

Nitric Oxide: A Promising Methodological Approach in Airway Diseases

G. Sahin^{a, b} L. Klimek^a J. Mullol^d K. Hörmann^b L.E. Walther^c O. Pfaar^{a, b}

^aCentre for Rhinology and Allergology, Wiesbaden, ^bDepartment of Otorhinolaryngology, Head and Neck Surgery, University Hospital Mannheim, Mannheim, and ^cORL Practice, Sulzbach (Taunus), Germany; ^dUnitat de Rinologia y Clínica de l'Olfacte, Department of Otorhinolaryngology, Hospital Clínic IDIBAPS, Barcelona, Spain

Key Words

Airway inflammation · Allergic rhinitis · Asthma · Chronic rhinosinusitis · Cystic fibrosis · Nasal polyps · Nitric oxide · Primary ciliary dyskinesia

Abstract

Nitric oxide (NO) is a potent biological mediator, and has a regulatory role in a wide variety of cellular and tissue functions. In the upper and lower airways, NO has been suggested to be involved in different functions with regulatory, protective, defensive or damaging effects. It is obvious that NO plays an important role in host defense, and is liberated in the nose and the paranasal sinuses. This review aims to highlight some aspects of the origin and function of NO in airway diseases, such as allergic rhinitis, chronic rhinosinusitis with and without nasal polyps, primary ciliary dyskinesia, and cystic fibrosis. In conclusion, NO measurement may be a promising noninvasive diagnostic marker for airway pathologies.

Copyright © 2011 S. Karger AG, Basel

Introduction

The physiological potential of nitric oxide (NO) in medicine was recognized during research in the cardiovascular area. In 1978, the pharmacologist Ferid Murad discovered that the substance NO, set free from organic nitrates, caused a vasodilatation of blood vessels [1]. In

1980, the pharmacologist Robert F. Furchgott found out that the endothelium of blood vessels produces an unknown substance that causes a relaxation of smooth muscles [2]. He named this substance endothelium-derived relaxing factor, which was only identified as NO in 1987 by two independent research teams [3, 4]. In 1998, Robert F. Furchgott, Ferid Murad and Louis J. Ignarro were awarded the Nobel Prize in Physiology or Medicine for their discoveries concerning NO as a signaling molecule in the cardiovascular system. Since 1991, when Gustafsson et al. [5] discovered that NO is present in exhaled breath of humans – and 2 years later, when Alving et al. [6] showed that exhaled NO is increased in patients with asthma – several aspects of exhaled and nasal NO have been studied [19]. Actually, the molecule NO was named ‘molecule of the year’ in 1992 by the journal *Science*.

Today, it is well known that NO plays a regulatory role in a broad range of cellular and tissue functions, e.g. vasoregulation, respiration, immune defense and others [19]. Further research has led to the understanding that nasal NO is affected by inflammation of the upper airways, and thus plays a significant role in host defense [7] and as a marker of inflammation.

Consequently, the measurement of NO in the upper airways seemed to be a simple non-invasive method for monitoring airway inflammation, and thus inspired a variety of further research studies. However, the possible use of nasal NO measurements in the diagnosis and treatment of airway diseases – such as allergic rhinitis, chron-

ic rhinosinusitis (CRS) with and without nasal polyps, primary ciliary dyskinesia (PCD) and cystic fibrosis (CF) – needs further evaluation because of the variable and also contradictory findings of nasal NO concentrations in these diseases. These contradictory findings seem to result from the complex anatomy of the upper airways on the one hand, and the various physiological properties of NO on the other hand – the lack of consensus on measurement techniques has also been an obstacle. This review aims to outline the potential future role of nasal NO in the diagnosis and management of airway diseases, and its acceptance into the everyday clinical life of physicians.

Origin of NO

In the atmosphere, the free radical NO is a colorless, odorless, poisonous gas [8]. As a result of its chemical structure being extremely reactive and having a very short half-life of a few seconds and a diffusion capacity in tissue of 200 μm , it is evident that NO production requires a carefully controlled enzymatic system. NO synthase is regulated through complex mechanisms of activation and inactivation [9]. In biological tissues, NO is synthesized from oxygen and the amino acid L-arginine by the enzyme nitric oxide synthase (NOS) [6, 7, 10]. Up until now, three isoforms of NOS have been described and named after the tissue in which they were first identified [10–13]. Two of these are constitutive NOS (cNOS), and are found in endothelial cells (eNOS or type 3) and in non-adrenergic non-cholinergic nerves (nNOS or type 1), respectively. nNOS and eNOS are calcium-calmodulin dependent, and are activated rapidly and transiently in response to a calcium signal [14]. Thus, the NO production is of small amounts and provides a rapid pulse-like signal. The third NO synthase is inducible (iNOS or type 2) through pro-inflammatory cytokines – such as tumor necrosis factor and interleukin-1 β as well as through bacterial lipopolysaccharides – and is found in phagocytes (monocytes, macrophages, neutrophils) [15–17]. Other than eNOS and nNOS the synthesis of iNOS is calcium-calmodulin independent, and once activated it produces up to 1,000 times more NO for prolonged periods [11, 18]. Besides the enzymatic NO synthesis, an alternative non-enzymatic pathway for NO production has been described [19].

It has been shown that in physiological states the major source of NO production in the airways originates from the nose and the paranasal sinuses, with only a minor contribution from the lower parts [6]. The NO concentration in the lower airways is reported to be 20 times lower

than in the upper airways in normal subjects [20]. However, the highest NO production site has been identified as the paranasal sinuses [6, 21–27], rather than the mucosa of the nasal cavity. In agreement with this, measurements in tracheotomized individuals obviously show that more than 90% of all exhaled NO comes from the nasal region [25, 27, 28]. On the other hand, other studies have suggested that sinuses only partly contribute to nasal NO production [19]. In fact, the occlusion of the ostiomeatal complex as well as the sphenoethmoidal recess reduced nasal NO concentrations by only around 12% in healthy volunteers, which indicates only a marginal role of the paranasal sinuses as a source of nasal NO [19, 29]. Therefore, the exact place of NO generation has not yet been decided, although the NO measured in exhaled air appears to be produced superficially in the mucosa [19].

All three NOS isoforms have been identified in the upper airways – the low-output constitutive NOS (nNOS and eNOS, both calcium-calmodulin dependent and steroid insensitive) and the high-output iNOS (classically calcium-calmodulin independent and steroid sensitive) [21, 22].

Immunohistochemical and *in situ* mRNA hybridization experiments verified the high occurrence of eNOS in healthy sinus epithelium, while this enzyme appears much less in the nasal mucosa [19, 84]. Several cells in the upper airways are supposed to express nNOS and eNOS, including parasympathetic neurons that innervate nasal vessels, endothelial cells, and ciliated epithelial cells [30]. iNOS has been detected in epithelial cells, macrophages, neutrophils, endothelial and vascular smooth muscle cells [30–32].

