allows the selection of good exhalations and the rejection of suboptimal exhalations by trained staff.

The problems of applying the single-breath on-line method to preschool children

Given the aforementioned reasons which led to the adoption of the on-line method as the gold standard, there are considerable difficulties in applying this method to young children [61]. BARALDI et al. [64] found that 50% of children aged 4–8 yrs could not perform the SBOL method adequately. Similarly, JÖBSIS et al. [57] reported that 30% of children aged 4–16 yrs could not achieve adequate SBOL manoeuvres. The difficulties are as follows. 1) Inability or unwillingness to consistently inhale to TLC and to continue exhaling until exhaled NO profiles show an adequate plateau. 2) Inability or unwillingness to inhale via the mouth. A noseclip can be tried to prevent nasal inspiration. 3) Inability or unwillingness to maintain flow or pressure within the required limits displayed to the subject.

Practical measures that can facilitate the single-breath on-line method

As with spirometry, there are certain measures that can help with the SBOL method in preschool children. 1) Trained and experienced staff with time and patience and adequate practice runs. 2) Audio-visual aids to facilitate inhalation to TLC and expiratory flow control, and incentives for good exhalations. 3) Dynamic flow restrictors have been proposed to allow children to exhale with a variable mouth pressure while maintaining a constant expiratory flow. They are simple manual or mechanical devices that vary their resistance depending on the blowing pressure. These could include mass-flow controllers, which maintain constant flow, servo mechanisms, which continuously sense mouth pressure and adjust a variable diaphragm to maintain flow, and a "starling resistor", which maintains constant flow within a range of driving pressures in adults [65]. Recently, a manually operated flow regulator that maintains constant flow by continuously varying expiratory resistance ("flow-driven

method") has been validated in children [64]. Using this method, only 7% of children aged 4–8 yrs were unable to perform the measurement. However, it is hard to conceive of any technology that could solve the problems of unwillingness to cooperate, failure to exhale long enough, or taking multiple breaths.

The SBOL method is considered to be the preferred method in all children who can cooperate. The use of dynamic flow restrictors that allow the maintenance of a constant expiratory flow is recommended.

On-line measurement of exhaled nitric oxide during spontaneous breathing

Children aged between 2–5 yrs present a special challenge. Unlike the infant, sedation for lung function testing is not feasible on a routine basis in older children. Reproducible cooperation in the awake child is limited. It is generally recognised that children can rarely cooperate for reproducible spirometric measurements before the age of 6 yrs [66]. SBOL of FENO is probably even more demanding and rarely successful in the young child. Use of various biofeedback systems and animated applications will certainly increase the acceptability in children aged >6 yrs and even in some below. However, there is a need to develop applicable techniques that are independent of verbal instruction or active cooperation of the child.

Observer-controlled exhalation flow during spontaneous breathing

Methodology. FENO can be measured on-line during spontaneous breathing, while the exhalation flow is adjusted by changing the exhalation resistance [67]. This allows for scrutiny of the breath-to-breath profiles and assures a stable and reproducible breathing pattern. The child breathes slowly and regularly through a mouthpiece connected to a two-way valve. NO-free air is continuously flushed through the inlet of the valve to allow the child to inhale NO-free air and to prevent collection of NO in the dead space from the previous expiration. Quiet breathing of normal frequency is attempted. The exhalation flow is targeted at 50 $mL \cdot s^{-1}$ (range 40–60) by continuously adapting the exhalation resistance. Adjustments of exhalation resistance to target a fixed exhalation flow can be carried out manually as previously described with the SBOL method [64] or by automatic flow controllers and servomechanisms (fig. 2). The imposed exhalation resistance assures that increased mouth pressures help to close the velum. The time that the child exhales within the target flow range is obviously essential to the success of this method.

