

Review article

Nasal nitric oxide

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The significance of nitric oxide (NO) in man was first investigated in the late 1980s, and NO has subsequently received great attention from biologists. Initially, this highly reactive gaseous molecule was seen as a mere noxious air pollutant. Closer investigation of its function in physiological processes, however, revealed that it took part in many different biologic processes. This multifunctionality led to its declaration as the molecule of the year in 1992.

We now know NO to be a smooth-muscle relaxant in blood vessels, an inhibitor of platelet aggregation, a neurotransmitter, and a mediator in local defense (2, 3). In the airways, NO is an important molecule with different functions such as stimulation of ciliary motility, mediation in inflammation, bacteriostatic and virostatic activity, and regulation of bronchial airway tone and even pulmonary vascular tone (4–7). Further studies on other systems will probably reveal more processes in which NO plays a key role.

Studies in healthy adults indicate that NO in nasal air is mainly produced in the epithelial cells of the nasal cavity, particularly in the paranasal sinuses (8). Many factors, such as smoking, drugs, physiological factors, and nasal and paranasal disorder, influence the level of NO measured in nasal air (6, 9, 10). The measurement technique is also of great importance (10, 11). NO measurement has begun to be used in experimental clinical settings, in order to clarify the clinical value of NO in diagnostic problems and therapeutic strategies for disorders such as primary ciliary dyskinesia (PCD) and various forms of sinusitis and allergy. The use of NO as a non-invasive diagnostic and therapeutic tool is the ultimate goal.

Production of NO

Many cells within the (upper and lower) respiratory tract can produce NO, including endothelial cells, epithelial cells, neutrophils, and (alveolar) macrophages (12). First, L-arginine is taken up by the cells via cationic transporters (CAT) (Fig. 1). CAT1 is constitutively expressed (housekeeping), while CAT2 is induced by cytokines. Second, L-arginine is *N*-hydroxylated into NG-hydroxy-L-arginine (NOHA). Subsequently, a three-electron oxidation takes place, resulting in NO and L-citrulline. While NO diffuses to the lumen, L-citrulline can be reconverted to L-arginine via argininosuccinate inside the cell (13).

This pathway of generation of NO is regulated by a family of enzymes called nitric oxide synthases (NOS). Three isoforms of NOS have now been identified in man and are differentially distributed in organs and tissues (14). Constitutively expressed nitric oxide synthase (eNOS) consists of two isoforms, nNOS (NOS type 1) and eNOS (NOS type 3), respectively expressed in neurons and vascular endothelium. The activity of nNOS and eNOS is regulated by intracellular calcium/calmodulin concentrations. These isoforms have been localized in human alveolar type II cells and in transformed and primary cultures of human bronchial epithelial cells (15). Inducible NOS (iNOS or NOS type 2) is probably present in every (epithelial) cell, and is activated by pro-inflammatory cytokines and/or bacterial products (2).

The inducible form of NOS is calcium independent. LPS alone increases the production of NO in human epithelial cells, but IFN- γ acts synergistically to enhance this response (15).

Immunohistochemical and mRNA *in situ* hybridiza-

tion show that NO synthase is expressed apically in the paranasal sinus epithelium, in contrast to the epithelium of the nasal cavity, where only weak NO synthase activity was found (16). The NOS of the paranasal sinuses most closely resembles the inducible isoform but has different characteristics from iNOS expressed elsewhere. These isoforms seem to be constantly expressed and active, and to be resistant to steroids. These properties are associated with constitutive, rather than with inducible, isoforms of NOS (16).

A new nonenzymatic pathway has been discovered in man that produces NO by reduction of inorganic nitrite under specific conditions (17). These nonenzymatic reactions take place in the stomach, on the surface of the skin, in the ischemic heart, and in infected nitrite-containing urine. NO generated by this mechanism is likely to play a role in similar biologic events, as when produced from L-arginine by NO synthases.

Origin of nasal NO

The exact origin of NO measured in nasal air and the relative contribution from other sources are not fully known. Not only is there the production within the nasal cavity and the paranasal sinuses, but there is also a contribution from other sources such as the ambient air and, more important, the lower respiratory tract (6–8, 10, 18, 19). Most studies indicate that the main production of nasal NO is in the paranasal sinuses (16, 20, 21). The first indication is the observation that there is a transient decrease in nasal NO measured from one nostril when air is continuously removed from one maxillary sinus, while air injected into the same sinus results in a transient elevation of nasal NO. This suggests a continuous flow of NO from the maxillary sinus to the nasal cavity (20). Another indication is the reduction of NO release from the paranasal sinuses by instillation of NO synthase inhibitor (L-NAME) into the maxillary sinus. Administration of L-NAME in the nasal cavity results in only a slight reduction of nasal NO levels (20).