In healthy upper airways, NO is supposed to be continuously produced via iNOS by the ciliated cells of the paranasal sinuses and nasal mucosa, and diffuses through the ostiomeatal complex to the nasal cavity. Thus, it is believed that the level of measured NO in the nasal air mainly depends on its production and the patency and size of the paranasal ostia [33].

Function of NO

As a result of its extreme reactivity and its direct and indirect effects, NO is involved in several regulatory functions – such as vasodilatation [3, 4] (as a result of smooth muscle relaxation), neuronal transmission, and inhibition of platelet aggregation. Furthermore, it could be shown that NO represents a first line of defense against microorganisms through its antiviral [34] and antibacterial [35]

activity, as well as by its upregulation of ciliary motility within the paranasal sinuses [11, 36, 37]. These findings could be supported by the fact that application of NO donors in nasal mucosa, such as L-arginine in vitro and sodium nitroprusside in vivo, significantly stimulated ciliary beat frequency [38]. On the other hand, NOS inhibitors have been shown to reverse the increase in ciliary beat frequency, which can be restored by sodium nitroprusside and L-arginine [38]. The role of NO as a biomarker in eosinophil inflammation could be demonstrated in several studies with asthmatics [6]. Consequently, NO measurement has developed both in the diagnosis and management of asthma to a standardized method.

In subjects without lung disease, Lundberg et al. [39, 40] have shown that compared with mouth breathing, nasal breathing reduces pulmonary vascular resistance and improves oxygen saturation. Also, infusion of nasal air into the tube of intubated patients improved their oxygen uptake [11, 40]. Furthermore, Lundberg et al. [40] have reported that the 'addition of 100 ppb NO during oral breathing mimicked the effect of nasal breathing, whereas moistened air during oral breathing had no effect'. They also added that 'some investigators could find clear effects on arterial oxygenation and pulmonary arterial pressure using concentrations of inhaled NO as low as 10–100 ppb' [28, 41, 42]. Other authors have demonstrated that endogenous NO produced by the paranasal sinuses is inhaled at similar concentrations during normal breathing [43–45]. These results led to the knowledge that NO derived from the upper airways is capable of improving ventilation/perfusion matching. This effect of endogenous NO in the human respiratory tract, derived from the upper airways, was termed 'aerocrine' to illustrate the airborne transport of a biological messenger [39]. Since it is known that exogenous NO gas delivered to inhaled air dilates vessels in the lung, leading to increased oxygen uptake and a reduction in pulmonary vascular resistance [46], the vasodilating effect of NO is used for the treatment of pulmonary hypertension [40, 47].

Thus, in the airways, NO provides many functions in host defense, ciliary activity and inflammation, and it is also supposed to be an aerocrine messenger between the upper and lower airways.

NO Measurement Methods

Exhaled NO has been proposed as a useful non-invasive marker of lower airway inflammation, and recommendations for standardization of measurement of both

exhaled and nasal NO have been given by the American Thoracic Society and the European Respiratory Society. There are two different methods for the measurement of NO. The most common method, regarded as the gold standard, is the chemiluminescence technique based on the photochemical reaction between NO and ozone generated in the analyzer. The stationary chemiluminescence analyzers, used in most of the published studies, are expensive. Recently, new handheld devices for NO measurement have been developed. These new analyzers, using an electrochemical sensor technology for NO measurement, have been tested in several studies. Comparisons of the stationary chemiluminescence device and the portable electrochemical analyzers for reproducibility and correlation have shown good results in both healthy subjects and patients with upper and lower airway diseases [48–50].

NO can be measured online during slow expiration or offline from samples of exhaled air collected in bags. The former method is applicable to patients who are able to cooperate; the latter method is particularly useful in children aged under 4 years.

The determination of reference values for a population is difficult because of the several interfering factors such as age, gender, race, smoking, dietary factors, medication, circadian rhythm and seasonal variation. In the largest study so far on reference values (involving 3,376 adults), Olin et al. [51] set a level of 24.0–54.0 ppb for exhaled NO, depending on age and height. Nasal NO levels are known to be higher, but there have been varying reports of the exact level. Struben et al. [52] reported a mean nasal NO level of 449 ppb in 340 investigated healthy children aged between 6 and 17 years. Similar results were demonstrated by Daya et al. [53] on a small number of children. Other authors have reported much higher mean nasal NO levels of between 898 and 1,024 ppb in children [54, 55]. The reported nasal NO levels in adults vary between 394 and 996 ppb [26, 56]. Studies on standardized reference values for nasal NO with large numbers of patients are not available.

The less expensive and more reliable handheld electrochemical analyzers have turned out to be a valuable addition to daily clinical practice, and will hopefully make NO measurement more commonly used and refined.

NO in Allergic Rhinitis

Allergic rhinitis is an IgE-triggered nasal inflammatory disorder involving the release of inflammatory mediators, as well as activation and tissue recruitment. Recently, Guilemany et al. [57] found out that in patients

with persistent allergic rhinitis, the levels of nasal NO correlated with the loss of smell – suggesting a mixed etiology (obstruction/inflammation) for smell disorders in allergic rhinitis.

Today, a range of approaches – such as nasal lavage, epithelial cytological examination and nasal biopsy – exist to monitor nasal airway inflammation. Recently, measurement of NO in nasal exhaled air as an indirect marker of allergic inflammation has been proposed. However, the role of NO in nasal allergic inflammation remains quite controversial. Some authors have shown increased nasal NO levels after exposure to allergens [58–62]. In the nasal lavage fluid of these patients, an overexpression of iNOS [30] and also an increase in nitrites and nitrates, derived from NO metabolism, have been noticed [19, 63]. Therefore, it is assumed that in an allergic inflammatory microenvironment, pro-inflammatory cytokines and ‘oxidative stress’ might upregulate the production of iNOS-derived NO.

On the other hand, other studies have observed no differences between patients with allergic rhinitis and controls. For instance, Palm et al. [64] found no change in nasal NO levels of patients with allergic rhinitis, whereas exhaled oral NO was increased. Also, other groups have not been successful in showing an evident increase in nasal NO in seasonal rhinitis, during as well as out of the pollen season [59, 65]. Similar results were observed by Valero et al. [66]. Although they reported an increase in nasal NO (of at least 1 h in duration) in subjects with asthma and rhinitis, no relationship between the pre-exercise and post-exercise values of nasal and exhaled NO and the bronchial and nasal responses was found. These contradictory results might be explained as a result of the biochemical pathway of NO. As we know, L-arginine and oxygen (O₂) are synthesized to NO by NOS. On the other hand L-arginine is also metabolized to L-ornithine and urea by arginase. In inflammatory conditions, cytokines might induce an increased activity of arginase, with the result of reducing the bioavailability of L-arginine for iNOS. As L-arginine is a substrate for arginase as well as for NOS, these enzymes might influence each other’s activity through substrate competition and might be a reason for the contradictory results. In some experimental studies, substrate competition between arginase and NOS was demonstrated [67–69], but it has not yet been proven that this competition affects measured NO levels in airways. Methodological differences in NO measurement between several studies might also be a reason for conflicting findings. Nevertheless, the validation of nasal NO as a marker of upper airway inflammation is eagerly anticipated.

