

PRIMARY CILIARY DYSKINESIA: NITRIC OXIDE, GENERAL DIAGNOSTICS

1: Ups J Med Sci. 2006;111(1):155-68.

Transmission electron microscopy in the diagnosis of primary ciliary dyskinesia.

Roomans GM, Ivanovs A, Shebani EB, Johannesson M.

Primary ciliary dyskinesia (PCD) is an autosomal recessive disease with extensive genetic heterogeneity. Dyskinetic or completely absent motility of cilia predisposes to recurrent pulmonary and upper respiratory tract infections resulting in bronchiectasis. Also infections of the middle ear are common due to lack of ciliary movement in the Eustachian tube. Men have reduced fertility due to spermatozoa with absent motility or abnormalities in the ductuli efferentes. Female subfertility and tendency to ectopic pregnancy has also been suggested. Headache, a common complaint in PCD patients, has been associated with absence of cilia in the brain ventricles, leading to decreased circulation of the cerebrospinal fluid. Finally, half of the patients with PCD has situs inversus, probably due to the absence of ciliary motility in Hensen's node in the embryo, which is responsible for the unidirectional flow of fluid on the back of the embryo, which determines sidedness. PCD, which is an inborn disease, should be distinguished from secondary ciliary dyskinesia (SCD) which is an acquired disease. Transmission electron microscopy is the most commonly used method for diagnosis of PCD, even though alternative methods, such as determination of ciliary motility and measurement of exhaled nitric oxide (NO) may be considered. The best method to distinguish PCD from SCD is the determination of the number of inner and outer dynein arms, which can be carried out reliably on a limited number of ciliary cross-sections. There is also a significant difference in the ciliary orientation (determined by the direction of a line drawn through the central microtubule pair) between PCD and SCD, but there is some overlap in the values, making this parameter less suitable to distinguish PCD from SCD.

2: Pediatr Pulmonol. 2006 Feb;41(2):158-63.

Impairment of nitric oxide output of conducting airways in primary ciliary dyskinesia.

Mahut B, Escudier E, de Blic J, Zerah-Lancner F, Coste A, Harf A, Delclaux C.

Nasal nitric oxide (NO) concentration is dramatically reduced in primary ciliary dyskinesia (PCD). The aims of this study were to apply a multiple-flow NO analysis to investigate whether NO output from the bronchial tree was affected in a similar way to nasal NO output, and to search for a relationship between flow-independent exchange parameters and airflow limitation. Multiple flow rate analysis of exhaled NO, allowing the calculation of maximum airway wall flux and alveolar NO concentration, was performed in 17 PCD patients (median age, 25-75th percentiles: 13.5, 12.1-17.6) with documented ultrastructural cilia

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abnormalities and 28 healthy subjects (16.0, 11.0-21.0). Median maximum airway wall flux and median alveolar NO concentration were significantly reduced in PCD patients compared to healthy subjects: 16.0, 7.5-29.5, vs. 25.0, 15.0-32.5 nl/min ($P<0.05$) and 2.5, 1.6-3.3, vs. 5.0, 3.6-6.5 ppb ($P<0.01$), respectively. Significant correlations between maximum airway wall flux and airflow limitation were found, i.e., resistance of respiratory system ($\rho=0.74$, $P<0.005$), forced expiratory volume in one second (FEV(1))/VC ($\rho=-0.61$, $P<0.05$), FEV(1) ($\rho=-0.52$, $P<0.05$), mid expiratory flow between 25 and 75% of forced vital capacity (MEF(25-75)) ($\rho=-0.54$, $P<0.05$), and maximal instantaneous expiratory flow at 50% of the vital capacity (MEF(50)) ($\rho=-0.55$, $P<0.05$). In conclusion, the impairment of NO output is less pronounced in the lower than in the upper (nasal) respiratory tract in PCD. A decrease in maximal NO output from conducting airways is associated with limited airflow impairment. (c) 2005 Wiley-Liss, Inc

3: Ann Med. 2005;37(6):439-49.

