

Exhaled nitric oxide in infants: a marker of inflammation?



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Summary

Exhaled nitric oxide (NO) is increasingly used for the diagnosis and monitoring of asthma. The standard single-breath technique cannot be applied in infants. Thus, three different methods have been developed: the tidal-breathing online method, the tidal-breathing off-line (or bag) method, and the single-breath method.

While it is not clear whether NO actually reflects airway inflammation in infants, the ability of NO measurements to differentiate between health and disease has been demonstrated for various disease groups. Comparable to older subjects, NO is elevated in atopic infants with and without wheezing episodes. Viral-triggered wheeze is less clear, partly due to overlapping phenotype definitions. In high-risk infants, NO may help to predict occurrence of respiratory symptoms.

The role of NO metabolism during normal and abnormal lung development seems important during infancy as evidence in animal models suggests. Results on NO in infants with bronchopulmonary dysplasia are less clear. However, NO seems to be useful to monitor environmental impact, *e.g.* tobacco smoke, particularly during rapid lung growth.

Taken together, NO measurements in infants are promising, especially as noninvasive estimates of environmental exposures, as predictors of subsequent respiratory diseases and in epidemiological studies to assess influences of maturational and inflammatory processes early in life.

Keywords: Asthma, exhaled nitric oxide, infants, inflammation, lung development, wheeze

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Acute and chronic airway inflammation are shared features of infant airway diseases, such as respiratory tract infection, asthma, bronchopulmonary dysplasia (BPD) and cystic fibrosis (CF). Many of the current concepts of therapy are targeted against inflammation; however, monitoring of anti-inflammatory treatment effects in infants has not yet been established in

clinical routine. Invasive techniques such as bronchoscopy with lavage and biopsy are suitable in difficult asthma [1], for example, but these techniques are not applicable for routine assessment. In the last decade noninvasive measurement techniques of airway inflammation have been extensively investigated, with one of the most promising being the measurement of exhaled nitric oxide fraction (F_{eNO}).

The standardised measurement of end-expiratory, single-breath F_{eNO} has been established for quite a long time and is commonly used in older children and adults, especially for asthma diagnosis and follow-up during treatment [2]. Its usefulness and application in this age group are summarised in previous chapters. As the measurement of end-expiratory F_{eNO} requires a single-breath exhalation (see previous chapters in this monograph), this test procedure is only feasible in older, cooperative subjects and is not possible in awake infants. Thus, other techniques have been developed and have been used in recent years by several groups [3]. While clear advantages, such as discriminative capacity and noninvasive sampling, of most of the techniques make nitric oxide (NO) measurements in infants a promising tool, there is still an ongoing debate about what exactly is measured by NO in infants and which technique is the best regarding easy application, reliable results, adjustment for expiratory flow, and usefulness for clinical purposes [4–6].

We are not able to answer these questions, but in this chapter we present the different measurement techniques and their advantages and disadvantages. We summarise current knowledge regarding the clinical application of NO measurements and provide an outlook on the possible relevance of the measurement techniques for different purposes.

Measurement techniques

Different measurement techniques have been described for measuring NO in infants. In contrast to older children and adults, however, only recommendations exist at this point for NO measurements in infants [3, 7]. This makes comparisons between centres and studies very difficult.

Three major techniques exist: the tidal online technique; the tidal off-line (or bag) technique; and the single-breath technique, with slight variations between different groups within these techniques. For example, some authors used a face mask that covered both the mouth and nose and, therefore, sampled NO from both lower and upper airway compartments (mixed NO), some authors tried to separate the airflows of upper and lower compartments (nasal NO and oral NO) or even obstructed the upper airways, and other authors used face masks that only covered the mouth.

Tidal techniques

NO measurements using the tidal techniques in infants can be performed without sedation. During online measurements, NO breath profiles and flow–volume loops are displayed in real-time with immediate feedback, whereas in the off-line technique exhalate is collected into a bag for delayed analysis.

Tidal online technique

Expirate is continuously sampled by the NO analyser and the resultant NO profile *versus* time or exhaled volume, together with other exhalation variables (*e.g.* airway flow rate and/or pressure), is recorded and displayed in real time (figs 1 and 2). In a very elegant study, HALL *et al.* [9] examined the exact characteristics of tidal NO, the flow dependence of tidal NO concentrations and calculated tidal NO output with the available flow values [9]. The authors suggested using the NO plateaus of the third quartile of the expiratory cycle in which, interestingly, the expiratory flow is close to $50 \text{ mL}\cdot\text{s}^{-1}$, the recommended flow for single-breath F_{eNO} measurements in older children and adults [3]. This method seems to be very accurate, especially due to the possibility to adjust the NO values for the expiratory flow and use NO output as results. NO output represents the amount of exhaled NO,



Figure 1. An example of tidal breathing measurement set-up. During natural sleep mixed nitric oxide is sampled.

diseases with possible flow restriction, which may influence the washout of NO from the lungs. As a very thorough technique, tidal online measurements allow sophisticated analysis and good repeatability between breaths which suggests that a few breaths are enough for valid results. The disadvantage is the need for the adequate stationary equipment, which makes measurements in the field impossible. Thus, the application of the tidal online technique seems to include those studies where exact values are needed and sophisticated analyses warranted.