NO in CRS with Nasal Polyps

Nasal polyposis is considered to be a chronic inflammatory condition in the nasal and paranasal sinus cavities, with symptoms such as nasal obstruction, anterior and posterior rhinorrhea, sneezing, loss of smell and facial pain [77]. It usually occurs in association with CRS, but the precise etiology of nasal polyps still remains unclear. It is supposed that multiple factors – such as immunological disorders, bacterial and viral infections, hormonal imbalance and dysfunction of the autonomic nervous system – may be involved in polyp formation. In nasal polyposis, high eosinophil recruitment has been shown – accordingly high levels of iNOS expression and activity have been found. Hence, it would seem likely that higher epithelial iNOS levels in nasal polyposis would be associated with higher nasal NO levels by upregulation. However, interestingly in these patients, nasal NO levels were lower compared to controls or to individuals with uncomplicated allergic rhinitis [70–73] and exhaled NO was increased, even in non-atopic patients [72, 74]. In patients with bronchiectasis, nasal NO was lower in CRS with nasal polyposis than in CRS without nasal polyposis, and inversely correlated with the occupancy of the ostiomeatal complex by CT scans in patients with nasal polyps [71]. These apparently controversial results are explained due to the blockade of the ostiomeatal complex, and to the consequent inability of newly generated NO to reach the nasal cavity. Actually, an inverse correlation between nasal NO levels and the extent of sinus disease and the number of occluded paranasal sinuses has been reported [71, 75, 76]. The hypothesis of occluded sinus ostia was supported by the fact that after medical [75, 77] and surgical [75] treatment of nasal polyps, nasal NO levels were significantly elevated. Especially in surgically treated patients, nasal NO levels were more elevated than in subjects who underwent pharmacological therapy [19]. Consequently, it was then suggested that nasal NO levels might constitute an objective and non-invasive assessment of nasal polyposis severity [72, 75, 76], and might also be used as an objective evaluation of polyposis therapy.

It has also been proposed that low levels of nasal NO detected in patients with nasal polyps might predispose sufferers to frequent sinus infections as NO of the respiratory tract is known to be a first-line host defense against inhaled micro-organisms.

Increased NO levels in the sinuses may be related to a sufficient host defence. On the other hand low NO levels may lead to a higher degree of vulnerability resulting in sinus infections.

NO in CRS without Nasal Polyps

CRS is an inflammatory disease of the nose and sinus mucosa. Similarly to nasal polyposis, reduced levels of nasal NO and iNOS expression have been reported in both acute and chronic rhinosinusitis [78–80]. It was demonstrated that due to a downregulation of iNOS within the ciliated epithelial cells of the maxillary sinus, NO concentration was reduced in the mucosa of patients with maxillary sinusitis [79, 81]. Accordingly, maxillary NO concentrations were found to be increased following sinusitis treatment [75, 80]. However, it is still uncertain whether the low NO levels in sinusitis are due to reduced maxillary NO production or result from a reduced passage of NO from the paranasal sinuses to the nasal cavity. Indeed local edema, nasal congestion and mucus accumulation – as seen in acute and chronic rhinosinusitis – might lead to a partial blockade of sinus ostia. On the other hand, the hypothesis suggesting reduced maxillary NO synthesis is supported by evidence that cytokines, which are known to reduce iNOS expression (both in vitro and in vivo) [82, 83], were found in the maxillary sinus mucosa of CRS patients. However, reported results regarding NO levels in sinusitis are also controversial. In fact, in two studies, no differences in nasal NO levels of patients affected by CRS were demonstrated [76, 84]. In other studies, NO metabolites in the maxillary or nasal lavage fluid of CRS patients were examined. Naraghi et al. [85] found out that NO metabolites were significantly higher in maxillary sinuses of patients with CRS. It was suggested that elevated levels of NO and NO metabolites in rhinosinusitis might damage healthy sinus epithelium. On the other hand, Deroe et al. [86] found that NO metabolite levels were decreased in nasal lavages of CRS patients, and reached normal levels after surgery along with improvement in the disease. They suggested that NO metabolite levels might be used as an indicator for the follow-up of patients after endoscopic sinus surgery.

In any case, it is now widely accepted that high local NO concentrations in airways and paranasal sinuses probably play an important role in protecting sinuses against disease and infections by reducing pathogen colonization and infection. The role of nasal NO in rhinosinusitis patients could be outlined as an indicator for disease monitoring, as well as for follow-up after medical and surgical treatments.

NO in Primary Ciliary Dyskinesia

PCD is a genetic disease characterized by abnormalities in ciliary structure and motility leading to progressive sinopulmonary disease [87]. It is associated with situs inversus in 50% of cases [94]. Clinical presentations may include neonatal respiratory distress, recurrent lower respiratory tract infections, CRS and male infertility. As diagnostic delay and inadequate therapy may result in a poor outcome, early diagnosis is required, including identification of ciliary dysmotility and specific electron microscopic analysis of ciliary ultrastructural defects. A distinction between primary and secondary ciliary abnormalities should be undertaken. Currently, the diagnosis of PCD is being delayed or missed or made incorrectly due to the lack of a simple screening test, since only a few specialized centers with equipment and expertise to measure ciliary beat frequency and to assess ciliary ultrastructure are available. Therefore, few patients seem to benefit from available preventive therapy [90].

Since Lundberg et al. [25] described very low levels of nasal NO in children with PCD, several other authors confirmed this phenomenon [76, 87–96]. NO levels in PCD were low despite the presence of open paranasal sinuses apparent on CT scan [76]. Karadag et al. [89] reported that in most children with PCD, nasal as well as exhaled NO levels were reduced. Therefore, measurement of NO as a relative simple screening tool has been proposed [90, 93]. Accordingly, nasal NO measurement is widely accepted as a screening test for PCD since specificity and sensitivity are very high [91]. However, the presence of normal exhaled and nasal NO concentration in the absence of lifelong oto-sino-pulmonary disease rules out PCD with a high degree of probability. Depending on measuring techniques, the normal range for nasal NO varies between 450 and 900 ppb [97]; for exhaled NO, measured normal levels range between 5 and 20 ppb for adults and 5 and 25 ppb for children [97]. Narang et al. [93] confirmed that in their study population nNO levels of <250 ppb were strongly suggestive of PCD; an exhaled NO level of >6 ppb excluded the diagnosis. Nevertheless, they concluded that exhaled NO cannot be used to distinguish between individual children with most chronic respiratory diseases, and therefore is less diagnostically useful. They added that these data were obtained in children and should not be extrapolated into the adult population, and confirmation of the diagnosis of PCD should always require further testing. Other authors suggest investigations for PCD at nasal NO levels <100 ppb [90, 94].