Primary ciliary dyskinesia: clinical presentation, diagnosis and genetics.

Van's Gravesande KS, Omran H.

Primary ciliary dyskinesia (PCD) is a phenotypically and genetically heterogeneous disorder with an autosomal-recessive inheritance pattern. Only rarely other modes of inheritance such as X-linked transmission are observed. The disease phenotype is caused by defects of respiratory cilia, sperm tails and the cilia of the embryonic node. The lack of mucociliary clearance contributes to recurrent respiratory tract infections, that might progress to permanent lung damage (bronchiectasis). The goal of therapy is prevention of bronchiectasis. Male infertility due to sperm tail dysmotility is another frequent finding in PCD. Half of affected individuals have situs inversus (Kartagener's syndrome) due to randomization of left/right body asymmetry. Currently three genes (DNAI1, DNAH5, DNAH11) that encode for dynein proteins have been linked to recessive PCD. Mutations in RPGR located on the X chromosome have been identified in males with retinitis pigmentosa and PCD. As a screening test nasal nitric oxide (NO) measurement is widely used. Establishment of diagnosis currently relies on electron microscopy, direct evaluation of ciliary beat by light microscopy, and/or the novel method of high-resolution immunofluorescent analysis of respiratory cilia.

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4: Ultrastruct Pathol. 2005 May-Aug;29(3-4):217-20.

Primary ciliary dyskinesia: a review.

Carlen B, Stenram U.

The entity sinusitis, bronchiectasis, and situs inversus is since long named Kartagener syndrome. Nowadays the designation used is primary ciliary dyskinesia(PCD), which implies cilia with decreased or total absence of motility, which may result in sinusitis, chronic bronchitis, bronchiectasis, and male infertility. A large number of deficiencies detectable on the ultrastructural level give rise to PCD. There may also be aberrations not detected up to the present. The normal left-right asymmetry of the body is thought to be due to the beating of the cilia in the embryonic (Hensen's) node. Total immotility of the cilia should therefore result in random asymmetry of the body that is situs inversus in 50% of the cases. It has also been claimed that 50% of cases with PCD have situs inversus. However, several deficiencies apparently do not cause total immotility, and all ultrastructural variants are not associated with situs inversus in 50% of the cases. Several of the deficiencies are difficult to detect. Optimal fixation and handling are therefore obligatory. The genetic changes behind the variants are now being studied in several laboratories. Patients with PCD have very low levels of nasal nitric oxide, which is of increasing diagnostic importance. Other established diagnostic methods are the saccharine test and determination of ciliary beat frequency.

5: Pediatr Pulmonol. 2005 Jun;39(6):551-7.

Correlation between cough frequency and airway inflammation in children with primary ciliary dyskinesia.

Zihlif N, Paraskakis E, Lex C, Van de Pohl LA, Bush A.

Cough is common in airway disease. We measured cough frequency in children with primary ciliary dyskinesia (PCD), to determine how accurately families assess this symptom; and to assess the relationship between cough frequency and airway inflammation, measured using induced sputum and exhaled nitric oxide (eNO). Twenty stable PCD children (7 boys), median age 10.8 years (interquartile range (IQR), 9-14), and 10 healthy control children, median age 12 years (IQR, 10.5-12.7), were recruited. ENO was measured using a chemiluminescence analyzer, with sputum induction with 3.5% saline. PCD children underwent ambulatory cough monitoring. Sputum neutrophils were higher in PCD (median, 70.3%; IQR, 55.3-78%) compared to controls (median, 27%; IQR, 24.5-33%; $P = 0.004$); cough frequency was higher (median episodes, 19; IQR, 11-22.5) compared to healthy children (median episodes, 6.7; IQR, 4.1-10.5; $P < 0.001$). Forced expiratory volume in 1 sec (FEV(1) percent predicted) and eNO were lower in PCD (median, 63%; IQR, 57-85%; $P < 0.0001$); eNO (median, 7.1 ppb (IQR, 4.8-19.1 ppb) vs.