The method assumes rapid-response analysers are analysers with a long response time and high-sampling flow rates are unable to track the NO changes during spontaneous breathing. The minimal time required to achieve steady state is primarily determined by the dead space of the upper airways (2–3 $mL \cdot kg^{-1}$) and equipment (rinse volume). Approximately 1 s of

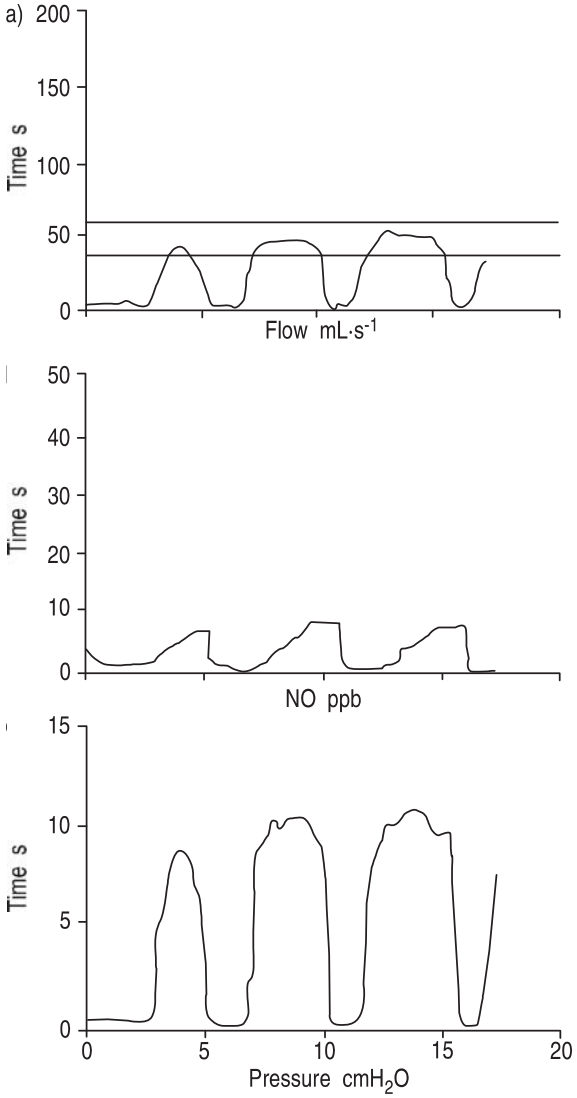

Fig. 2.–a) Flow (airflow), b) fractional exhaled nitric oxide, and c) pressure (airway pressure) tracings for a 3-yr-old male using the controlled spontaneous breathing method. NO: nitric oxide; ppb: parts per billion.

exhalation at the target flow of 50 $mL \cdot s^{-1}$ is therefore required to flush the rinse volume. The mean NO concentration within the target flow range in the subsequent exhalation may be used to reflect the NO concentration of the conducting airways. Exhalation flow can be prolonged and targeted to the desired flow in most preschool children by adjusting the exhalation resistance. In a recent study of young children aged $\geq$2 yrs, FENO was measured during spontaneous breathing with observer-controlled exhalation flow. FENO was estimated from the last part of those exhalation flow profiles providing $\geq$1 s within target flow [67].

Limitations. The method still requires passive cooperation in as much as the child needs to breathe

slowly and regularly through a mouthpiece, which is a limiting factor. Use of biofeedback visualizing the tidal breathing pattern may facilitate a slow and regular respiration. Measurements during spontaneous breathing may be biased, as there is no control over the lung volume at which the flow is measured [67]. The starting lung volume affects the exhaled NO concentration and the FRC will probably increase due to the imposed expiratory resistance. In addition, the time within the target flow may be too short to allow FENO to reach an equilibrium, similar to that seen in the SBOL method. For these reasons, NO levels measured during spontaneous breathing may not equate with SBOL measurements. Separate characterization is therefore required, including definition of normal values in healthy children.

Off-line methods with variable or constant flow

Off-line collection of exhaled air with variable flows employs two different techniques: uncontrolled single exhalations [15, 56, 57, 68–70] and tidal breathing into an NO-inert reservoir [71–73].