Table 1. Nasal NO levels in different upper and lower airway diseases compared to controls

Disease	First author	nNO level in ppb patients	nNO level in ppb controls	Number of patients
Allergic rhinitis	Arnal, 1997 [58]	right nose: 382 ± 20 left nose: 396 ± 28	right nose: 235 ± 11 left nose: 225 ± 9	controls n = 19; patients n = 36
	Kharitonov, 1997 [60]	$1,527 \pm 87$	996 ± 39	controls n = 46; patients n = 12
	Palm, 2003 [64]	160 ± 75	153 ± 36	controls n = 18; patients n = 18
	Henriksen, 1999 [59]	1,105 (median)	1,014 (median)	controls n = 12; patients n = 46
	Colantonio, 2002 [70]	659.8 ± 304.8	740.9 ± 148.1	controls n = 20; patients n = 44
Nasal polyps	Colantonio, 2002 [70]	Significantly low NO levels compared to controls or allergic rhinitis, rise of NO levels after conservative or surgical treatment	740.9 ± 148.1	controls n = 20; patients n = 23
	Gilain, 2002 [72]	596.4 ± 102.06	$2,251.6 \pm 288.6$	controls n = 21; patients n = 18
	Guilemany, 2009 [71]	347 ± 62	no controls	no controls; patients n = 22
	Arnal, 1999 [76]	150 ± 20	223 ± 6	controls n = 42; patients n = 20
CRS	Lindberg, 1997 [78]	96.4 ± 72.8	233.2 ± 66.8	controls n = 66; patients n = 12
	Baraldi, 1997 [80]	70 ± 8.7	245 ± 15	controls n = 16; patients n = 16
	Deja, 2003 [79]	31 ± 10	$2,554 \pm 385$	controls n = 11; patients n = 11
	Arnal, 1999 [76]	172 ± 30	223 ± 6	controls n = 42; patients n = 10
PCD	Arnal, 1999 [76]	14 ± 2	223 ± 6	controls n = 42; patients n = 6
	Lundberg, 1994 [25]	4 ± 1	221 ± 14	controls n = 20; patients n = 4
	Noone, 2004 [94]	19 ± 17	376 ± 124	controls n = 27; patients n = 64
	Wodehouse, 2003 [90]	64 ± 36.6	759 ± 145.8	controls n = 16; patients n = 42
	Karadag, 1999 [89]	97 ± 193 eNO 2.17 ± 1.18	664 ± 298 eNO 5.94 ± 3.49	controls n = 60; patients n = 21
	Narang, 2002 [93]	60.3 eNO 2.0	716 eNO 4.85	controls n = 53; patients n = 31
CF	Narang, 2002 [93]	491.3 eNO 2.6	716 eNO 4.85	controls n = 53; patients n = 17
	Grasemann, 1999 [88]	438 ± 225 eNO 2.3 ± 1.4	$1,195.5 \pm 618.2$ eNO 4.7 ± 2.2	controls n = 11; patients n = 11
	Balfour-Lynn, 1996 [55]	460 eNO 4.7	1,024 eNO 4.8	controls n = 57; patients n = 50
	Grasemann, 1997 [104]	eNO 5.9 ± 2.6	eNO 9.1 ± 3.6	controls n = 30; patients n = 27
	Wodehouse, 2003 [90]	447.5 ± 162.6	759 ± 145.8	controls n = 16; patients n = 15

As demonstrated in several studies, exhaled and nasal NO levels are altered in certain disorders. Nasal NO is two- to threefold higher than NO from lower airways. High exhaled or nasal NO levels are consistent with eosinophilic airway inflammation. Very low exhaled or nasal NO values on the other hand may be an in-

dicator for primary ciliary dyskinesia or cystic fibrosis. NO values are not disease specific, but may be clinically useful in the diagnosis and treatment monitoring. In table 2, exhaled and nasal NO values for children and adults with clinical aspects are collocated.

The reason for the low nasal NO in PCD is still not completely understood. The low levels of nasal NO in PCD may be due to several possible reasons – primarily, impaired NO synthesis. As already mentioned, it has been shown that the application of NO donors into the

human nasal mucosa stimulates ciliary beat [98], and that low NO levels result in an impaired muco-ciliary function in the upper airways [19, 99]. In fact, Loukides et al. [100] demonstrated that in patients with PCD, L-arginine (a substrate for NO production) induced an increase in

Table 2. Clinical relevance of exhaled and nasal NO values in both adults and children, taken from [97]

Exhaled NO (ppb)			Nasal NO (ppb)	
adults	children	clinical relevance	adults/children	clinical relevance
5–20	5–25	normal/absence of significant eosinophilic inflammation	<100	very low: consider primary ciliary dyskinesia or cystic fibrosis
25–50	20–35	consistent with mild eosinophilic airway inflammation	<450	low: may reflect obstruction at the sinus ostium
>50	>35	consistent with significant eosinophilic airway inflammation	450–900	normal range; does not exclude significant nasal disease
			>900	consistent with nasal mucosal inflammation, sinuses probably patent

nasal NO levels, as well as improved ciliary beating frequency and nasal mucociliary clearance.

Another hypothesis suggests a genetic origin [101]. The hypothesis states there might be a close linkage between the gene for PCD and the gene for iNOS, with co-inheritance of defects [93] resulting in a reduced or absent production of NO. To date, 6 genes have been clearly linked with PCD [102]. A further hypothesis arises from the observation of low NO production from myocytes in patients with Duchenne's muscular dystrophy (DMD) [93]. To date, it is known that DMD is associated with decreased total NO, despite the fact that muscles of DMD patients retain all three NOS isoforms with an upregulation of the inducible NOS isoform [103]. However, the mechanisms leading to NO deficiency with progressive muscle cell degeneration remain still unclear [103]. Like myocytes, cilia also contain mechanochemical ATPases, and it is thus possible that a loss of ciliary function in PCD patients could cause a reduction in NOS activity resulting in low NO production.