In patients who have impaired ostial patency, significantly lower nasal NO levels are measured. Impairment of ostial patency and thus lower nasal NO levels are seen in disorders such as Kartagener's syndrome and cystic fibrosis. In these cases, there is probably a lower contribution of NO flowing from the paranasal sinuses into the nose, in addition to a possibly decreased production of NO (8, 22).

Moreover, nasal NO levels are high in man and other primates with paranasal sinuses, while, in contrast, the baboon, a primate which lacks paranasal sinuses, has very low nasal NO levels (21). The strong constitutive expression of iNOS in the sinus epithelium and the lack of expression in the nasal epithelium are another indication (16). There are indications that nasal NO levels in children rise until the age of 10 years, when they reach

the normal value as in adults. This may be a sign of increasing pneumatization of the developing paranasal sinuses in growing children (16, 23).

The role of bacteria in the production of nasal NO has also been suggested; however, most studies showed nasal NO release to be independent of the presence of bacteria, since systemic antibiotics had no effect on the nasal NO values of healthy adults, and the sterile nasal cavities of neonates delivered by cesarean section had measurable nasal NO levels (7, 24, 25).

NO: a local defense mechanism

As, in recent years, a wide variety of physiological processes in which NO is involved have been thoroughly investigated, it became clear that NO is important within the system where it is produced. Although initially considered a noxious air pollutant, many scientists now agree on the important roles of NO in different organ systems, such as those of a neurotransmitter in the nervous system, a smooth-muscle relaxant, and an inhibitor of platelet aggregation in the cardiovascular system (6, 16, 26).

In the airways, NO seems to be of great importance in local host defense and is a major mediator in many physiological and pathophysiological events, although the exact role of this pluripotent gas is far from fully known. It participates in host defense and inflammation, and as an airborne messenger in bronchial tone and pulmonary vascular resistance.

Inflammation

The role of NO in inflammation is contradictory. Some studies indicate a harmful role of NO in inflammation, whereas others indicate a positive influence (18). There is evidence that NO production is enhanced at sites of inflammation, leading to local increased NO levels, as in asthma, cystitis, and inflammatory bowel disease (18, 27). The harmfulness of NO may be due to extensive production of NO by iNOS in some inflammatory circumstances such as pertussis and asthma, leading to autotoxicity in the affected area (18). However, basal NO production in the upper respiratory tract by a constitutively expressed iNOS, leading to fairly high NO levels, has no destructive effect on local airway epithelium, and is even physiological (16). On the contrary, NO production in the upper respiratory tract seems to serve as an important protection against local attack, not as a mere inflammatory mediator, but as a regulator of various protective activities in host defense.

A remarkable illustration of the positive role of NO in inflammation was given by McCafferty et al., who found worse inflammation in iNOS knockout mice than in wild-type mice in an animal model of colon inflammation (28).

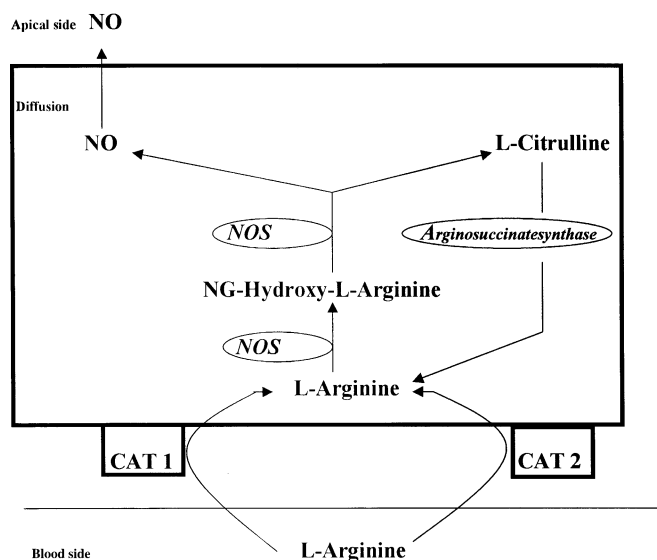


Figure 1. NO metabolic pathway (13) (reproduced with permission).

Host defense

The enhanced production of NO during local aggression against the airway epithelium suggests a role of NO in host defense. NO concentration in normal paranasal sinuses and even in the nasal cavity exceeds greatly NO concentrations that are bacteriostatic (i.e., 100 ppb) (6, 16, 29). Children who have low NO production, as in primary ciliary dyskinesia (PCD) and cystic fibrosis, also have recurrent airway infections, a fact which may be an indication of the (host) protective effect of NO. NO may also have virostatic activities, as indicated in a mouse model (30). There are also indications that NO is active against fungi and parasites, and it may also protect against tumor cells (31).