Off-line uncontrolled single exhalations into a reservoir

Methodology. The child blows air by a mouthpiece into an NO-inert balloon, while nasal contamination is prevented by closing the velum by exhaling against 5 cmH_2O oral pressure [56, 57]. The gas-collection equipment is inexpensive, consisting of inert Mylar or Tedlar balloons. Resistance tubing should also be NO inert. The size of the balloon is not critical, but should preferably be similar or larger than the subject's vital capacity. The results correlate with, but are not necessarily identical to, on-line measurements [56, 57]. Wearing a noseclip and breath-holding are not recommended, as they potentially affect nasal contamination [70]. NO concentrations in balloons can be stable for several hours [15, 56, 70, 74], although formal evaluation of factors that might affect stability, including temperature, light exposure, unperceived damage to bags and pressure changes, has not been carried out.

Advantages. The technique is simple, and feasible in children aged as young as 2–3 yrs. It does not require active flow regulation by either the child or the investigator. The gas-sampling equipment is inexpensive. Measurements can take place at a distant site, e.g. school or home. Reproducibility appeared to be fair or good with intrasubject coefficients of variation down to 5% [56, 57, 68, 69].

Disadvantages. There are certain imprecisions in this technique. As FENO is flow dependent, there will be a scatter of data due to variation in flows. Contamination of inspired air with high NO levels from the nose or environment can partly be overcome by measuring whilst ambient NO is <10 ppb, or, preferably, by using NO-free air or scrubber systems to clean ambient air for inhalation [75].

Possible fields of application. Groups of children with and without mild airway disease show significantly different values of FENO using uncontrolled single exhalations [70]. Therefore, this method may be suitable for epidemiological studies, where it may help in defining phenotypes.

Future developments. Discarding dead-space volume is a controversial issue. Anatomical dead space, although by definition not significantly involved in oxygen/carbon dioxide exchange, is an important site for NO production as the airways and not the alveoli are the source of exhaled NO. It seems likely that, with the development of simple systems that allow for passive flow control (dynamic flow restriction, see later), methods employing uncontrolled variable flow may become obsolete in due time.

Off-line tidal breathing into a collection bag

Methodology. Exhaled air can be sampled during tidal breathing in an NO-inert bag and stored for later analysis [15, 57, 73]. A similar method is the tidal-breathing method against a resistance that makes use of a collecting breathing circuit from which exhaled air is sampled during the procedure [71, 72]. The equipment consists of a mouthpiece or face mask attached to a two-way non-rebreathing valve, the expiration port of which is coupled to a balloon or a circuit via NO-inert material. NO-free air or an NO scrubber may be used for inspiration to avoid contamination with ambient NO [75]. This may be especially important when ambient levels are >10 ppb, although formal comparisons of measurements with and without NO-free inspiratory air using these techniques are not available.

Advantages. This sampling method only requires passive cooperation and no "blowing skills". It can be applied to infants and newborns [73] as well as patients with neuromuscular disease. The gas-collecting equipment is simple and inexpensive. Normative data for the age range 6–15 yrs have been published [76].

Disadvantages. Nasal contamination cannot be avoided during inspiration, when any measurement to close the soft palate and isolate the nasopharynx based on positive mouth pressure cannot work. A scatter of NO data due to variation in flows can occur.

Future developments. Techniques that separate nasal air from oral air could reduce contamination. One possible and somewhat radical solution is to apply a negative pressure to the nose. This will, however make the method more complicated and less tolerable. Attempts to standardize exhalation flow by dynamic restriction during tidal breathing are being developed.

Off-line single exhalations with constant flow using biofeedback or dynamic-flow restrictor: the off-line method of choice

Flow standardisation can improve the reproducibility of off-line methodology, with results similar to those of on-line constant-flow methods in schoolchildren and adolescents [62, 77]. In children who are able to perform constant-flow manoeuvres, off-line measurements can be performed using biofeedback signals (fig. 3). Several such systems have been proposed, including a mechanical or electronic manometer display attached to the tubing of the collection device [62, 77–79]. As with on-line systems, it requires considerable skill and is difficult for children aged <6–7 yrs.