Efforts to recognize the disease better and earlier, and to standardize clinical care for the problems associated with the disease, should be made. New handheld devices with the ability to measure NO even in babies are promising in this regard. NO could be a simple non-invasive screening test for PCD, but nasal NO measurement is still not standardized and available in the clinical setting, and it does not replace the evaluation of ciliary beat frequency on nasal brushing or biopsy to confirm the diagnosis and elucidate its ultrastructural nature. For a reliable PCD diagnosis either a ciliary ultrastructure analysis or a genetic mutation analysis or both are required.

NO in Cystic Fibrosis

CF is a disorder caused by a variety of mutations in the CF transmembrane regulator (*CFTR*) gene. The pathogenesis of this disease is still not fully understood, and despite great improvements in diagnosis and therapy the outcome is poor. Patients experience recurrent respiratory tract infections, and their paranasal sinuses are chronically infected and often blocked. Infections are associated with neutrophil inflammation, and there are elevated concentrations of several cytokines in both sputum and bronchoalveolar lavage. Considering the inflammatory nature of cystic fibrosis, it is surprising that nasal NO levels are not as low as in PCD, albeit still very low [104]. Accordingly, iNOS expression was reduced in both the upper and the lower airways of CF patients, and cytokines did not induce any increase in iNOS mRNA in CF cells [105, 106]. The mechanism underlying this defect is unclear. It is supposed that a cellular protein metabolism is one reason [107]. Other possible reasons – such as impaired diffusion, increased metabolism of NO [108, 109], destruction of epithelial cells [109] and defects in ciliary function [93] – were discussed for reduced nasal NO levels in CF. Kelley and Drumm [106] have demonstrated that the NOS activity is impaired in CF airways. As a consequence of the reduced NO concentration in CF airways, defensive mechanisms against host infections and mucociliary clearance might be disturbed [55]. Hence, some authors [97] suggest that nasal NO measurement could possibly be recommended in screening for CF. However, the NO metabolism in CF airways is complex and not yet completely understood, and is therefore of limited value in the diagnosis of CF. The gold standard in CF diagnosis is still the sweat chloride test.

NO Levels and Their Clinical Relevance

Table 1 displays nasal NO levels in different upper and lower airway diseases compared to controls, while table 2 explains the clinical relevance of such levels.

Conclusion

NO plays an important role in the pathophysiology of the airways. Therefore, NO measurement has been suggested and partly established as a valuable tool for airway diseases, as it is a simple and completely non-invasive method that provides instant results.

New portable and reliable NO analyzers using an electrochemical sensor are becoming less expensive and more easily available. The possibility of taking NO measurements in children under 5 years of age is also very promising for detecting PCD in the early stages.

In the diagnosis and management of asthma, exhaled NO is of great value as it is a valid and reproducible non-invasive marker with a high specificity for the disease in both children and adults.

Pathological conditions – such as allergic rhinitis, CRS with or without nasal polyps, and CF – result in altered nasal NO concentrations. In rhinosinusitis and nasal polypsis, NO might serve as an indicator of disease severity, as well as being used during follow-up to gauge improvement after medical and surgical therapy. Nasal NO measurement in CF could be recommended for screening and monitoring of inflammation. The clinical importance for nasal NO measurement in the above mentioned diseases should be further established.

In disorders such as PCD, nasal NO measurement represents a useful screening tool with high specificity and sensitivity, and will hopefully be better available in general routine healthcare once it is standardized.

In this regard, future studies will be primarily aimed at better exploiting the different factors affecting the nasal levels of NO and its involvement in biological and pathological mechanisms in order to assist the diagnosis, treatment and monitoring of some relevant respiratory disorders.

References

- 1 Murad F, Mittal CK, Arnold WP, Katsuki S, Kimura H: Guanylate cyclase: activation by azide, nitro compounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin. *Adv Cyclic Nucleotide Res* 1978;9:145–158.
- 2 Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288:373–376.
- 3 Ignarro L, Buga G, Wood K, Byrns R, Chaudhuri G: Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 1987;84:9265–9269.
- 4 Palmer RM, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987;327:524–526.
- 5 Gustafsson I, Leone A, Persson M, Wiklund N, Moncada S: Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem Biophys Res Commun* 1991;181:852–857.
- 6 Alving K, Weitzberg E, Lundberg JM: Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 1993;6:1368–1370.
- 7 Moncada S, Higgs A: The L-arginine-nitric oxide pathway. *N Engl J Med* 1993;329:2002–2012.
- 8 Nathan C, Xie QW: Nitric oxide synthases: roles, tolls, and controls. *Cell* 1994;78:915–918.
- 9 Kelm M: Nitric oxide metabolism and breakdown. *Biochim Biophys Acta* 1999;1411:273–289.
- 10 Nathan C: Nitric oxide as a secretory product of mammalian cells. *FASEB J* 1992;6:3051–3064.
- 11 Djupesland P, Chatkin J, Qian W: Nitric oxide in the nasal airway: a new dimension in otorhinolaryngology. *Am J Otolaryngol* 2001;22:19–32.
- 12 Förstermann U, Closs E: Nitric oxide synthase isoenzymes: characterisation, purification, molecular cloning, and functions. *Hypertension* 1994;23:1121–1131.
- 13 Nathan C: Inducible nitric oxide synthase: what difference does it make? *J Clin Invest* 1997;100:2417–2423.
- 14 Nathan C, Xie QW: Regulation of biosynthesis of nitric oxide. *J Biol Chem* 1994;269:13725–13728.
- 15 Hibbs JB Jr: Infection and nitric oxide. *J Infect Dis* 2002;18(suppl 1):S9–S17.
- 16 Rimele TJ, Sturm RJ, Adams LM, Henry DE, Heaslip RJ, Weichman BM, Grimes D: Interaction of neutrophils with vascular smooth muscle: identification of a neutrophil-derived relaxing factor. *J Pharmacol Exp Ther* 1988;245:102–111.
- 17 Jorens PG, Van Overveld FJ, Bult H, Vermeire PA, Herman AG: L-Arginine-dependent production of nitrogen oxides by rat pulmonary macrophages. *Eur J Pharmacol* 1991;200:205–209.
- 18 Lundberg JO, Weitzberg E, Rinder J, Rudehill A, Jansson O, Wiklund NP, et al: Calcium-independent and steroid-resistant nitric oxide synthase activity in human paranasal sinus mucosa. *Eur Respir J* 1996;9:1344–1347.
- 19 Maniscalco M, Sofia M, Pelaia G: Nitric oxide in upper airways inflammatory diseases. *Inflamm Res* 2007;56:58–69.
- 20 Kharitonov S, Alving K, Barnes PJ: Exhaled and nasal nitric oxide measurements: recommendations. The European Respiratory Society Task Force. *Eur Respir J* 1997;10:1683–1693.
- 21 Lundberg J, Rinder J, Weitzberg E, Lundberg JM, Alving K: Nasally exhaled nitric oxide in humans originates mainly in the paranasal sinuses. *Acta Physiol Scand* 1994;152:431–432.
- 22 Lundberg JO, Farkas-Szallasi T, Weitzberg E, Rinder J, Lidholm J, Anggaard A, Hökfelt T, Lundberg JM, Alving K: High nitric oxide production in human paranasal sinuses. *Nat Med* 1995;1:370–373.