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12.4 ppb (IQR, 10.3-17.3 ppb), $P = 0.043$). Parental scoring of day and night cough correlated with recorded cough ($r = 0.930$, $P < 0.0001$, daytime; $r = 0.711$ for nighttime, $P = 0.002$). Visual analogue score and cough episodes also correlated positively ($r = 0.906$; $P < 0.0001$). There was a positive correlation between cough frequency and sputum neutrophil count in PCD (Spearman's $r = 0.693$, $P < 0.002$), but not percent FEV(1) or eNO. Stable PCD children have increased cough frequency and neutrophilic airway inflammation. In conclusion, cough frequency correlated with sputum neutrophils but not with FEV1 or eNO. Copyright 2005 Wiley-Liss, Inc.

6: Eur Respir J. 2004 Nov;24(5):881-3.

Nasal nitric oxide is low early in life: case study of two infants with primary ciliary dyskinesia.

Baraldi E, Pasquale MF, Cangiotti AM, Zanconato S, Zacchello F.

Nasal nitric oxide levels are low in patients with primary ciliary dyskinesia, but it is not known whether this defect is already present in the first months of life. The current authors measured nasal nitric oxide in two infants with situs inversus and primary ciliary dyskinesia, diagnosed by electron microscopy at 4 and 6 months of age, and in five healthy control infants. Nasal nitric oxide values in the primary ciliary dyskinesia infants (85 and 115 parts per billion (ppb)) were markedly lower than in the healthy controls (mean: 295 ppb, range: 225-379 ppb). This is the first report to show that nasal nitric oxide values are already low in early life in primary ciliary dyskinesia children, supporting the hypothesis that a reduced production of nasal nitric oxide is an intrinsic feature of this disease. The current authors suggest that the nasal nitric oxide test may be a useful, noninvasive method for screening young children for primary ciliary dyskinesia in clinical practice.

7: Chest. 2004 Oct;126(4):1054-9.

Nasal nitric oxide measurements to screen children for primary ciliary dyskinesia.

Corbelli R, Bringolf-Isler B, Amacher A, Sasse B, Spycher M, Hammer J.

STUDY OBJECTIVE: To examine the usefulness of exhaled and nasal nitric oxide (NO) measurements to detect primary ciliary dyskinesia (PCD) in children.
DESIGN AND METHODS: The study population consisted of 34 children with symptoms suggestive of PCD who were previously referred to our pediatric university respiratory disease clinic for a diagnostic workup including analysis of ciliary structure and function by respiratory mucosal biopsy. PCD was diagnosed in

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17 of the 34 children according to the ciliary biopsy results. Measurements of nasal and exhaled NO were performed according to European Respiratory Society and American Thoracic Society guidelines in the patients with and without biopsy-proven PCD, and also in 24 healthy age-matched subjects. RESULTS: Nasal NO was significantly lower in those children with proven PCD (geometric mean; 13.7 parts per billion [ppb]), compared to those who had negative biopsy results (132.7 ppb) and healthy control subjects (223.7 ppb). The measurement of nasal NO in our study population showed, below a cut-off level of < 105 ppb, a specificity of 88% for PCD, and positive predictive value of 89%. Nasal NO above a cut-off level of 105 ppb excluded PCD with a 100% certainty. The lower levels of exhaled NO in patients with PCD did not reach statistical significance. CONCLUSION: The measurement of nasal NO appears to be a useful tool to screen children for PCD and to exclude this disease in those with high nasal NO levels.

8: Chest. 2003 Aug;124(2):633-8.

Nitric oxide metabolites are not reduced in exhaled breath condensate of patients with primary ciliary dyskinesia.

Csoma Z, Bush A, Wilson NM, Donnelly L, Balint B, Barnes PJ, Kharitonov SA.