A major improvement in off-line collection can be expected from the incorporation of a dynamic-flow restrictor (see Single-breath on-line measurements section) in the collection system. Dynamic-flow restrictors are already incorporated into the newest prototype commercial on-line NO-analyser. This technique has proven highly feasible in children as young as 4 yrs (M. Pijnenburg and J.C. de Jongste, Sophia Children's Hospital, Rotterdam, the Netherlands, personal communication).

When using dynamic-flow restrictors, there seems to be no justification in previous ATS recommendations [2] for using higher flows with off-line collection than with on-line collection. Therefore, the group reached a working consensus and proposed flows of 50 $mL \cdot s^{-1}$ for both off- and on-line collection [62, 77].

Exhaled nitric oxide in infants

Wheezing and cough are extremely common symptoms in infants and can be due to different mechanisms, including viral infections and early-onset asthma. Only a minority of wheezy infants have symptoms that persist to school age and are consistent with the diagnosis of asthma. Clearly there is a need for diagnostic tests that help identify infants who wheeze as a consequence of chronic airway inflammation, and would allow for better targeting of inhaled corticosteroids in this age group. For the determination of levels of exhaled NO during tidal breathing both on-line and off-line methods as well as an SBOL technique have been used in a limited number of studies in infants [73, 80, 81].

Tidal breathing techniques

Both on-line and off-line methods have been applied in infants and neonates without the use of sedatives [55, 73, 81].

Off-line. Exhaled air can be collected via a face mask, which covers the mouth, which is connected to a non-rebreathing valve that allows inspiration of NO-free air from a NO-inert reservoir to avoid contamination by ambient NO. Exhaled breath samples are collected into a NO-inert bag fitted with the expiratory port once a stable breathing pattern is present. The expiratory port of the valve provides an expiratory resistance, resulting in an airway opening pressure that exceeds 2 cmH_2O during most of expiration [73] (fig. 4). Such a resistance will only help to avoid nasal contamination if the mask does not cover the nose.

On-line. There is limited experience with on-line tidal NO measurements in infants and young children [81]. Infants breathe into a face mask that covers the nose and mouth, or only the mouth. Teflon tubing is connected to an NO analyser either via a small hole in the mask or a side-port in the expiratory part of a valve system that separates inspiration from expiration. NO concentrations should be recorded during phases of quiet tidal breathing only. The reproducibility of this technique both throughout different times of the day and between days has been shown to be satisfactory [81].

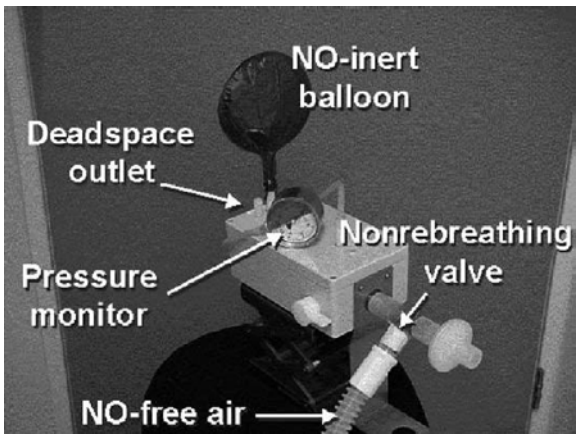

Fig. 3.–Off-line collection of single breath, end-expiratory air in a Mylar balloon, using a dynamic-flow restrictor to obtain a constant flow of 50 $mL \cdot s^{-1}$. NO: nitric oxide.