- 23 Kimberly B, Nejadnik B, Giraud GD: Nasal contribution to exhaled nitric oxide at rest and during breathholding in humans. *Am J Resp Crit Care Med* 1996;153:829–836.
- 24 Dillon WC, Hampl V, Shultz PJ, Rubins JB, Archer SL: Origins of breath nitric oxide in humans. *Chest* 1996;110:930–938.
- 25 Lundberg J, Weitzberg E, Nordvall S: Primarily nasal origin of exhaled nitric oxide and absence in Katagener's syndrome. *Eur Resp J* 1994;7:1501–1504.
- 26 Schedin U, Frostell C, Persson MG, Jakobsson J, Andersson G, Gustafsson LE: Contribution from upper and lower airways to exhaled endogenous nitric oxide in humans. *Acta Anaesthesiol Scand* 1995;39:327–332.
- 27 Kramer MF, Olzowy B, Bihler A, de la Motte D, Nowak D, Jorres RA, Dressel H: Exhaled and nasal nitric oxide in laryngectomized patients. *BMC Pulm Med* 2010;10:4.
- 28 Lundberg JO, Weitzberg E: Nasal nitric oxide in man. *Thorax* 1999;54:947–952.
- 29 Haight J, Djupesland P, Qjan W, Chatkin J, Furlott H, Irish H: Does nasal nitric oxide come from the sinuses? *Otolaryngol* 1999;28:197–204.
- 30 Kawamoto H, Takeno S, Yajin K: Increased expression of inducible nitric oxide synthase in nasal epithelial cells in patients with allergic rhinitis. *Laryngoscope* 1999;109:2015–2020.
- 31 Kawamoto H, Takumida M, Takeno S, Watanabe H, Fukushima N, Yajin K: Localization of nitric oxide synthase in human nasal mucosa with nasal allergy. *Acta Otolaryngol Suppl* 1998;539:65–70.
- 32 Giannessi F, Fattori B, Ursino F, Giambelluca MA, Soldani P, Scavuzzo MC, Ruffoli R: Ultrastructural and ultracytochemical study of the human nasal respiratory epithelium in vasomotor rhinitis. *Acta Otolaryngol* 2003;123:943–949.
- 33 Kirihe RK, Rees G, Wormald PJ: The influence of the size of the maxillary sinus ostium on the nasal and sinus nitric oxide levels. *Am J Rhinol* 2002;16:261–264.
- 34 Croen KD: Evidence for antiviral effect of nitric oxide: inhibition of herpes simplex virus type 1 replication. *J Clin Invest* 1993;91:2446–2452.
- 35 Mancinelli RL, McKay CP: Effects of nitric oxide and nitrogen dioxide on bacterial growth. *Appl Environ Microbiol* 1983;46:198–202.
- 36 Jain B, Rubinstein I, Robbins RA, Leise KL, Sisson JH: Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. *Biochem Biophys Res Commun* 1993;191:83–88.
- 37 Alberty J, Stoll W, Rudack C: The effect of endogenous nitric oxide on mechanical ciliostimulation of human nasal mucosa. *Clin Exp Allergy* 2006;36:1254–1259.
- 38 Runer T, Lundberg S: Effects of nitric oxide on blood flow and mucociliary activity in the human nose. *Ann Otol Rhinol Laryngol* 1998;107:40–46.
- 39 Lundberg Jo, Lundberg JM, Settergreen G, Alving K, Weitzberg E: Nitric oxide, produced in the upper airways, may act in an 'aerocrine' fashion to enhance pulmonary oxygen uptake in humans. *Acta Physiol Scand* 1995;155:467–468.
- 40 Lundberg JO, Settergren G, Gelinder S, Lundberg JM, Alving K, Weitzberg E: Inhalation of nasally derived nitric oxide modulates pulmonary function in humans. *Acta Physiol Scand* 1996;158:343–347.
- 41 Gerlach H, Rossaint R, Pappert D, Falke KJ: Time-course and dose-response of nitric oxide inhalation for systemic oxygenation and pulmonary hypertension in patients with adult respiratory distress syndrome. *Eur J Clin Invest* 1993;23:499–502.
- 42 Puybasset L, Roubly JJ, Mourgeon E, Stewart TE, Cluzel P, Arthaud M, Poète P, Bodin L, Korinek AM, Viars P: Inhaled nitric oxide in acute respiratory failure: dose-response curves. *Intensive Care Med* 1994;20:319–327.
- 43 Busch T, Kuhlen R, Knorr M, Kelly K, Lewandowski K, Rossaint R, Falke KJ, Gerlach H: Nasal, pulmonary and autoinhaled nitric oxide at rest and during moderate exercise. *Intensive Care Med* 2000;26:391–399.
- 44 Gerlach H, Rossaint R, Pappert D, Knorr M, Falke KJ: Autoinhalation of nitric oxide after endogenous synthesis in nasopharynx. *Lancet* 1994;343:518–519.
- 45 Tornberg DC, Marteus H, Schedin U, Alving K, Lundberg JO, Weitzberg E: Nasal and oral contribution to inhaled and exhaled nitric oxide: a study in tracheotomized patients. *Eur Respir J* 2002;19:859–864.