Tidal off-line technique

NO measurements can be made from exhaled gas collected in a reservoir (bag or balloon) and subsequently analysed for NO concentrations within 12 h (fig. 3). Exhaled air from five breaths is sampled into a mylar bag while infants are breathing quietly [11]. This technique allows fast sampling of NO without the need of natural sleep or sedation; however, breathing pattern or agitation during the procedures may influence results.

To avoid nasal NO contamination in tidal online and off-line measurements, DANIEL *et al.* [12] used a face mask separating nasal and oral airflow in healthy children aged ≥ 2 yrs. Higher NO levels were found when mixed NO was measured. Better agreement between on- and off-line

is denoted by NO output ($V'\text{NO}$) and is calculated from the product of NO concentration in $\text{nL}\cdot\text{L}^{-1}$ and expiratory flow rate in $\text{L}\cdot\text{min}^{-1}$. $V'\text{NO}$ may be particularly useful in situations where NO excretion is measured over longer periods of time and during varying flow situations.

BUCHVALD *et al.* [10] presented a tidal online technique with operator-controlled expiratory flows in children aged ≥ 2 yrs. NO adjusted for expiratory flow had reasonable agreement with the reference method while mixed NO did not [3]. Although repeatability was not reported, and further studies validating the method are not available, this method might be suited when sophisticated analysis, *e.g.* partitioning pulmonary NO, are applied.

The main advantage of the tidal online techniques lies in the fact that compared to the tidal off-line or bag method, it is possible to record flow simultaneously and thus correct results for the expiratory flow. This is especially important in obstructive airway

measurements was found in those using a face mask with a valve separating airflows compared to those using a face mask without a valve. However, repeatability of the different techniques was inconsistent. Following recommendations from older children, BARALDI *et al.* [13] suggested a measurement method in which nasal contamination is avoided during NO sampling by using a face mask occluding the nostrils. However, this technique has limitations as occluded nostrils are hardly accepted by infants, especially if infants are not sedated.

The advantage of the off-line technique lies in the potential for expirate collection at sites remote from the analyser. Thus, the main relevance of this technique seems to be in large epidemiological and field studies.

Although small differences between disease groups may be detected [11], predictive value for individuals seems to be limited. Potential problems with off-line methods include errors introduced by sample storage and reduced capacity to allow for instantaneous feedback and assessment of technique. However, the main disadvantage of this technique is the impossibility to control for the expiratory flow or to adjust results for differences in the flow [14]. This is believed to be especially sensitive when measuring NO in lung diseases with altered airflows. It seems intuitive to critically interpret results obtained by the off-line tidal technique without knowing or adjusting expiratory flow.

Single-breath technique

WILDHABER *et al.* [15] developed a method for the measurement of gentle, flow-controlled, single-breath F_{eNO} by compression of the thorax with an inflatable jacket in sedated infants. It was shown that this technique has better discriminative capacity between wheezy and healthy infants than the off-line tidal breathing technique [16]. Agreement between these techniques was poor (fig. 4). The single-breath technique using positive expiratory pressure allows F_{eNO} to be investigated at different flows [17]. Nasal contamination can be avoided by adjusting expiratory resistance to achieve vellum closure [15–17]. These strengths of the method make it the only method likely to

