Eur Respir J 1999; 13: 1402–1405
Printed in UK – all rights reserved

European Respiratory Journal
ISSN 0903-1936

Nasal and lower airway level of nitric oxide in children with primary ciliary dyskinesia

B. Karadag, A.J. James, E. Gültekin, N.M. Wilson, A. Bush

Nasal and lower airway level of nitric oxide in children with primary ciliary dyskinesia. B. Karadag, A.J. James, E. Gültekin, N.M. Wilson, A. Bush. ©ERS Journals Ltd 1999.

ABSTRACT: Exhaled nitric oxide can be detected in exhaled air and is readily measured by chemiluminescence. It is thought to be involved in both the regulation of ciliary motility and host defence. Recently, upper airway NO has been found to be reduced in a small number of children with primary ciliary dyskinesia (PCD) and its measurement has been recommended as a diagnostic test for this condition.

The aim of this study was to compare the levels of NO in the upper and lower airways in a larger number of children with proven PCD with those found in healthy children. Exhaled NO was measured in the upper airway by direct nasal sampling during a breath-hold and in the lower airway as the end-tidal plateau level, using a chemiluminescence NO analyser.

Upper airway NO levels were significantly lower in PCD (n=21) than in the healthy children (n=60) (mean±SD, 97±193, 664±298 parts per billion (ppb), respectively, $p<0.0001$). In PCD, the lower airway NO levels were also reduced (2.17±1.18, 5.94±3.49 ppb, respectively, $p<0.0001$). The levels were not associated with steroid use and did not correlate with lung function.

Although there was some overlap between normal children and those with primary ciliary dyskinesia with regard to lower airway NO, nasal NO discriminated between the two groups in all but one child in each group. Measurement of nasal NO therefore may be a useful screening test for primary ciliary dyskinesia.

Eur Respir J 1999; 13: 1402–1405.

Dept of Paediatrics, Royal Brompton Hospital, London, UK.

Correspondence: A. Bush
Dept of Paediatrics
Royal Brompton Hospital
Sydney Street
London SW3 6NP
UK
Fax: 44 1713518763

Keywords: Child
nitric oxide
primary ciliary dyskinesia

Received: May 18 1998
Accepted after revision November 17 1998

Recent evidence has shown that the concentration of nitric oxide detected in exhaled air plays a significant role in the pathophysiology of airway disease [1]. NO is synthesized from L-arginine by the enzyme NO synthase (NOS) which exists in at least three isoforms, two being constitutive (endothelial and neuronal) and one being inducible (iNOS) [2]. In healthy subjects, the substantial proportion of NO found in exhaled air is derived from the upper airway, with only a minor contribution from the lower respiratory tract, unless steps are taken to exclude upper airway contribution [3, 4]. Nasal NO produced by commensal organisms is negligible, so nasal NO is not reduced after antibiotic treatment [5]. An increasing number of studies suggest that NO is a marker of airway inflammation in asthma [4, 6, 7]. This may reflect expression of iNOS in epithelial cells [8], the induction of which is inhibited by glucocorticoids [9].

NO is thought to be involved in both the regulation of ciliary motility and host defence [10–12]. Primary ciliary dyskinesia (PCD) is a genetic disease characterized by the lack of effective ciliary motility, due to an ultrastructural and/or functional defect of cilia. The result is chronic infection in the upper and lower airways. Early diagnosis is important in order to prevent the occurrence of bronchiectasis or inappropriate otorhinological procedures [14]. Recently, marked reduction of nasal NO was reported in a small number of children with Kartagener's syndrome [5]. The purpose of this study was to extend these observations by studying a larger group of children with PCD and comparing the results to healthy control subjects; a further aim was to assess the use of NO measurements as a diagnostic tool in PCD.

Methods

Subjects

The PCD group consisted of 21 children with proven PCD, who were recruited from the PCD clinic of the Royal Brompton Hospital (table 1). PCD was diagnosed by nasal brushing with estimation of ciliary beat frequency (CBF) [14] and electron microscopy [15].