- 46 Frostell C, Fratacci MD, Wain JC, Jones R, Zapol WM: Inhaled nitric oxide: a selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation* 1991;83:2038–2047.
- 47 Roberts JD Jr, Fineman JR, Morin FC 3rd, Shaul PW, Rimar S, Schreiber MD, Polin RA, Zwass MS, Zayek MM, Gross I, Heymann MA, Zapol WM: Inhaled nitric oxide and persistent pulmonary hypertension of the newborn. The Inhaled Nitric Oxide Study Group. *N Engl J Med* 1997;336:605–610.
- 48 Alving K, Janson C, Nordvall L: Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. *Respir Res* 2006;7:67.
- 49 Menzies D, Nair A, Lipworth BJ: Portable exhaled nitric oxide measurement: comparison with the 'gold standard' technique. *Chest* 2007;131:410–414.
- 50 Khalili B, Boggs PB, Bahna SL: Reliability of a new hand-held device for the measurement of exhaled nitric oxide. *Allergy* 2007;62:1171–1174.
- 51 Olin A, Bake B: Fraction of exhaled nitric oxide at 50 ml/s: reference values for adult lifelong never-smokers. *Chest* 2007;131:1852–1856.
- 52 Struben VM, Wieringa MH, Mantingh CJ, Bommeljé C, Don M, Feenstra L, de Jongste JC: Nasal NO: normal values in children age 6 through to 17 years. *Eur Respir J* 2005;26:453–457.
- 53 Daya H, Qian W, McClean P, Haight J, Zamel N, Papsin BC, Forte V: Nasal nitric oxide in children: a novel measurement technique and normal values. *Laryngoscope* 2002;112:1831–1835.
- 54 Cobos Barroso N, Reverté Bover C, Gartner S, Liñán Cortés S, Quintó Domech L: Exhaled and nasal nitric oxide in normal and asthmatic children (in Spanish). *An Esp Pediatr* 1998;49:241–247.
- 55 Balfour-Lynn IM, Lavery A, Dinwiddie R: Reduced upper airway nitric oxide in cystic fibrosis. *Arch Dis Child* 1996;75:319–322.
- 56 Kharitonov SA, Chung KF, Evans D, O'Connor BJ, Barnes PJ: Increased exhaled nitric oxide in asthma is mainly derived from the lower respiratory tract. *Am J Respir Crit Care Med* 1996;153:1773–1780.
- 57 Guilemany JM, García-Piñero A, Alobid I, Cardelús S, Centellas S, Bartra J, Valero A, Picado C, Mullol J: Persistent allergic rhinitis has a moderate impact on the sense of smell, depending on both nasal congestion and inflammation. *Laryngoscope* 2009;119:233–238.
- 58 Arnal J: Nasal nitric oxide is increased in allergic rhinitis. *Clin Exp Allergy* 1997;27:358–362.
- 59 Henriksen A, Sue-Chu M, Holmen T, Langhammer A, Bjerner L: Exhaled and nasal NO levels in allergic rhinitis: relation to sensitization, pollen season and bronchial hyperresponsiveness. *Eur Respir J* 1999;13:301–306.
- 60 Kharitonov S: Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *Allergy Clin Immunol* 1997;99:58–64.
- 61 Martin U, Bryden K, Devoy M, Howarth P: Increased levels of exhaled nitric oxide during nasal and oral breathing in subjects with seasonal rhinitis. *J Allergy Clin Immunol* 1996;97:768–772.
- 62 Takeno S, Osada R, Furukido K, Chen JH, Yajin K: Increased nitric oxide production in nasal epithelial cells from allergic patients – RT-PCR analysis and direct imaging by a fluorescence indicator: DAF-2 DA. *Clin Exp Allergy* 2001;31:881–888.
- 63 Sato M, Fukuyama M: Increased nitric oxide in nasal lavage fluid and nitrotyrosine formation in nasal mucosa – indices for severe perennial nasal allergy. *Clin Exp Allergy* 1998;28:597–605.
- 64 Palm JP, Alving K, Lundberg JO: Characterization of airway nitric oxide in allergic rhinitis: the effect of intranasal administration of L-NAME. *Allergy* 2003;58:885–892.
- 65 Maniscalco M, Sofia M, Carratu L, Higenbottam T: Effect of nitric oxide inhibition on nasal airway resistance after nasal allergen challenge in allergic rhinitis. *Eur J Clin Invest* 2001;31:462–466.
- 66 Valero A, Serrano C, Valera JL, Barberá A, Torrego A, Mullol J, Picado C: Nasal and bronchial response to exercise in patients with asthma and rhinitis: the role of nitric oxide. *Allergy* 2005;60:1126–1131.
- 67 Meurs H, Hamer MA, Pethe S, Vadon-Le Goff S, Boucher JL, Zaagsma J: Modulation of cholinergic airway reactivity and nitric oxide production by endogenous arginase activity. *Br J Pharmacol* 2000;130:1793–1798.