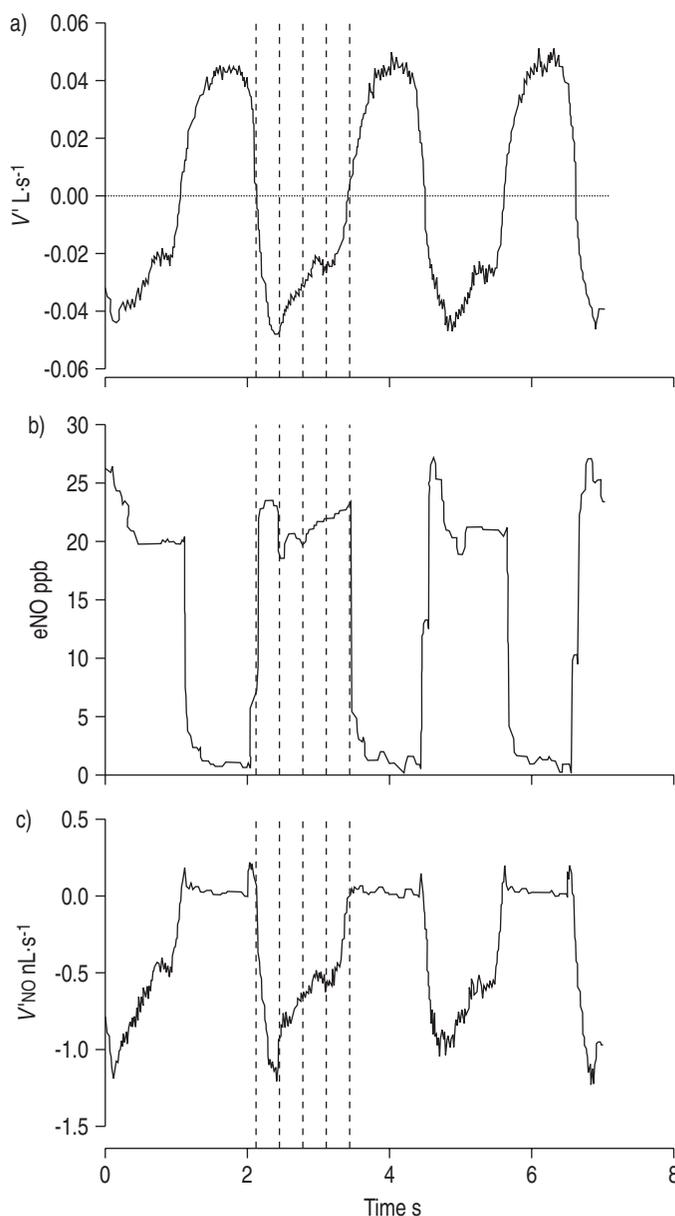


Figure 2. Representative example of a) online flow (V'), b) exhaled nitric oxide (eNO) and c) NO output ($V'NO$). The levels of eNO show a steep increase at the beginning of expiration and then approximate a plateau towards the end of expiration. By multiplying V' and eNO, $V'NO$ can be calculated ($V'NO = eNO \times V'$). V' rapidly approximates zero at the end of expiration. Reproduced from [8] with permission from the publisher.

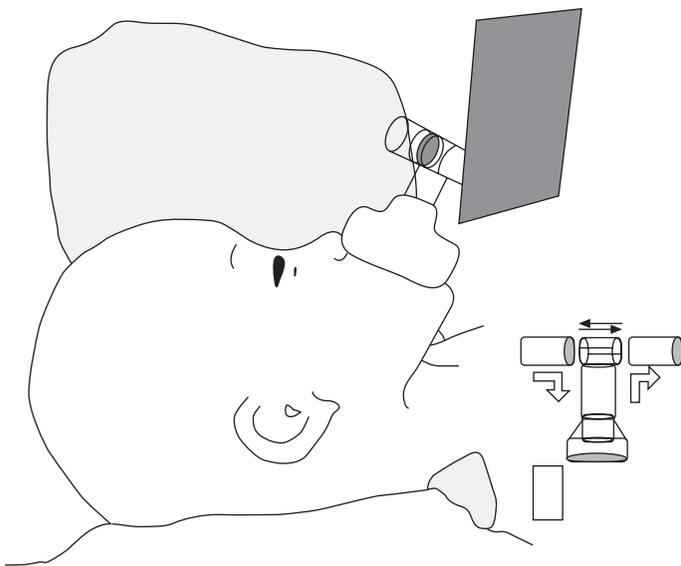


Figure 3. Schematic illustration of exhaled nitric oxide fraction measurement using the off-line tidal breathing method in an infant. The mask is connected to a non-rebreathing valve. Modified from [10] with permission from the publisher.

be comparable to the standardised single-breath technique in older children, although longitudinal studies comparing both techniques are lacking. However, the technique is highly sophisticated and its application in large study settings and clinical routine does not seem to be feasible. Furthermore, thoracoabdominal compression and sedation are not physiological and may change the washout of the NO gas from the lungs.

Taken together, while no standardisation of NO measurements in infants exists, the methods do not need to be regarded as a “methodological nightmare” [5], but need to be considered in conjunction with their respective advantages and disadvantages depending on the purpose of the measurement, the overall aim of the study and the age and cooperation of the child.