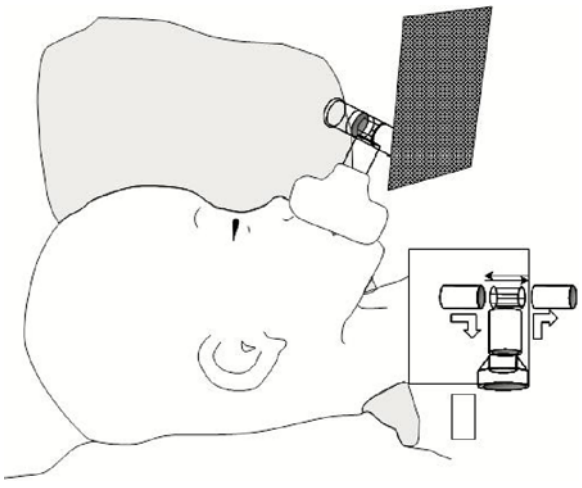

Fig. 4.–Schematic illustration of fractional exhaled nitric oxide measurement with the off-line tidal-breathing method in an infant. The mask is connected to a non-rebreathing valve.

Limitations of tidal-breathing techniques in infants. The disadvantage of mixed expiratory air is that it is contaminated by ambient NO and NO from the upper and lower airway. Whilst the inspiratory NO contamination can be limited by inhalation of NO-free air, there are currently no data on the relative contribution of upper airway NO to the mixed expiratory NO concentration in infants. Limited data suggest that nasal NO concentrations are higher than lower airway NO, particularly in newborns and young infants [51, 52, 81]. Therefore upper airway contamination could potentially have a significant impact on mixed exhaled NO concentrations in infancy, although paranasal sinuses are less well developed. The variable expiratory flows will produce a scatter of exhaled NO.

Recommendations for measurements. Measurements should be performed during quiet, regular tidal breathing. Until more is known about the contribution of upper airway NO to FENO levels, it seems sensible to try and exclude nasal NO by collecting only orally-exhaled air. This can be accomplished by using a face mask that covers the mouth alone, with nostrils occluded [73]. However, small infants may not tolerate nasal occlusion. If possible, infants should inhale NO-free air or measurements should be performed when ambient NO is <10 ppb [2]. If ambient levels are >10 ppb, measurements should be made after breathing NO-free air in order to permit washout of the dead space and lungs. Ten NO-free breaths seems reasonable for this purpose. With a fast response NO analyser, small samples of exhaled air (*e.g.* five breaths) are sufficient for analysis.

Single-breath technique

There is limited experience with single-breath methods in infants. A modification of the raised-volume rapid thoraco-abdominal compression technique (RVRTC) [82] has been used to measure FENO during a single forced exhalation [80]. NO levels are measured on-line and the plateau of NO achieved during constant expiratory flow is then determined. Flows as low as 10 $mL \cdot s^{-1}$ have been used to obtain a measurable plateau (unpublished data). The technique can be incorporated into standard protocols that use the RVRTC methodology. Sedation is needed. It is currently unclear how this technique compares with the single-breath technique used in older children and adults, or to tidal-breathing techniques in the same age range.

Nasal nitric oxide measurements

Nasal nitric oxide measurements with constant flow

The reader is referred to recent recommendations for nasal NO measurements with constant flow in adults and cooperative children [2, 83].

Nasal nitric oxide measurements with variable flow

In noncooperative children, nasal NO can be measured during normal tidal breathing if sedation is not to be used [81]. Two methods have been proposed. 1) Nasal mask. A soft and tightly fitting nose mask connected to a non-rebreathing valve is placed over the nose, and the mouth is closed by gently pressing the chin upwards. NO-free air is used for inhalation and the sample port is placed as close as possible to the valve. The rebreathing dead-space volume in the system should be minimised. For maximal sensitivity, on-line breath-by-breath analysis of nasally exhaled NO is recommended. Mean peak levels of at least five breaths is calculated. Alternatively, nasally-exhaled air is collected in a reservoir for off-line analysis. 2) Nasal olive (nostril adapter). A soft olive-shaped sampling tip with a standardized inner diameter is gently introduced into one nostril during tidal breathing through the nose when the child is in the supine position. Nasal air is sampled directly into the NO analyser with a sample flow rate that is lower than the minute volume of the child. An NO plateau should be achieved with only small variations due to breathing. Mean NO or mean NO peak values are calculated for a stable plateau of $\geqslant$10 s or five breaths [81].

