



EXHALED NITRIC OXIDE IN CHILDHOOD ASTHMA: METHODOLOGICAL ISSUES AND CLINICAL APPLICATIONS

WINNING ABSTRACT: The work described herein was presented as an oral, invited presentation at the European Respiratory Society (ERS) Congress 2006 and was awarded the European Respiratory Society Annual Award for Paediatric Respiratory Research in Europe; the work consists of two parts. The first part is on methodological issues of measuring exhaled nitric oxide fraction ($F_{e,NO}$) in children. The second part includes four studies on clinical applications of $F_{e,NO}$ measurements in asthmatic children.



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Airway inflammation is a hallmark of asthma. Since the 1990s there has been considerable interest in nitric oxide (NO) in exhaled breath. NO is produced in the airways and its fractional concentration in exhaled air ($F_{e,NO}$) is elevated in patients with steroid-naïve atopic asthma. Higher $F_{e,NO}$ separates untreated asthmatics from normal subjects with minimal overlap. In atopic asthmatic adults and children, $F_{e,NO}$ correlates with eosinophil counts in induced sputum and with eosinophil infiltration of the airway wall, and this makes $F_{e,NO}$ the first noninvasive, valid marker of asthmatic airway inflammation. Treatment with inhaled corticosteroids (ICS) reduces $F_{e,NO}$ in a (partly) dose-dependent way.

$F_{e,NO}$ MEASUREMENTS IN CHILDREN: METHODOLOGICAL ISSUES

In 1999 and 2002, the American Thoracic Society (ATS) and the European Respiratory Society (ERS) published recommendations for standardised procedures to measure $F_{e,NO}$. Despite the available guidelines, several questions remain and were tackled in four separate studies.

Exhaled nitric oxide measurements with dynamic flow restriction in children aged 4–8 yrs

$F_{e,NO}$ strongly depends on exhalation flow; however, children often are unable to perform controlled flow procedures. We

developed a device for off-line $F_{e,NO}$ sampling, in which dynamic flow restriction kept the exhalation flow constant at $50 \text{ mL}\cdot\text{s}^{-1}$ with minimal cooperation of the child, and tested this in 4–8-yr-old children [1]. There was an excellent correlation and good agreement between $F_{e,NO}$ measured with this off-line technique and on-line values. The procedure was highly feasible in children aged 4–8 yrs. Normal values were obtained in 34 children and were $4.9 (\text{SEM } \pm 1.2)$ ppb for boys and 7.6 ± 1.1 ppb for girls. We concluded that off-line $F_{e,NO}$ measurements with dynamic flow restriction are feasible in young children and correspond to on-line values.

Exhaled nitric oxide in mylar balloons: influence of storage time, humidity and temperature

In the study discussed previously, we collected exhaled air in mylar balloons. In a further study [2], we examined the influence of different storage conditions on the stability of NO in mylar balloons. NO remained stable for 9 h irrespective of ambient temperatures, without silica gel. NO increased between 9 and 48 h, but only with low initial $F_{e,NO}$, and silica gel increased variability.

We concluded that mylar balloons are suitable for off-line collection of exhaled breath samples and that NO should be analysed within 9 h.

Exhaled nitric oxide in healthy subjects aged 4–17 yrs

In a large international study [3], normal values for $F_{e,NO}$ in 4–17-yr-old children were obtained. The geometric mean $F_{e,NO}$ in all 405 children was 9.7 ppb, and the upper 95% confidence limit was 25.2 ppb. However, $F_{e,NO}$ increased significantly with age, and higher $F_{e,NO}$ values were seen in children with self-reported rhinitis/conjunctivitis or hay fever.

The effect of spirometry and exercise on exhaled nitric oxide in asthmatic children

In a study of 24 asthmatic children [4], we examined if, and to what extent, spirometry or exercise could affect $F_{e,NO}$. A small drop in $F_{e,NO}$ was found 5 and 15 min after spirometry; after exercise, $F_{e,NO}$ values showed a larger drop after 5 and 15 min, irrespective of baseline $F_{e,NO}$, and values returned to baseline within 30 min. We recommend that children should refrain from physical exercise for ≥ 30 min before $F_{e,NO}$ measurements and that $F_{e,NO}$ measurements can best be performed before spirometric manoeuvres.

Fe,NO MEASUREMENTS IN CHILDREN: CLINICAL APPLICATIONS

Fe,NO as a noninvasive marker of airway inflammation might be particularly useful in monitoring asthma in children. We addressed several clinical questions concerning how Fe,NO can be used in everyday paediatric pulmonary practice.

High fractional concentration of NO in exhaled air despite steroid treatment in asthmatic children

A substantial proportion of asthmatic children treated with ICS have elevated Fe,NO values. There may be several reasons for this; we examined two of these [5]. First, we hypothesised that optimising inhalation technique would reduce Fe,NO in children who had elevated Fe,NO while using ICS. Then, in children with persistently elevated Fe,NO despite optimal inhalation technique, we increased ICS doses. We found that improving inhalation technique did not reduce Fe,NO, and increasing ICS from a daily median dose of 800 to 1,200 µg budesonide had no significant effect on Fe,NO. Possible explanations for these results may be that our patients had high median ICS doses and the maximal effect on Fe,NO was already attained, and that only 16 out of the 41 children included showed inadequate inhalation technique.

Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission

Remission of asthma is common in children, but it is difficult to determine the best time-point at which to reduce or stop ICS. Currently there is no objective parameter to predict the probability of asthma relapse following steroid withdrawal. We hypothesised that Fe,NO might be a predictor of asthma relapse in children in whom ICS are discontinued [6]. In total, 40 children were studied and Fe,NO was measured before and 2, 4, 12 and 24 weeks after withdrawal of steroids. Nine patients relapsed. Fe,NO 2 and 4 weeks after withdrawal of steroids predicted which children were about to relapse with the best combination of sensitivity (71%) and specificity (93%) for Fe,NO of 49 ppb 4 weeks after discontinuation of steroids.