Specific issues regarding exhaled NO measurement in infants

Biology of NO in the developing lung

During infancy the airways and the lungs are still in the process of growth and development, which is determined by various pre- and post-natal factors [18, 19]. One of these factors is NO, with various functions in the maturing lung [20]. Amongst others, pulmonary vascularisation, alveolarisation and airway branching are thought to be influenced by NO. NO also alters apoptosis of several different lung cell types. Regulation of lung liquid production and peripheral contractile elements in the perinatal phase of lung development, and pulmonary vasomotor tone are mediated by NO [21–25]. During fetal development, NO synthase (NOS) expression in the lung is thought to be upregulated in the third trimester, as suggested by animal models [26]. In children it is believed that changing expression of inducible NOS (iNOS) is the major determinant of NO variability [27]. Whether this is also true for infants or whether other isoforms of NOS (neuronal and endothelial, nNOS and eNOS, respectively) known to influence branching morphogenesis in rat lungs [28] also contribute to exhaled NO concentrations is unknown. Thus, NO in infants may not only reflect some kind of eosinophilic airway inflammation as in older subjects, but may also be regarded as a potential surrogate marker of the developmental course of the lung.

Confounding factors

Factors that influence NO measurement results can be divided into physiological factors, such as breathing pattern, and measurement factors, such as ambient NO concentration, expiratory flow or sampling rate of NO. These confounders have been summarised previously in this monograph by ALVING and MALINOVSKI [29]. While most of the cofounders are similar during infancy and later in life, herein we focus on some issues that seem to be especially important during infancy (table 1), either because they are unique to infants or because they need to be considered especially in the measurement techniques used in infants.

Physiological factors

Breathing pattern and sighs

Changes in breathing pattern and sighs occur often in infants and may change airway mechanics, the wash-out of NO gas from the lungs and, thus, resulting NO values. Furthermore, if an infant is agitated just before the NO measurement, a significant effect on NO washout and thus NO values due to “breathing manoeuvres” cannot be excluded, although to our knowledge no studies on this issue have been performed yet. Thus, in tidal breathing techniques it is crucial to measure NO during quiet sleep or normal tidal breathing, especially if flow–volume loops are not recorded, which would allow feedback on breathing pattern.

Nasal contamination

While nasal and paranasal cavities are known to significantly produce NO, sinuses are not fully developed and ventilated; therefore, nasal contribution to mixed NO is less clear in infants than in older children. Looking at online NO washout curves recorded during tidal online breathing, some authors suggest that NO peaks before plateaus reflect nasal contamination [10, 15]. Dynamically adjusted flow resistance might be a potential approach to apply sufficient respiratory resistance to close the soft palate during tidal breathing and, therefore, minimise nasal NO contribution. However, as infants physiologically breathe through their nose, these methods disturb the infants’ natural breathing pattern, are not accepted very well by infants and, as such, possibly influence NO values. Depending on the purpose of the study, the exact source of NO may be more or less important to know.

Food intake

Breast milk or formula are usually low in nitrite content. The timing of food intake should not have a large influence on NO values. Breastfeeding 5 min prior to sampling does not seem to have a significant effect on NO [4].

Age, smoke exposure and atopic status

As mentioned previously in this monograph, NO values are, amongst others, influenced by age, atopic status and smoke exposure. While it can be assumed that this is similar in early childhood, in infants, especially newborns, the atopic status of the parents and smoke exposure during