The healthy group consisted of 60 healthy children without a history of chronic or recent respiratory disease, who were recruited from siblings of children attending the asthma or a general clinic at either the Hammersmith or Royal Brompton Hospitals, London, or from children of the staff.

Nitric oxide measurement

Exhaled NO was measured using a chemiluminescence analyser (LR 2000 series; Logan Research, Rochester, UK) according to the method recommended by the European

Table 1. – Clinical and spirometric findings in children with primary ciliary dyskinesia (PCD) and healthy controls

	PCD	Healthy control
Age yrs	10.8±3.2	10.8±3.5
Males %	47	42
Inhaled steroids %	71	-
FEV_1 % pred	75±15	97 (12)
FVC % pred	90±15	-
$FEF_{25–75\%}$ % pred	59±26	-
CBF* Hz	0 (0–11.1)	-

Data are presented as mean±SD unless otherwise stated. FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; $FEF_{25–75\%}$: forced mid-expiratory flow; CBF: ciliary beat frequency. *: median (range).

Respiratory Society (ERS) Task Force Report [16]. This equipment was sensitive to NO from 1 to 5,000 parts per billion (ppb), and gave continuous online recordings with a resolution of about 0.3 ppb, with a response time of 0.4 s. In addition to NO, the analyser also measured CO_2 (resolution 0.1% CO_2, response time 200 ms) and exhalation pressure and volume (or flow) in real time. The analyser was calibrated using certified NO mixtures (90–500 ppb) in N_2 (BOC Special Gases, Surrey Research Park, Guildford, UK). Ambient air NO levels were also recorded.

Exhaled lower airway sampling

This was attempted in all the children. After maximum inspiration, subjects exhaled for as long as possible (slow vital capacity manoeuvre) into a wide-bore tube. A fine-bore Teflon tube, connected directly to the analyser, continuously sampled the exhaled air adjacent to the mouthpiece at 0.25 $L \cdot min^{-1}$. In all these manoeuvres, subjects wore a nose clip and kept the flow during expiration within a constant range by the use of auditory and visual guides (lights, the flapping ears of a plastic dog and a musical sound), which were activated when exhalation was within the required range of expiratory flow (200–280 $mL \cdot s^{-1}$). Thus, the pressure generated (5 cm H_2O) was sufficient to exclude nasal NO production by elevating the soft palate and prevent nasal contamination. The trace was observed until the end-tidal CO_2 reached a plateau and the NO concentration at the end of exhalation was recorded at this plateau value. The test was repeated five times and the mean value was calculated. A result was only excluded if it was not possible to determine a plateau value because expiration time or control of expiratory flow was inadequate.

Exhaled upper airway sampling

Nasal NO was measured in all the PCD and 20 of the healthy children. It was not attempted in the first 40 healthy children, who underwent lower airway NO measurements only. A Teflon tube was inserted just inside one nostril whilst the contralateral nostril was left open. Air was sampled at 250 $mL \cdot min^{-1}$ continuously from one nostril *via* the other nostril during a breath-hold, maintained as long as possible. NO concentrations were recorded when the values reached a plateau. Nasal CO_2 was also monitored to ensure exhalation was not taking place. This test was repeated three times in each nostril, and the mean value was calculated.

Within-subject repeatability

Within-subject repeatability of NO was measured in subsets of children, 10 with asthma and 10 control, in whom five tidal measurements were repeated within 15 min and the means compared. For nasal NO, mean values obtained from the left and right nostril were compared.

Statistical analysis

Statistical analysis was performed using SPSS for Windows Software (SPSS, Chicago, IL, USA). Comparison of NO levels between groups was performed using a two-sample Student′s t-test or Kruskal–Wallis, as appropriate according to data distribution. Correlations were made using Spearman′s rank test. Repeatability has been expressed as the coefficient of variation as variation varied with the mean. A p-value of <0.05 was considered significant. The results are expressed as mean±SD, the 95% confidence interval (CI) of means or median and range.