Titrating steroids on exhaled nitric oxide in asthmatic children: a randomised controlled trial

Anti-inflammatory treatment with ICS is based mainly on symptoms reported by the child or parents. However, symptoms are not closely related to the presence and severity of airway inflammation. Therefore, the question we addressed in another of our studies [7] is whether asthma treatment in children should be targeted at symptoms or airway inflammation. We included 85 allergic asthmatic children prescribed inhaled steroids, who were randomly allocated to two groups. In the Fe,NO group (n=39), treatment decisions were made on both Fe,NO and symptoms, in the symptom group (n=46) on symptoms only. At the end of the study, there was no difference in median steroid dose, the primary end-point, between both groups. However, in the Fe,NO group, airway hyperresponsiveness improved more than in the symptom group. We concluded that ICS dose titration using Fe,NO improved important objective end-points in children with moderate-to-severe allergic asthma.

Daily ambulatory exhaled nitric oxide measurements in asthma

Recently, a new hand-held NO analyser (NIOX MINO; Aerocrine, Solna, Sweden) was developed, which offers the possibility of measuring Fe,NO at home. Home monitoring of Fe,NO has the potential to detect inflammation at an early stage and adapt treatment with ICS accordingly. We evaluated the feasibility and variability of Fe,NO measurements in the home situation [8]. Secondly, we investigated the correlation between symptoms and Fe,NO during the 2 weeks of the study. Fe,NO was measured in 21 stable asthmatics twice daily for 2 weeks with a success rate of 93%. We found a significant diurnal variation in Fe,NO and marked individual fluctuation. Fe,NO and cumulative symptom scores did not correlate. We concluded that home Fe,NO measurements are feasible and offer the possibility of airway inflammation assessment on a daily basis.

IMPACT FOR PAEDIATRIC ASTHMA MANAGEMENT

Asthma is a common disease in children and the work presented here has direct practical relevance to asthma management in children. The use of Fe,NO measurements enables more effective and efficient administration of ICS. Fe,NO provides us with a practical tool to distinguish patients who will benefit from ICS from those who will not, and patients who require additional therapy from those whose medication dose could feasibly be reduced.

As an “inflammometer”, Fe,NO provides the clinician with hitherto unavailable information regarding the nature of underlying airway inflammation, thus complementing conventional physiological testing, including the measurement of airway hyperresponsiveness. The studies presented and discussed here pave the way towards better treatment of childhood asthma using inflammometry and we feel that the time has come to incorporate Fe,NO into the routine assessment of asthmatic children.

MY JOB AND THE UNIT IN WHICH I WORK

In 1999 I started training as a fellow in Paediatric Pulmonology at the Sophia Children's Hospital (Rotterdam, the Netherlands), under the supervision of Prof. Johan de Jongste. The Dutch Asthma Fund funded this fellowship. It was during this fellowship that I started the research culminating in my PhD thesis in 2006. An additional grant from the Foundation Sophia Children's Hospital Fund and the Kröger Foundation made it possible to finish this research. Since 2004, I have been a staff member of the Dept of Paediatrics/Paediatric Pulmonology. Our medical team consists of four paediatric pulmonologists with Johan de Jongste as our head. We have one fellow in training for Paediatric Pulmonology. Our clinical work concentrates on children with asthma and cystic fibrosis, and on children on home ventilation because of neuromuscular disease. As one of the centres for Paediatric Pulmonology in the Netherlands, we see a wide variation in congenital abnormalities, rare infections, interstitial diseases and so on. We have a fully equipped lung function laboratory including the option of infant lung function testing and a bronchoscopy unit.

Our research focuses on asthma (birth cohort studies, non-invasive measurement of inflammation, intervention studies),

cystic fibrosis (focusing on imaging, inhalation therapy and infections) and lung function testing in children.

REFERENCES

- 1 Pijnenburg MW, Lissenberg ET, Hofhuis W, *et al.* Exhaled nitric oxide measurements with dynamic flow restriction in children aged 4–8 years. *Eur Respir J* 2002; 20: 919–924.
- 2 Bodini A, Pijnenburg MW, Boner A, De Jongste JC. Stability of NO in mylar balloons: effect of storage conditions. *Mediators Inflamm* 2003; 12: 47–49.
- 3 Buchvald F, Baraldi E, Carraro S, *et al.* Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. *J Allergy Clin Immunol* 2005; 115: 1130–1136.
- 4 Gabriele C, Monti F, Pijnenburg MW, Hop WC, De Jongste JC. The effect of exercise and forced expiratory manoeuvres on exhaled nitric oxide in children. *Pediatr Allergy Immunol* 2005; 16: 243–247.
- 5 Pijnenburg MW, Bakker EM, Ghio L, Lever S, De Jongste JC. High FeNO in steroid treated asthmatics. *Clin Exp Allergy* 2005; 35: 920–925.
- 6 Pijnenburg MW, Hofhuis W, Hop WC, De Jongste JC. Exhaled NO predicts asthma relapse in asymptomatic asthmatic children. *Thorax* 2005; 60: 215–218.
- 7 Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating steroids on exhaled nitric oxide in asthmatic children: a randomized controlled trial. *Am J Respir Crit Care Med* 2005; 172: 831–836.
- 8 Pijnenburg MW, Floor SE, Hop WC, De Jongste JC. Daily ambulatory exhaled nitric oxide measurements in asthma. *Pediatr Allergy Immunol* 2006; 17: 189–193.