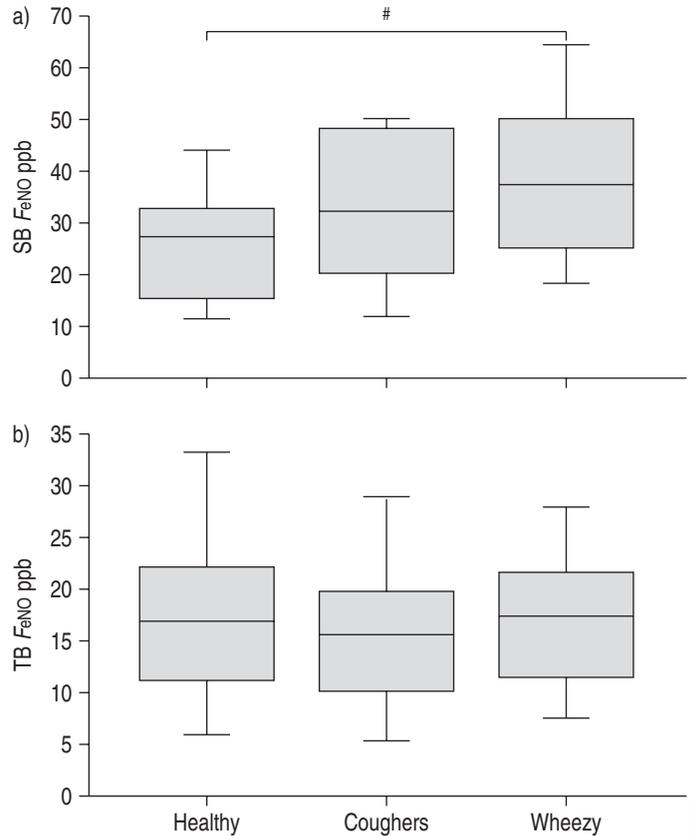


Figure 4. Box plots of exhaled nitric oxide fraction (F_{eNO}) in 23 healthy, 16 recurrent coughing and 32 recurrent wheezy infants measured with a) the single breath (SB) method and b) the tidal breathing (TB) online method. Infants were sedated prior to measurements. The box represents the median, 25th and 75th percentiles and the whiskers represent the 10th and 90th percentiles. #: $p=0.01$. Reproduced from [16] with permission from the publisher.

Table 1. Confounding factors of exhaled nitric oxide (NO) measurement in infants

Impact on exhaled NO values	Physiological and environmental factors	Measurement factors
High	Atopic parents Parental smoke exposure Timing and intensity of smoke exposure Combination of factors, smoke exposure and atopic parents	Ambient NO Expiratory flow Mixed oral and nasal NO sampling
Low	Gestational age Sex	
None	Anthropometrics Breast milk	Sedation Forced expiratory manoeuvres preceding NO sampling
Unknown	Breathing pattern (<i>e.g.</i> sighs and crying) Development of nasal and paranasal sinuses Mutations (<i>e.g.</i> cystic fibrosis transmembrane conductance regulator carrier) Food other than breast milk	Thoracoabdominal compression Nasal obstruction Sampling rate Sample storage

pregnancy must also be considered as these factors have been shown to significantly modify certain effects on NO [30].

Measurement factors

Ambient NO

Ambient NO levels are regarded as a critical confounder of exhaled NO values, thus, subjects should inhale NO free air prior to NO sampling in order to washout NO from the airways [3]. Unfortunately, instead of using NO-filters during inhalation, it has been recommended [7] that NO measurements should be discarded on days when ambient NO levels are >10 ppb, and is being implemented in some studies [31]. This is not accurate, as it cannot be excluded that varying ambient NO levels <10 ppb also influence NO results and introduce a non-systematic bias [6]. Thus, always using NO filters, to make sure NO free air is inhaled, seems justified.

Expiratory flow-rate dependence

The influence of expiratory flow on NO levels has been well-known for a long time. Adjustment of NO for flows seems important in order to obtain accurate results, especially in infants.

Application in clinics and research

The following section summarises studies in infants where NO was measured and used to differentiate disease groups, to obtain information on disease processes or to help in making clinical decisions. NO measurements in infants are performed in only a few centres and are not part of the clinical routine, thus, study designs of existing publications clearly overlap between its potential clinical application and its use for research purposes.

Wheezing disorders

While a huge amount of data on F_eNO in adults and older children with established allergic asthma exist, relatively few studies to date have investigated NO in infants with different forms of wheezing disorders [2]. In infants, wheezing episodes are mostly related to viral infections. The disease entity “asthma” in older children is not comparable to the different phenotypes of recurrent wheezing during infancy.

Thus, for a better overview we divided studies in wheezing infants into “atopic wheeze” and “viral infection and wheeze”. However, on an individual level, the distinction between both phenotypes is not as clear and a large overlap exists between groups.

Atopic wheeze

In several studies using different techniques, it was shown that infants with recurrent wheezing episodes had higher NO levels compared to healthy controls [11, 13, 15, 16, 32]. High-quality evidence exists in older subjects that F_eNO levels decrease after a course of inhaled corticosteroids (ICS) [33] or leukotriene receptor antagonist combined with ICS [34]. Thus it is not surprising that comparable findings have been found in preschool children [35, 36].

Atopic dermatitis in infants is known to be associated with asthma in later childhood [37]. In this regard, it is interesting that infants with eczema both with [38] and without a history of wheezing [38, 39] and with normal forced expiratory flows [40] have been shown to have elevated NO levels compared to healthy controls. Thus, elevated NO seems to reflect changes other than those detected by measurements of airway mechanics also early in life [6].