Results

Nasal NO measurements were obtained in all children with PCD and in all the 20 healthy children in whom it was attempted; lower airway NO could not be measured in 3/18 children with PCD, and 3/60 healthy children could not cooperate with the controlled exhalation necessary. In the PCD group, the mean coefficient of variation of repeated measurements was 10% for upper and 13% for lower airway NO compared to 8% for healthy children.

Exhaled lower airway NO

The range for lower airway NO in healthy children was 2.53–22.7 ppb. There was some overlap with PCD (range 0.22–5.26 ppb) (fig. 1), but the mean NO concentration in PCD was significantly lower than that in the healthy children (mean (95% CI) 2.17 (1.01–3.3), 5.94 (5.03–6.9) ppb, $p<0.001$, respectively) (table 2).

Exhaled upper airway NO

Upper airway NO was found to be markedly reduced in children with PCD compared to healthy control subjects (table 2). There was overlap between the groups in one child in each group (fig. 1). In the child with PCD who had a normal nasal NO level (959 ppb), the lower airway NO was also low at 1.06 ppb; this patient had dextrocardia and an inner dynein arm defect with a measured CBF of 11.1 Hz on the few cilia that were apparent; the movements were dyskinetic.

There was no relation between CBF and NO levels (nasal $r^2= 0.01$; tidal $r^2=0.01$), between lung function

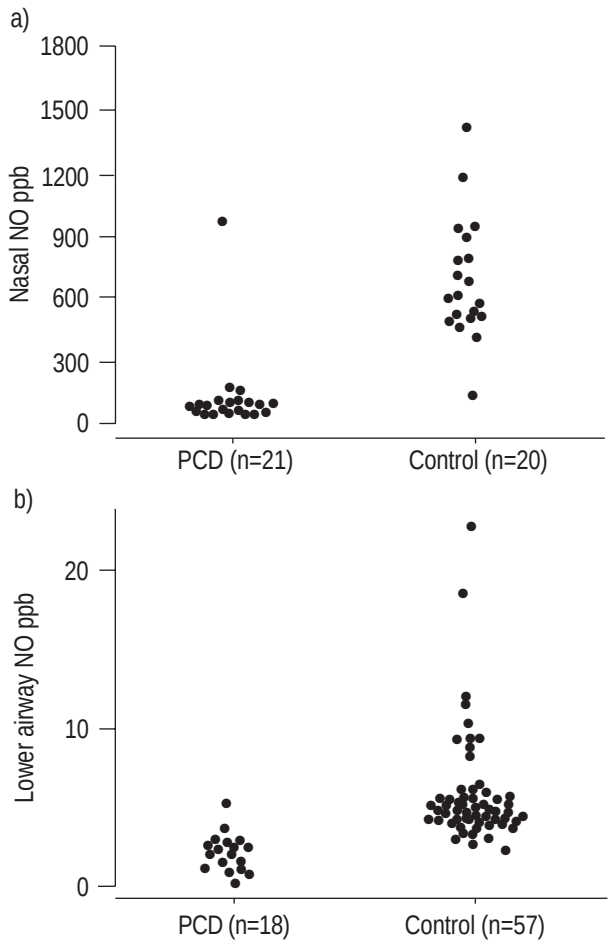

Fig. 1. – Upper (a) and lower (b) airway NO in healthy control children and those with primary ciliary dyskinesia (PCD). The difference in scale between upper and lower airway NO should be noted. ppb: parts per billion.

(FEV1) and either upper or lower levels (r^2=0.002; r^2=0.02, respectively) or between lower and upper airway NO (r^2=0.02). In PCD children, there was no difference in either the lower or upper airway NO in those with and without reported acute infections in the preceding 2 weeks. Upper airway NO was significantly higher than lower airway NO in the PCD group (p=0.03), and in the healthy children (p<0.001; table 2). Neither upper nor lower airway NO values were significantly related to ambient NO levels (nasal r^2=0.24; tidal: r^2=0.03). There was no significant difference in tidal NO in PCD children treated with (n=15) and without (n=6) inhaled steroids (median (range) 1.12 (0.8–2.8), 2.28 (0.22–5.26) ppb, respectively, p=0.71. Lower airway NO in nonsteroid treated PCD was significantly lower than that in healthy control subjects (median (range) 5.0 (2.5–23), p<0.001). NO was unrelated to age in either group for upper (p=0.9) and lower (p=0.2) airways.