STUDY OBJECTIVES: To investigate whether nitric oxide (NO) metabolites would be reduced in children affected by primary ciliary dyskinesia (PCD). DESIGN: Single-center observational study. PATIENTS: Fifteen children with PCD (seven boys; mean [± SEM] age, 10.3 ± 0.7 years; mean FEV₁, 73 ± 2.1% predicted) were recruited along with 14 healthy age-matched subjects (seven boys; mean age, 11.5 ± 0.4 years; mean FEV₁, 103 ± 5% predicted). INTERVENTIONS: We assessed the levels of nitrite (NO₂(-)), NO₂(-)/NO₃(-) (NO₂(-)/NO₃(-)), and S-nitrosothiol in exhaled breath condensate, exhaled NO, and nasal NO from children with PCD compared to those in healthy children. MEASUREMENTS AND RESULTS: The mean exhaled and nasal NO levels were markedly decreased in children with PCD compared to those without PCD (3.2 ± 0.2 vs 8.5 ± 0.9 parts per billion [ppb], respectively [p < 0.0001]; 59.6 ± 12.2 vs 505.5 ± 66.8 ppb, respectively [p < 0.001]). Despite the lower levels of exhaled NO in children with PCD, no differences were found in the mean levels of NO₂(-) (2.9 ± 0.4 vs 3.5 ± 0.3 microM, respectively), NO₂(-)/NO₃(-) (35.2 ± 5.0 vs 34.3 ± 4.5 microM, respectively), or S-nitrosothiol (1.0 ± 0.2 vs 0.6 ± 0.1 microM, respectively) between children with PCD and healthy subjects. CONCLUSION: These findings suggest that NO synthase activity may not be decreased as much as might be expected on the basis of low exhaled and nasal NO levels.

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9: Eur Respir J. 2003 Jan;21(1):43-7.

Nasal nitric oxide measurements for the screening of primary ciliary dyskinesia.

Wodehouse T, Kharitonov SA, Mackay IS, Barnes PJ, Wilson R, Cole PJ.

Primary ciliary dyskinesia (PCD) presents to general practitioners with symptoms pertinent to a variety of specialists because of the involvement of ciliated epithelium in the upper/lower respiratory tract, ears, eyes and genital tract. There is no easy, reliable screening test for PCD, and thus, the majority of patients remain undiagnosed. Nitric oxide (NO) is measurable in nasal air of normal subjects and found to be low in cystic fibrosis (CF) and very low in PCD. Recently, it was suggested to play an important role in regulating ciliary motility. The aim of this study was to evaluate whether measurements of nasal NO could be used to screen for PCD. Nasal NO was measured from the nasal cavity by a chemiluminescence analyser in subjects with PCD, healthy controls, CF, idiopathic bronchiectasis, Young's syndrome and lone sinusitis. Nasal NO was significantly lower in PCD (64.0+/-36.6) compared with normal controls (759+/-145.8), idiopathic bronchiectasis (734+/-163.7), CF (447.5+/-162.6), lone sinusitis (1487+/-734) and Young's syndrome (644+/-129.9). Nasal NO was also significantly lower in PCD than CF patients. Measurement of nasal nitric oxide may therefore be used clinically in various specialities to screen suspected patients for primary ciliary dyskinesia.

10: Thorax. 2003 Jan;58(1):68-72.

Comparison of exhaled and nasal nitric oxide and exhaled carbon monoxide levels in bronchiectatic patients with and without primary ciliary dyskinesia.

Horvath I, Loukides S, Wodehouse T, Csiszer E, Cole PJ, Kharitonov SA, Barnes PJ.