Taken together, elevated NO has been consistently reported to be elevated in atopic infants independent of NO measurement techniques. In fact, different NO levels found in atopic and healthy infants seem to be due to biological variability and this difference seems to be significant enough to fairly outbalance inter-technical variability. However, it remains questionable as to whether a single NO measurement is useful in a clinical setting as considerable overlap of NO levels between groups were found (fig. 4) and predictive values of NO have yet to be determined.

Moreover, it is not clear whether elevated NO actually reflects ongoing airway inflammation in atopic infants with a history of recurrent wheeze. Airway biopsy studies in asthmatic school-aged children [41–43] as well as in wheezy preschool children [41, 44] revealed evidence of eosinophilic inflammation in the airway walls and airway remodelling, *e.g.* thickening of bronchial epithelial reticular basement membrane and epithelial loss. However, these histological changes were not found in biopsy studies in wheezy infants, even in the presence of atopy [45], whereas elevated inflammatory markers in bronchoalveolar lavage of those wheezy infants have been reported [46, 47].

To our knowledge no study in atopic infants has confirmed the hypothesised causal relationship of inflammation in the airway lumen and airway wall and NO. Until recently it was not clear whether elevated NO in atopic infants “snapshots atopy” [48], depends on certain NOS genotypes [27, 49–51], or reflects NOS upregulation due to unknown mechanisms. More importantly, it is unknown whether elevated NO in infancy relates to later development of asthma in these children; ongoing follow-up in birth cohort studies may answer the latter question.

Viral infections and viral-induced wheeze

Most infants and preschool wheezers lose their symptoms by the time they reach school age. Only a minority develops unremitting, multiple trigger wheeze and are diagnosed with asthma later in life [52]. Therefore, several authors investigated cross-sectionally whether infants with viral wheeze have different NO levels compared to asymptomatic infants.

In infants with viral-induced respiratory tract illness, nasal, oral [53] and mixed NO [16, 31, 54] was decreased compared with healthy controls. However, the difference of NO levels between

groups was dependent on the NO measurement technique in one study [16]. Infants with mild upper respiratory symptoms had either decreased [54] or similar [31] NO values compared with healthy controls. A history of recurrent cough in infants did not affect NO levels in one study [16].

Taken together, viral respiratory tract infections, with or without wheeze, seem to modulate NO metabolism to some extent. This relationship might be explained by downregulated NO production, impaired NO release due to secretions and rather neutrophil dominated airway inflammation [55].

Several methodological constraints limit current information on NO in infants with viral-induced respiratory symptoms. While a minority of reports accounted, for example, for different breathing patterns [16, 53], most of the studies did not report other influencing factors, such as atopy or environmental-smoke exposure. Moreover, viral-induced respiratory symptoms were diagnosed clinically, with large overlap between group definitions and virus species not being reported. This might have introduced increased disease heterogeneity of studied infant populations.

Thus, it seems clear that atopy alone and wheezing episodes in atopic infants leads to elevated NO and viral infections decrease NO levels. However, despite some preliminary data [56], it is unclear whether NO can help distinguishing those wheezers who only have viral-induced wheeze from those who tend to have multiple-trigger wheeze and will possibly continue to develop asthma later in life. This uncertainty is mainly due to lack of longitudinal NO data in ongoing cohorts, rather than methodological issues of NO measurements *per se*.

Prediction of wheezing

In a birth cohort study, the association between NO measured online during tidal breathing in unsedated infants shortly after birth and subsequent respiratory symptoms later in infancy was studied [30]. In infants of atopic and smoking mothers, elevated levels of NO were associated with an increased risk of subsequent respiratory symptoms. The association was strongest in infants of mothers who were both atopic and smoking. Recent data confirmed that elevated NO levels may precede respiratory symptoms in older preschool children with moderate-to-severe intermittent wheezing [57]. Parental history of atopy was not associated with NO levels in infants, and smoke exposure was not reported in the latter study [57].

In another study, NO was measured in children between 3–47 months of age with recurrent episodes of cough and wheeze using the off-line bag technique [56]. The authors found that those wheezing children with a stringent index for the prediction of asthma at school age have higher levels of NO than those with a loose index or those with recurrent cough [56]. Although these studies suggest that NO in early life may help to predict the risk for later asthma development, clearly more evidence and longer follow-up times of these children are needed before prospective NO typing of infants can be performed in clinical practice [58].

Cystic fibrosis

CF is characterised by chronic neutrophil inflammation of airways, mucous plugging and bronchial infection and colonisation with specific pathogens [59]. While low $FeNO$ [60] and higher exhaled NO metabolites [61] were found in school-aged children with CF, NO levels in infants with CF were not different from levels in healthy controls [11, 62], irrespective of the NO measurement technique used. Moreover, NO was not associated with airway inflammation [62, 63]. However, there is good evidence [64] that iNOS protein expression is lowered in airway epithelial cells from infants with CF compared with healthy controls.