Table 2. – Values of upper and lower airway NO (parts per billion (ppb)) in children with primary ciliary dyskinesia (PCD) and healthy control subjects

	Upper airway	Lower airway
PCD	55 (3.3–959)	2.3 (0.2–5.3)
Healthy control	553 (116–1437)	5.0 (2.5–23)
p-value	<0.001	<0.001

Data are presented as median (range).

Discussion

In this larger group of children with PCD, earlier reports that NO in the upper airway is reduced [5, 17] have been confirmed. In addition, it is reported for the first time that NO concentration is also reduced in the lower airways in most children with PCD. An important finding in this study was of a normal nasal NO concentration in one child with PCD and a low nasal NO in a healthy child; the implications of this are discussed below.

The readings were made using strictly standardized conditions in order to ensure accurate measurements. It was ensured that the nasal samples were not contaminated by expired gas from the lower airway by checking that there was no carbon dioxide signal. For the tidal measurements, a resistor in the expiratory circuit prevented nasal contamination of the expirate by preventing flow from the nasopharynx [3]. Standardization of the respiratory flow rate with auditory and visual signals minimized flow-dependent variability of the measurements [18]. Ambient NO levels had no effect on lower airway NO levels, as has been previously noted [16], and nor was there any correlation between ambient and nasal values even in the children with PCD. It is therefore believed that the present measurements represent uncontaminated nasal and lower airway samples, and that the differences between the two groups reflect real differences and not methodological artefacts.

The finding of low NO in a condition like PCD, which is characterized by chronic infection and airway inflammation, is at first sight surprising. One possible explanation is the presence of a "barrier" preventing diffusion into the airway: in the upper airway, from infection leading to chronic obstruction of the paranasal sinuses, the major site of NO production, and, in the lower airway, due to airway surface mucus [19]. If this were so one might have expected a correlation between NO and lung function or recent infections, which was not the case. The poor correlation with recent infection might be because it was difficult to distinguish when these children with chronic symptoms had a superimposed acute infection. Furthermore, the levels of NO in cystic fibrosis (CF) are normal or only slightly reduced [20], despite the fact that in CF upper airways are also affected and the lower airway disease usually much more severe than in PCD. However, in a recent study, nitrite levels in breath condensate were increased in CF, suggesting increased NO production undetected by exhaled NO [21].

Another possible explanation is that impaired NO production is in some way related to the underlying molecular pathology of the disease, either primary or secondary. PCD is usually characterized by structural ciliary abnormalities (including absent dynein arms, radial spoke defects and tubular problems), and it is difficult to imagine how low levels of NO could produce such a multiplicity of structural abnormalities. NO has certainly been implicated in control of ciliary function [12], but no relation between NO and CBF could be shown. This may be because CBF was only

measured at diagnosis, and not at the time of NO measurement.

The diagnosis of PCD may be difficult; secondary functional and structural changes in cilia may be confused with the primary disease [22]. Furthermore, children in particular find that nasal brushing is unpleasant, and a completely noninvasive screening test is appealing. Although for the group tidal NO is reduced in PCD, the overlap with healthy children was too great for it to be useful to screen individuals. For nasal NO, however, using a level <250 ppb as diagnostic, both the positive and negative predictive values were 0.95 (20/21 and 19/20 respectively); unlike previous reports, the separation was not complete [5, 17]. Nevertheless it would seem reasonable to proceed to a nasal brushing in all children with a low NO and to exclude the diagnosis of PCD in all with normal nasal values, unless the index of suspicion is high. A prospective study would be needed to assess this approach. It should also be noted that many cases of PCD will present before they are old enough to cooperate with a breath-hold, a manoeuvre necessary to measure nasal NO using current techniques; in these children nasal NO could obviously not be used to screen for PCD.