It should be noted that the diagnostic sensitivity and specificity of nasal NO measurements in noncooperative children have not been studied.

Factors influencing nasal nitric oxide levels

Factors influencing the nasal nitric oxide levels include the following. 1) Sex. There are presently no data available on the effect of sex on nasal NO output. 2) Age. Nasal NO-output increases until the age of 10–12 yrs and then stays at the same level in healthy individuals [53]. 3) Food intake. Intake of nitrate in, for example, lettuce, does not influence nasal-NO levels [84]. This indicates a lack of nonenzymatic NO formation in the nasal airways. Conversely, intake of large amounts of L-arginine may increase nasal NO levels [85]. 4) Medication. The use of topical nasal decongestants reduce nasal NO levels [86], and should be avoided on the day of NO measurements. 5) Physical characteristics of inspired air. Changes in temperature and humidity have been reported not to affect nasal NO [87].

Clinical applications of exhaled and nasal nitric oxide in childhood airway disease

Exhaled and nasal NO may be useful to guide both diagnostic and therapeutic clinical decisions in individuals. The lack of standardization of FENO measurements has, until now, made it difficult to pool the evidence that exhaled NO is clinically useful. The purpose of this Task Force was to address this problem.

Diagnostic utility

High FENO levels are commonly found in untreated asthmatics. Recently, no overlap was found between asthmatic children and healthy controls with the use of a standardized on-line method with 50 $mL \cdot s^{-1}$ [88, 89]. In most previous studies [69, 90, 91], there is considerable overlap between the group results for normals and asthmatics and it seems that differences in methodological factors and patient selection are the main reasons for this inconsistency. Values of FENO in CF [92–95], primary ciliary dyskinesia (PCD) [96, 97] and non-CF bronchiectasis [98] have shown overlap with those of normals and may therefore be of limited use as a diagnostic tool.

Increased nasal NO levels have been shown in allergic rhinitis and this elevation can be restored to normal levels by topical glucocorticoids [99–101]. These changes in nasal NO are probably due to up- and downregulation, respectively, of the expression of iNOS in nasal epithelium, events that seem to be closely related to eosinophilic inflammation [102]. However, an increase in nasal NO may not always be found in allergic rhinitis [93, 103]. Reductions in nasal NO levels have also been described in PCD, CF, and acute and chronic sinusitis [13, 85, 92, 93, 96, 97, 104, 105]. This could be due to a diffusion block for NO. In addition, the expression of iNOS in airway epithelium has been shown to be lower in patients with CF [31], and nasal NO is markedly reduced in PCD, in spite of the presence of open paranasal sinuses [106]. Preliminary data suggest that nasal NO is already reduced before the onset of respiratory symptoms in infants with CF [107]. Nasal NO is so much lower in children with PCD than in normals or other disease groups, that the measurement can probably be used to exclude the diagnosis of PCD, unless clinical suspicion is very high [13, 96, 97]. Although groups of patients with CF have low nasal NO, the overlap with normals is too great to use nasal NO as a diagnostic test for CF [54, 92–94, 108]. However, high levels make CF less probable. The effects of allergic rhinitis on nasal NO are controversial. Nasal NO may be reduced by chronic sinusitis [105]. In acute sinusitis, nasal NO is also low, and the values rise after antibiotic treatment [104].