Although NO measurements do not help to assess airway inflammation in infants with CF, the association of polymorphisms in NOS genes with $FeNO$, spirometry and bacterial colonisation in adult CF patients suggests that NO metabolism may have a disease modifying role [65, 66]. Whether this is already true for early life is still to be determined.

Primary ciliary dyskinesia

Primary ciliary dyskinesia (PCD) lung disease is characterised by chronic infections and inflammation of the upper and lower respiratory tract due to defective ciliary motility [67]. Nasal NO (nNO) and FeNO are low in children with PCD [68, 69] but the mechanisms are not understood. Measurement of nNO, which is more discriminatory than FeNO, is a good screening test as discussed previously [68].

Some case reports of low nNO values in infants with PCD also suggest a certain value of nNO in infants [70, 71]. Clearly, methodological issues have to be solved and larger studies are needed before measuring nNO can be seen as a suitable screening test for PCD and implemented into the clinical routine in infants.

BPD and chronic lung disease

BPD is a common adverse outcome of premature birth and is a major cause of chronic lung diseases (CLD) [72]. In contrast to atopic asthma, airway inflammation in BPD is predominated by neutrophil granulocytes [72]. However, due to the function of NO during airway development and regulation of vascular tone, NO metabolism might play an important role during the development of BPD [20, 26, 73]. Some authors reported [74, 75], that infants with BPD have increased NO levels compared to controls, while others found decreased NO values in infants [8] and preschool children [76, 77] with BPD. Interestingly, NO production appears to change in infants developing BPD [78].

Taken together, studies on NO in infants with BPD are scarce and inconsistent findings have been reported. Different measurement techniques, different gestational ages, lack of control for variable breathing patterns [11, 75, 78], smoke exposure and atopy [78] may have confounded the findings. From a clinical point of view, it is not clear whether the different findings between studies of NO in BPD are due to methodological issues or biological heterogeneity in infants with BPD.

NO during lung development

The regulation of different NOS isoforms in developing healthy lungs and in CLD is complex. In a baboon CLD model [73], decreased eNOS and nNOS protein, and increased iNOS protein were detected in proximal respiratory epithelial cells compared to term-born baboons. Furthermore, NO measurements were performed consecutively every day during the first 2 weeks of life in a ventilated baboon CLD model [26]. In this model, NO levels remained stable during the first 7 days of life and subsequently showed a 2.5-fold increase from baseline until the end of the second week of life. nNOS and eNOS protein expression and enzymatic activity increased during 140–160 days of gestation but fell again from 160–175 days of gestation. In contrast, a marked rise in iNOS expression and activity during 160–175 days of gestation was observed.

Changes in the expression of the different NOS isoforms seem to play a major role during lung development. These data suggest that NO metabolism in CLD is altered and can be partly explained by the induction of iNOS and downregulation of constitutional NOS isotypes in respiratory epithelial cells [26, 73, 78]. Consequently, the timing of NO measurements, especially in preterm infants, seems to be crucial. Further studies are necessary to characterise the role of NO in BPD. If NO measurements in BPD could be regarded as a noninvasive proxy for lung maturational processes, longitudinal measurements of NO could help to predict the disease course in BPD infants.

Environmental exposure

In the context of fluctuating regulation of NOS isotypes during normal and abnormal lung development, the impact of environmental exposures on NO levels in infants strongly depends on timing and intensity of stimuli during the developmental process [6].

In a cohort study [79], NO levels were found to relate to parental smoke in a dose–response relationship. Infants exposed to pre- and post-natal smoke [9, 31] had decreased levels of NO, and only post-natal smoke-exposed infants had increased levels of NO compared to non-exposed infants (fig. 5) [31]. Interestingly, FREY *et al.* [32] showed that maternal but not paternal history of asthma modified the effect of pre-natal and early post-natal factors on NO levels in infants. Pre-natal smoke exposure was associated with higher NO in infants of asthmatic mothers and with lower NO in infants of non-asthmatic mothers compared to unaffected infants.

The possible effect of air pollution on NO levels in infants was examined in another cohort study from Switzerland [80]. Elevated NO was found in infants of mothers exposed to high levels of ambient NO₂ during pregnancy.

Whether environmental factors alter NOS activity and NO reflects early airway inflammation remains unknown [81], but may provide a possible link for the well-known association between exposure to air pollution and subsequent evolution of asthma. Despite different measurement techniques and different cohort characteristics, the effect size was very comparable between the studies, suggesting that NO measurement is a promising tool to assess the impact of environmental exposures on the developing lung [6], be it smoke exposure or air pollution [82].