- 68 Chicoine LG, Paffett ML, Young TL, Nelin LD: Arginase inhibition increases nitric oxide production in bovine pulmonary arterial endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L60–L68.
- 69 Maarsingh H, Leusink J, Bos IS, Zaagsma J, Meurs H: Arginase strongly impairs neuronal nitric oxide-mediated airway smooth muscle relaxation in allergic asthma. *Respir Res* 2006;7:6.
- 70 Colantonio D, Brouillette L, Parikh A, Scadding GK: Paradoxical low nasal nitric oxide in nasal polyposis. *Clin Exp Allergy* 2002;32: 698–701.
- 71 Guilemany J, Angrill J, Alobid I: United airways again: high prevalence of rhinosinusitis and nasal polyps in bronchiectasis. *Allergy* 2009;64:790–797.
- 72 Gilain L, Bedu M, Jouaville L, Guichard C, Advenier D, Mom T, Laurent S, Caillaud D: Analysis of nasal and exhaled nitric oxide concentration in nasal polyposis (in French). *Ann Otolaryngol Chir Cervicofac* 2002;119: 234–242.
- 73 Cekin E, Ipcioglu OM, Erkul BE, Kapucu B, Ozcan O, Cincik H, Gungor A: The association of oxidative stress and nasal polyposis. *J Int Med Res* 2009;37:325–330.
- 74 Prieto L, Seijas T, Gutierrez V, Uixera S, Bruno L, Lopez R: Exhaled nitric oxide levels and airway responsiveness to adenosine 5'-monophosphate in subjects with nasal polyposis. *Int Arch Allergy Immunol* 2004;134: 303–309.
- 75 Ragab SM, Lund VJ, Saleh HA, Scadding G: Nasal nitric oxide in objective evaluation of chronic rhinosinusitis therapy. *Allergy* 2006;61:717–724.
- 76 Arnal J, Flores P, Rami J, Murriss-Espin M, Bremont F, Pasto I: Nasal nitric oxide concentration in paranasal sinus inflammatory diseases. *Eur Respir J* 1999;13:307–312.
- 77 Delclaux C, Malinvaud D, Chevalier-Bidaud B, Callens E, Mahut B, Bonfils P: Nitric oxide evaluation in upper and lower respiratory tracts in nasal polyposis. *Clin Exp Allergy* 2008;38:1140–1147.
- 78 Lindberg S, Cervin A, Runer T: Nitric oxide (NO) production in the upper airways is decreased in chronic sinusitis. *Acta Otolaryngol* 1997;117:113–117.
- 79 Deja M, Busch T, Bachmann S, Riskowski K, Campean V, Wiedmann B, Schwabe M, Hell B, Pfeilschifter J, Falke KJ, Lewandowski K: Reduced nitric oxide in sinus epithelium of patients with radiologic maxillary sinusitis and sepsis. *Am J Respir Crit Care Med* 2003; 168:281–286.
- 80 Baraldi E, Azzolin NM, Biban P, Zacchello F: Effect of antibiotic therapy on nasal nitric oxide concentration in children with acute sinusitis. *Am J Respir Crit Care Med* 1997; 155:1680–1683.
- 81 Degano B, Genestal M, Serrano E, Rami J, Arnal JF: Effect of treatment on maxillary sinus and nasal nitric oxide concentrations in patients with nosocomial maxillary sinusitis. *Chest* 2005;128:1699–1705.
- 82 Min Y, Lee C, Rhee C: Increased expression of IL-4, IL-5, IFN-gamma, IL-6, IL-8 and TGF-beta mRNAs in maxillary mucosa of patients with chronic sinusitis. *Am J Rhinol* 1999;13:339–343.
- 83 Zamora R, Vodovotz Y, Billiar TR: Inducible nitric oxide synthase and inflammatory diseases. *Mol Med* 2000;6:347–373.
- 84 Lundberg JO, Maniscalco M, Sofia M, Lundblad L, Weitzberg E: Humming, nitric oxide, and paranasal sinus obstruction. *JAMA* 2003;289:302–303.
- 85 Naraghi M, Deroee AF, Ebrahimkhani M, Kiani S, Dehpour A: Nitric oxide: a new concept in chronic sinusitis pathogenesis. *Am J Otolaryngol* 2007;28:334–337.
- 86 Deroe A, Naraghi M, Sontou AF, Ebrahimkhani MR, Dehpour AR: Nitric oxide metabolites as biomarkers for follow-up after chronic rhinosinusitis surgery. *Am J Rhinol Allergy* 2009;23:159–161.
- 87 Bush A: Primary ciliary dyskinesia. *Acta Otorhinolaryngol Belg* 2000;54:317–324.
- 88 Grasemann H, Gartig S, Wiesemann H: Effect of L-arginine infusion on airway NO in cystic fibrosis and primary ciliary dyskinesia syndrome. *Eur Respir J* 1999;13:114–118.
- 89 Karadag B, James AJ, Gultekin E, Wilson NM, Bush A: Nasal and lower airway level of nitric oxide in children with primary ciliary dyskinesia. *Eur Respir J* 1999;13:1402–1405.
- 90 Wodehouse T: Nasal nitric oxide measurements for the screening of primary ciliary dyskinesia. *Eur Respir J* 2003;21:43–47.
- 91 Horvath I, Loukides S, Wodehouse T, Csiszer E, Cole PJ, Kharitonov SA, Barnes PJ: Comparison of exhaled and nasal nitric oxide and exhaled carbon monoxide levels in bronchiectatic patients with and without primary ciliary dyskinesia. *Thorax* 2003;58: 68–72.
- 92 Csoma Z, Bush A, Wilson NM: Nitric oxide metabolites are not reduced in exhaled breath condensate of patients with primary ciliary dyskinesia. *Chest* 2003;124:633–638.
- 93 Narang I, Ersu R, Wilson NM, Bush A: Nitric oxide in chronic airway inflammation in children: diagnostic use and pathophysiological significance. *Thorax* 2002;57:586–589.
- 94 Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, Zariwala MA, Knowles MR: Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 2004;169:459–467.
- 95 Corbelli R, Bringolf-Isler B, Amacher A, Sasse B, Spycher M, Hammer J: Nasal nitric oxide measurements to screen children for primary ciliary dyskinesia. *Chest* 2004;126: 1054–1059.
- 96 Mahut B, Escudier E, de Blic J, Zerah-Lancner F, Coste A, Harf A, Delclaux C: Impairment of nitric oxide output of conducting airways in primary ciliary dyskinesia. *Pediatr Pulmonol* 2006;41:158–163.
- 97 Scadding G, Scadding K: Update on the use of nitric oxide as a non-invasive measure of airways inflammation. *Rhinology* 2009;47: 115–120.
- 98 Runner T, Cervin A, Lindberg S: Nitric oxide is a regulator of mucociliary activity in the upper respiratory tract. *Otolaryngol Head Neck Surg* 1998;119:278–287.
- 99 Lindberg S, Cervin A, Runer T: Low levels of nasal nitric oxide (NO) correlate to impaired mucociliary function in the upper airways. *Acta Otolaryngol* 1997;117:728–734.
- 100 Loukides S, Kharitonov S, Wodehouse T, Cole PJ, Barnes PJ: Effect of arginine on mucociliary function in primary ciliary dyskinesia. *Lancet* 1998;352:371–372.
- 101 Jeganathan D, Chodhari R, Meeks M, Faeroe O, Smyth D, Nielsen K, Amirav I, Luder AS, Bisgaard H, Gardiner RM, Chung EM, Mitchison HM: Loci for primary ciliary dyskinesia map to chromosome 16p12.1–12.2 and 15q13.1–15.1 in Faroe Islands and Israeli Druze genetic isolates. *J Med Genet* 2004;41:233–240.
- 102 Escudier E, Duquesnoy P, Papon JF, Amselem S: Ciliary defects and genetics of primary ciliary dyskinesia. *Paediatr Respir Rev* 2009;10:51–54.
- 103 Buchwalow IB, Minin EA, Muller FU, Lewin G, Samoilova VE, Schmitz W, Wellner M, Hasselblatt M, Punkt K, Müller-Werdan U, Demus U, Slezak J, Koehler G, Boecker W: Nitric oxide synthase in muscular dystrophies: a re-evaluation. *Acta Neuropathol* 2006;111:579–588.
- 104 Grasemann H, Michler E: Decreased concentrations of exhaled nitric oxide (NO) in patients with cystic fibrosis. *Ped Pulmol* 1997;24:173–177.
- 105 Zheng S, Xu W, Bose S, Banerjee AK, Haque SJ, Erzurum SC: Impaired nitric oxide synthase-2 signaling pathway in cystic fibrosis airway epithelium. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L374–L381.
- 106 Kelley T, Drumm M: Inducible nitric oxide synthase expression is reduced in cystic fibrosis murine and human airway epithelial cells. *J Clin Invest* 1998;102:1200–1207.
- 107 Shori DK, Dormer RL, Goodchild MC, McPherson MA: Defective phosphorylation of a calmodulin-binding protein in cystic-fibrosis submandibular glands. *Biochem J* 1989;263:613–616.
- 108 Grasemann H, Ratjen F: Cystic fibrosis lung disease: the role of nitric oxide. *Pediatr Pulmonol* 1999;28:442–448.
- 109 Grasemann H, Storm van's Gravesande K, Buscher R, Knauer N, Silverman ES, Palmer LJ, Drazen JM, Ratjen F: Endothelial nitric oxide synthase variants in cystic fibrosis lung disease. *Am J Respir Crit Care Med* 2003;167:390–394.