Therapeutic monitoring

A single randomised controlled clinical trial in adults has shown that monitoring bronchial hyperreactivity (BHR) and treating an asymptomatic elevation in BHR, as an addition to standard clinical methods, produced a superior outcome in terms of fewer asthma exacerbations and less airway remodelling [110]. Initial studies suggested that FENO could be used as a nonspecific inflammometer in asthma, with high levels in untreated asthma [69, 90, 91, 93] falling with anti-inflammatory treatment [69, 71, 91]. However, asymptomatic elevation in FENO has been documented during clinical remission of atopic asthma [89]. FENO has also been shown to fall after treatment of asthmatics with oral steroids [71, 111, 112], inhaled steroids [91] and leukotriene antagonists [113, 114], with no change after long- or short-acting bronchodilators, which have no known anti-inflammatory effects [71, 114, 115]. This makes NO a promising clinical tool for evaluating steroid-treatment effects in asthma. FENO gives additional and complementary information to clinical symptoms and lung function in asthma, i.e. on airway inflammation. More importantly it is the only marker that easily measures airway inflammation.

Future research: the important questions

What is the best way to make FENO measurements? There are several questions relating to the technique of FENO measurement in children that still need to be addressed. For instance, while flow dependency of FENO measurements is widely recognised, the effect of diet, age, sex, puberty and body size have not been clearly defined. These unknown factors have contributed to difficulties in standardisation and the establishment of normal values for comparison between laboratories [62, 76]. Currently, adequate reference values for children with standardised methods are lacking. In addition, the timing of measurements in relation to sputum induction [116], bronchodilators and spirometry [117, 118], needs to be defined and the existence of diurnal or circadian rhythms determined.

Large population-based studies, using standardised techniques, are required to determine the effects of physiological changes and diagnostic and therapeutic modalities (outlined earlier) on FENO measurements.

Fractional exhaled nitric oxide concentration and atopy. The relationship between asthma, atopy and FENO is arguable [111,119–122], underscoring the need for careful use of standardized techniques. There is controversy in the literature about the similarity of immunopathology in atopic and nonatopic asthma [123, 124]. Some studies have shown that adults and children who are atopic and asthmatic have higher FENO levels than nonatopic asthmatic subjects [121, 122, 125], suggesting that this instantaneous test could help develop understanding about the different phenotypes of childhood asthma. However, the relationship between atopy, FENO and airway immunopathology needs further clarification, with close attention paid to the definition of the different asthma phenotypes. It is therefore important that the atopic status of individuals be stated in studies dealing with exhaled NO.

Fractional exhaled nitric oxide concentration and airway histology. The relationship between FENO and airway eosinophilia, and whether the relationship is changed by therapy with inhaled corticosteroids is still under debate. In a preliminary study in steroid-naïve subjects, it was proposed that there is a correlation between FENO and blood eosinophil count [126], but not lung function. FENO correlated the best with eosinophils in sputum in steroid-naïve asthmatic children, but the correlation disappeared in those treated with inhaled corticosteroids [127]. Another group found good

correlations between sputum eosinophil cationic protein (ECP) and FENO in children treated with inhaled steroids [128]. In biopsy studies in children and adolescents, there was a weak correlation between FENO and mucosal eosinophil count and eosinophil activation, as evidenced by deposition of major basic protein in the mucosa [89, 129, 130]. Therefore, the relationship between FENO and inflammatory changes in bronchial biopsies, as well as the impact of different treatments on this relationship, need to be defined. Studies are also needed to determine whether FENO is related to the events driving remodelling or to the actual presence of remodelling [131].

Fractional exhaled nitric oxide concentration and symptoms. FENO has been suggested to be a marker of good symptomatic control in asthmatic children [132] and another study has shown that baseline FENO predicted the degree of exercise-induced asthma [88]. Recently, JONES et al. [133] showed an increase in the FENO in asthmatic subjects experiencing loss of control, when inhaled glucocorticoid therapy was stopped, documenting the usefulness of FENO for predicting and diagnosing poorly-controlled asthma. Furthermore, one group found that patients apparently in complete remission from atopic asthma, on no treatment and with no physician visits, had raised FENO. Interestingly, on bronchial biopsy they had eosinophilic inflammation [89, 130]. The relationship between symptoms, compliance with treatment, and FENO is an interesting field to be explored further.