Perspectives

Measurement techniques

Due to different advantages and disadvantages of the respective measurement techniques, it is probable that no single technique will be exclusively used. Known confounding factors should be better standardised in the future such as, for example, using NO filters for inhalation of NO free air in all techniques. Furthermore, the exact influence of some of those factors should be clarified. One example is the influence of the expiratory flow on NO measurements using the tidal off-line

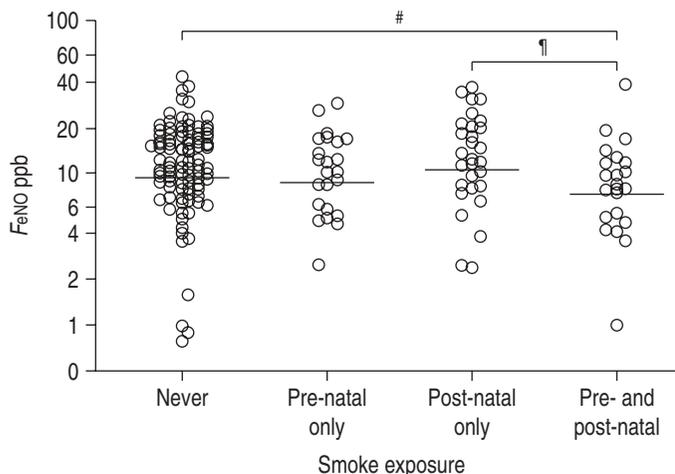


Figure 5. Pre- and post-natal maternal smoking exposure and exhaled nitric oxide fraction (F_{eNO}) values in infants. Never-exposed: $n=113$; pre-natal exposure only: $n=23$; post-natal exposure only: $n=30$; exposed pre- and post-natally: $n=21$. Horizontal lines represent geometric mean F_{eNO} estimated with multivariable linear regression and adjusted for sex, birth weight, maternal atopy, age at study date, ambient nitric oxide, lower respiratory symptoms and upper respiratory symptoms. #: $p=0.052$; ¶: $p=0.042$. Reproduced from [31] with permission from the publisher.

bag method. It would be easy to monitor expiratory flow during NO sampling in order to assess the impact of expiratory flow on subsequently measured NO. A study comparing the tidal off-line bag method with the tidal online method would further highlight this issue. Furthermore, there remains a lack of knowledge concerning the influence of the nasal contamination in infants, who on the one hand mostly breathe through the nose and on the other hand whose nasal sinuses are not yet fully developed.

Clinical application

From a clinical perspective, nasal NO measurements in infants seem important in early diagnosis of PCD. However, again some methodological issues need to be addressed before nasal NO measurement can be recommended as a screening tool for PCD. Are nasal NO measurements

valid during tidal breathing? How can oral contamination be excluded in non-cooperating subjects? Would it be enough to measure mixed NO in infants? At what age can reduced nasal NO values be measured?

Another important clinical question to answer is the ability of NO measurements to predict the occurrence of subsequent respiratory disease or the development of later asthma. While few preliminary studies with short follow-up time seem promising [30, 56], clearly longer follow-up of the children and other patient groups are needed to answer this important issue.

Research purposes

For epidemiological studies, NO measurements seem promising in assessing the influence of different extrinsic (such as smoke exposure or air pollution) and intrinsic (such as genetic predisposition or regulation of NOS isoforms) factors on normal and abnormal lung development [6]. In this context, longitudinal studies with regular NO measurements and ongoing recording of influencing factors are probably needed to accurately assess possible effects. Although these are time consuming, expensive and, not to mention, hard to justify from an ethical point of view, it would be interesting to better disentangle the exact role of the respective NOS isoforms during different time-points of normal lung development and disease pathogenesis [26, 64]. This could lead to new attractive therapeutic options.

Conclusion

It is increasingly recognised that the measurement of exhaled biomarkers in general, and NO in particular, constitute a novel way to monitor separate aspects of lung diseases, such as asthma, which cannot be assessed by other means or lung function. While it is less clear whether NO actually reflects airway inflammation in infants, the intrinsic role of NO during lung development has been widely accepted. The additional information on the pathogenesis of respiratory diseases, their modification by therapy and the increasing awareness of the life-long importance of events occurring early in life justifies further research on NO in infants. Moreover, recent evidence highlights the potential of NO measurements in infants to estimate the impact of environmental exposures on developing lungs. In this context NO may become increasingly important in monitoring the health and disease of the developing lungs.

Statement of interest

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