In conclusion, it has been demonstrated that children with primary ciliary dyskinesia have a negligible amount of nasally derived NO and also a reduced level in their lower airways. The reason is not clear and further studies are needed to determine whether these low values are primary or secondary to the ciliary defect. Measurement of nasal, but not tidal, NO may be a useful alternative screening test for primary ciliary dyskinesia.

References

1. Barnes PJ, Belvisi MG. Nitric oxide and lung disease. *Thorax* 1993; 48: 1042–1043.
2. Moncada S, Higgs A. The L-arginine–nitric oxide pathway. *N Engl J Med* 1993; 329: 2002–2012.
3. Kharitanov SA, Barnes PJ. Nasal contribution to exhaled nitric oxide during exhalation against resistance or during breath hold. *Thorax* 1997; 52: 540–545.
4. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 1993; 6: 1368–1370.
5. Lundberg JON, Weitzberg E, Nordvall SL, *et al*. Primarily nasal origin of exhaled nitric oxide and absence in Kartagener′s syndrome. *Eur Respir J* 1994; 7: 1501–1504.
6. Borland C, Cox Y, Higenbottam T. Measurement of exhaled nitric oxide in man. *Thorax* 1993; 48: 1160–1162.
7. Kharitonov SA, Yates D, Robbins RA, *et al*. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994; 343: 133–135.
8. Hamid Q, Springall DR, Riveros-Moreno V, *et al*. Induction of nitric oxide synthase in asthma. *Lancet* 1993; 342: 1510–1513.
9. Di Rosa M, Radomski M, Carnuccio R, *et al*. Glucocorticoids inhibit the expression of an inducible, but not the constitutive nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci USA* 1990; 87: 10043–10049.
10. Liew FY, Cox FF. Nonspecific resistance mechanisms: the role of nitric oxide. *Immunol Today* 1991; 12: A17–A21.
11. Jain B, Rubenstein I, Robbins RA, *et al*. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. *Biochem Biophys Res Commun* 1993; 191: 83–88.
12. Xue C, Botkin SJ, Johns RA. Localisation of endothelial NOS at the basal microtubule membrane in ciliated epithelium of rat lung. *J Histochem Cytochem* 1996; 44: 463–471.
13. Hadfeild PJ, Rome-Jones JM, Bush A, Mackay IS. Treatment of otitis media with effusion in children with primary ciliary dyskinesia. *Clin Otolaryngol* 1997; 22: 302–306.
14. Teichtal H, Wright PL, Kirsner RLG. Measurement of *in vitro* ciliary beat frequency: a television modification of the transmitted light technique. *Med Biol Eng Comput* 1986; 24: 193–196.
15. Rutland J, Dewar A, Cox T, Cole PJ. Nasal brushing for the study of ciliary ultrastructure. *J Clin Pathol* 1982; 35: 356–359.
16. Kharitonov S, Alving K, Barnes PJ. ERS Task Force Report. Exhaled and nasal nitric oxide measurements: recommendations. *Eur Respir J* 1997; 10: 1683–1693.
17. Nordvall SL, Lundberg JON, Alving K. Low nasal nitric oxide secretion in children with Kartagener′s syndrome. *Eur Respir J* 1996; 9: Suppl. 23, 247s.
18. Byrnes CA, Dinarevic S, Busst EA, Bush A. Effect of measurement conditions on measured levels of peak exhaled nitric oxide. *Thorax* 1997; 52: 697–701.
19. Lundberg JON, Farkas-Szallasi T, Weitzberg E, *et al*. High nitric oxide production in human paranasal sinuses. *Nat Med* 1995; 1: 370–373.
20. Balfour-Lynn IM, Laverty A, Dinwiddie R. Reduced upper airway nitric oxide in cystic fibrosis. *Arch Dis Child* 1996; 75: 319–322.
21. Ho LP, Innes JA, Greening AP. Nitrite levels in in breath condensate of patients with cystic fibrosis is elevated in contrast to exhaled nitric oxide. *Thorax* 1998; 53: 680–684.
22. Carson JZ, Collier AM, Hu SS. Acquired ciliary defects in primary ciliary dyskinesia in nasal epithelium of children with acute viral respiratory tract infections. *N Engl J Med* 1985; 12: 463–468.