BACKGROUND: Primary ciliary dyskinesia (PCD) is associated with chronic airway inflammation resulting in bronchiectasis. **METHODS:** The levels of exhaled Nitric oxide (eNO), carbon monoxide (eCO) and nasal NO (nNO) from bronchiectatic patients with PCD (n=14) were compared with those from patients with non-PCD bronchiectasis without (n=31) and with cystic fibrosis (CF) (n=20) and from normal subjects (n=37) to assess the clinical usefulness of these measurements in discriminating between PCD and other causes of bronchiectasis. **RESULTS:** Exhaled NO levels were lower in patients with PCD than in patients with non-PCD non-CF bronchiectasis or healthy subjects (median (range) 2.1 (1.3-3.5) ppb v 8.7 (4.5-26.0) ppb, p<0.001; 6.7 (2.6-11.9) ppb, p<0.001, respectively) but not lower than bronchiectatic patients with CF (3.0 (1.5-7.5) ppb, p>0.05). Nasal levels of nNO were significantly lower in PCD patients than in any other subjects (PCD: 54.5 (5.0-269) ppb, non-PCD bronchiectasis without CF: 680

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(310-1000) ppb, non-PCD bronchiectasis with CF: 343 (30-997) ppb, control: 663 (322-1343) ppb). In contrast, eCO levels were higher in all patient groups than in control subjects (PCD: 4.5 (3.0-24.0) ppm, $p < 0.01$, other bronchiectasis without CF: 5.0 (3.0-15.0) ppm, $p < 0.001$; CF: 5.3 (2.0-23.0) ppm, $p < 0.001$ v 3.0 (0.5-5.0) ppm). Low values in both eNO and nNO readings (< 2.4 ppb and < 187 ppb, respectively) identified PCD patients from other bronchiectatic patients with a specificity of 98% and a positive predictive value of 92%. CONCLUSION: The simultaneous measurement of eNO and nNO is a useful screening tool for PCD.

11: Acta Otorhinolaryngol Belg. 2000;54(3):271-80.

Nasal nitric oxide.

Lefevere L, Willems T, Lindberg S, Jorissen M.

Nitric oxide (NO) has witnessed an explosion of interest of scientists all over the world during the last decade. This small gaseous molecule is produced in many systems such as the nervous system, cardiovascular system, the upper and lower airways. In all of these it contributes to a number of (patho)physiological processes. Concerning the airways, NO concentrations in the upper respiratory tract are much higher (i.e. ranging from 200 to 2000 parts per billion (ppb)) than NO levels in the lower respiratory tract (i.e. ranging from 4 to 160 ppb). NO is most frequently measured using a chemiluminescence method, based on a reaction of NO with O₃ resulting in the emission of light. In the airways NO exerts many functions in host defense, ciliary activity, inflammation and it is also an aerocrine messenger between the upper and lower airways. Nasal NO concentrations are influenced by age, physical exercise, smoking and certain drugs. Nasal NO is conveniently measured in all ages and can be used for screening of disease or monitoring the effects of treatment. Pathological conditions, as in allergic rhinitis, sinusitis, nasal polyps, cystic fibrosis and primary ciliary dyskinesia, result in altered nasal NO concentrations. The clinical relevance for measurement of nasal NO in different conditions, however, remains to be established.

12: Eur Respir J. 1999 Jan;13(1):114-8.

Effect of L-arginine infusion on airway NO in cystic fibrosis and primary ciliary dyskinesia syndrome.

Grasemann H, Gartig SS, Wiesemann HG, Teschler H, Konietzko N, Ratjen F.

Airway nitric oxide concentrations in patients with cystic fibrosis or primary ciliary dyskinesia syndrome have been shown to be lower than in healthy subjects. Decreased NO concentrations may contribute to impaired ciliary clearance, respiratory tract infections, or obstructive lung disease in these

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conditions. Nasal and exhaled NO concentrations were compared before and after infusion of 500 mg x kg⁻¹ L-arginine, the substrate of NO synthases, in 11 cystic fibrosis (CF) patients, seven primary ciliary dyskinesia (PCD) syndrome patients, and 11 control subjects. Baseline nasal and exhaled NO concentrations were significantly lower in both CF and PCD syndrome patients than in controls ($p < 0.01$). In controls, the maximum increase of NO was seen immediately after L-arginine infusion in the upper airways (1.8-fold) and 3 h after the infusion in the lower airways (1.4-fold). Although NO concentrations also increased significantly in both CF (1.9-fold and 1.6-fold, respectively) and PCD syndrome patients (1.4-fold and 1.8-fold, respectively), concentrations remained subnormal compared with baseline values of controls. Pulmonary function remained unchanged in both patient groups. In conclusion, the low airway nitric oxide formation in both cystic fibrosis and primary ciliary dyskinesia syndrome patients can be augmented by L-arginine administration. The finding that pulmonary function remained unchanged in both conditions may be due to the fact that normalization of airway nitric oxide concentrations could not be achieved.