Fractional exhaled nitric oxide and monitoring of allergen exposure/avoidance. Experimental evidence suggests that FENO may represent a useful tool in monitoring exposure to relevant allergens in sensitised patients [134]. A longitudinal study reported that FENO levels were increased in grass-pollen allergic asthmatic children during the pollen season, even in the absence of significant changes in airway function [72]. In addition, a prompt decrease in the FENO levels was observed when mite-allergic asthmatic children moved to a mountain mite-free environment [135]. However, an increase in FENO was found after a short period of natural re-exposure to mite [136].

Is fractional exhaled nitric oxide useful in monitoring therapy? The fact that FENO is reduced by steroid treatment has been well established in asthmatics over a wide-age range. Despite the administration of oral prednisolone for 5 days to children with acute asthma, FENO was still elevated, implying residual inflammation [69, 71]. A similar finding was reported in a study measuring interleukin (IL)-5, sIL-2r, and ECP [137]. Future research in exhaled NO in children should try to determine the onset of anti-inflammatory effects and the time of maximum anti-inflammatory effects of existing and new anti-asthma medications.

Whether FENO can be used as an inflammometer and how it relates to other surrogate markers of airway inflammation needs to be determined. One study comparing the change in FENO with ECP and sIL2R with steroid therapy for asthma suggested that FENO was the most useful indicator [111]. It is also not known whether such monitoring will improve outcome pending prospective trials. The relationship between FENOFENO and other markers is poorly defined, and it is not known whether particular markers may be more helpful in different circumstances. Recently, FENO has been shown to be as useful as induced sputum analysis and airway hyperresponsiveness in assessing airway inflammation, with the advantage that FENO is easy to perform [133]. The clinical utility of measurements of FENO, other inflammatory markers, and clinical outcome is unknown. Key priorities are to determine whether FENO, as an inflammometer, is of real benefit in terms of clinical outcome and whether it is cost-effective.

What does fractional exhaled nitric oxide teach us about asthma pathophysiology? There has been interest in using FENO to differentiate between different phenotypes of asthma. Children with a non-inflammatory phenotype may perhaps be identified, thus influencing the treatment to be offered [138]. In addition, FENO may prove useful for identifying the large group of preschool children with chronic cough, some of whom may suffer from eosinophilic airway inflammation ("cough variant asthma") and might benefit from inhaled corticosteroid therapy. The relationship between FENO and asthma phenotypes demands further study.

Can measurement of fractional exhaled nitric oxide predict prognosis for infants who wheeze? FENO levels were higher in a group of wheezing infants and fell after 5 days prednisolone therapy [73]. There was overlap between the wheezers and the normal range. It needs to be determined whether FENO measurements in infants, either at the time of acute exacerbation or during remission, can be used to predict long-term outcome of wheeze in children.

The role of fractional exhaled nitric oxide in preterm infants. Little is known about the role of NO in the developmental biology of the lung. Undoubtedly, there is much to learn about the developmental biology of NO. The function of exhaled NO in infants, and whether FENO can be used to predict the development of chronic lung disease in premature infants, remains to be determined.

Fractional exhaled nitric oxide and cystic fibrosis. The low FENO levels in CF are puzzling, and some have speculated that this relates to diffusion barriers, such as airway mucus. However, the absence of an inverse correlation between lung function and FENO in CF militates against this hypothesis [108] and recent data show that iNOS expression is reduced in CF human airway epithelial cells [31]. The positive correlation between pulmonary function and both FENO and sputum NO metabolites that was observed in CF patients may support the hypothesis that reduction of FENO in CF may, at least in part, result from decreased NO formation in the airways [95, 139, 140]. However, the mechanisms that result in the low FENO levels seen in undoubted inflammatory lung diseases such as CF, remain to be determined.

What about other diseases?. FENO may be relevant to a number of other diseases, including sickle-cell disease, chronic liver disease, chronic inflammatory bowel disease, lung transplantation and panbronchiolitis [141]. More information regarding FENO in diseases with secondary pulmonary involvement is needed.

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