13: Eur Respir J. 1999 Jun;13(6):1402-5.

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

Karadag B, James AJ, Gultekin E, Wilson NM, Bush A.

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 \pm 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.

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14: Eur Respir J. 1999 Feb;13(2):307-12.

Nasal nitric oxide concentration in paranasal sinus inflammatory diseases.

Arnal JF, Flores P, Rami J, Murriss-Espin M, Bremont F, Pasto I, Aguilla M, Serrano E, Didier A.

In normal upper airways, nitric oxide is generated by the paranasal sinus epithelium and then diffuses into the nasal cavities. This study examined whether or not nasal NO concentration is affected by paranasal sinus inflammatory diseases. The influence of obstruction (nasal polyposis) and/or inflammation (allergy or chronic sinusitis) of the paranasal sinuses on nasal NO concentration was evaluated in nasal allergic (n=7 patients) or nonallergic (n=20) polyposis, nonallergic chronic sinusitis (n=10) and Kartagener's syndrome (n=6) and compared with control subjects (n=42). A score of alteration of the paranasal sinus (number of altered and occluded sinuses) was determined by a computed tomography scan. The nasal NO concentration in nasal nonallergic polyposis (150±20 parts per billion (ppb)) was significantly decreased compared with both controls (223±6 ppb, p=0.01) and polyposis with allergy (272±28 ppb, p<0.0001). In each group, the nasal NO concentration was inversely correlated with the extent of tomodensitometric alteration of the paranasal sinuses. In Kartagener's syndrome, the nasal NO concentration (14±2 ppb) was drastically decreased compared with all other groups, despite the presence of open paranasal sinuses. Thus, the nasal NO concentration in patients with nasal polyposis appeared to be dependent on both the allergic status and the degree of obstruction of the paranasal sinuses.

15: Lancet. 1998 Aug 1;352(9125):371-2.

Effect of arginine on mucociliary function in primary ciliary dyskinesia.

Loukides S, Kharitonov S, Wodehouse T, Cole PJ, Barnes PJ.

16: Eur Respir J. 1994 Aug;7(8):1501-4.

Primarily nasal origin of exhaled nitric oxide and absence in Kartagener's syndrome.

Lundberg JO, Weitzberg E, Nordvall SL, Kuylensstierna R, Lundberg JM, Alving K.

The exact origin of nitric oxide (NO) in exhaled air is not known. We wanted to further investigate at what site exhaled NO is produced and to determine whether children with Kartagener's syndrome exhibited altered levels of exhaled NO. NO was measured by chemiluminescence technique in air sampled directly from the

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nose and in normally exhaled air of four children (2.5-13 years old) with Kartagener's syndrome, 20 healthy children, four healthy adults, and four conscious tracheostomized adults. NO was almost absent (98% reduced) in air sampled directly from the nose in four children with Kartagener's syndrome (4 +/- 1 parts per billion (ppb)), compared to age-matched controls (221 +/- 14 (ppb)). Tracheostomized adult subjects had considerably higher NO values in nasally (22 +/- 3 ppb) and orally (14 +/- 2 ppb) exhaled air, compared to levels in air exhaled through the tracheostomy (2 +/- 0 ppb). Treatment with intranasal corticosteroids for 14 days, or with antibiotics for 1 week, did not affect exhaled NO. These results clearly show that, basically, all NO in exhaled air of healthy subjects originates from the upper respiratory tract, with only a minor contribution from the lower airways. Furthermore, the absence of nasal NO in children with Kartagener's syndrome could be of use as a simple noninvasive diagnostic test.