NO is also a regulator of ciliary beat frequency in the upper airway epithelium (4, 5, 32). The lack of NO in nasal air in diseases caused by profound ciliary dysfunction, such as PCD, strongly suggests a relation between NO and ciliary motility with clinical implications. For example, in infection, increased NO production can lead to enhanced ciliary activity, resulting in an effective clearance of aggressive organisms and potentially noxious metabolic products. This can have beneficial results in host defense.

Other findings suggest that NO enhances blood flow in the human nasal mucosa (33). Although its possible protective effect is not clear yet, further studies on this subject may elucidate the meaning of this finding.

Aerocrine messenger

NO produced in the upper respiratory tract follows the airstream to the lower airways and lungs with inhalation. This supports the hypothesis that NO derived from the upper airways has physiological effects in the lung and acts as an aerocrine messenger. There is some evi-

dence that inhaled (exogenous) NO, at concentrations as low as 100 ppb, significantly decreases pulmonary vascular resistance and improves arterial oxygenation in subjects with severe pulmonary disease (33). Other studies suggest that NO helps to decrease the bronchial tonus, although this might be a central rather than a peripheral airway effect (7).

Detection of NO

NO in gas phase at low concentrations, as in the human airways, is fairly stable and therefore can be detected and quantified. The most widely used technique for measurement of NO in exhaled air is the chemiluminescence method. This highly sensitive technique is based on the emission of electromagnetic radiation from excited NO_2^* . NO reacts with an excess of ozone (O_3), resulting in NO_2 with an electron in an excited state (NO_2^*), which returns to its basic energy by emitting a photon. The quantity of light emitted is proportional to the NO concentration and can be displayed online on-screen. The lower limit of measurement is 1 ppb. Nasal NO measurement is based on the same method as exhaled NO, but sampling can be done directly or indirectly from the nose (6, 10). Other methods that have been used to measure NO in human exhaled air are mass spectrometry and gas chromatography-mass spectrometry (6).

The measurement technique that is used in a particular experiment is very important for the eventual value of the nasal NO level (10, 34). Even in the same population the NO level is dependent on the measurement technique (11). The most important factors are ambient NO; the method of measuring (i.e., sampling while breathholding or tidal breathing, soft palate closure, etc.); and the characteristics of the chemiluminescence analyzer, the sampling flow, and the intranasal flow (10, 11).

For comparison of different values, it is important to have a notion of these factors. In 1997, the European Respiratory Society Task Force tried to determine a standard method in order to obtain more comparable and reliable values (10). However, scientists continue to use different experimental settings, and one should be aware of this in order to interpret and compare NO values from different studies.

Normal values of NO and physiological variations

The values of oral and nasal NO in the exhaled air of controls measured by the chemiluminescence method vary among laboratories: oral NO ranges from 4 to 160 ppb, while nasal NO varies from 200 to 2000 ppb (12, 22, 23, 35–38). Another remarkable feature is that NO levels are always higher in the upper respiratory tract than in the lower airways in normal subjects (6, 8, 10, 12, 22, 24, 36, 38). The variety of NO values in

Table 1. Influences on nasal NO

Type of influence	Effect on nasal NO
Physical variations	
Age	
In children up to age of 10 years	+
In adults	0
Sex	0
Menstrual cycle	0
Physical exercise	—
External factors	
Smoking	(—)
Drugs (in healthy persons)	
Topical glucocorticoids	0
Systemic glucocorticoids	0
Antibiotics	0
Topical nasal decongestants	—
Histamine	0
Pathologic variations	
Upper respiratory tract infection	0
Allergic rhinitis	?
Asthma	0
Sinusitis	—?
Nasal polyps	
With allergy	(+)
Without allergy	—
Cystic fibrosis	—
Primary ciliary dyskinesia	—

+: Leads to higher nasal NO levels; —: leads to lower nasal NO levels; 0: no effect on nasal NO; ?: contradictory results; (—): result is not statistically significant.
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different studies is due to different factors such as measurement techniques, physiological variations, and pathologic changes (9–11, 16, 23, 34, 39–41). A summary of the influences on nasal NO is given in Table 1.

Age

Nasal NO levels rise from birth until the age of 10 years, when they reach the normal adult level. This finding supports the paranasal origin of nasal NO, as in children development of paranasal sinuses results in higher nasal NO levels until the age of 10 years, when they reach their final constitution (16, 23, 43). Interestingly, Schedin et al. found nasal NO already present at birth, including those neonates delivered by cesarean section (25). When nasal NO levels were correlated with body surface, the concentration in children around 10 years of age was approximately twice as high as the nasal NO concentration in adults. The following two possible explanations have been proposed:

- 1) the surface of paranasal sinuses in children develops faster than the body surface
- 2) children excrete a larger proportion of NO in the nasal mucosa (16).

Another study found that nasal NO levels in adults between 20 and 90 years of age were similar (23). Artlich et al. related levels of nasal NO to the body surface in preterm children and found that the NO excretion is similar to that of adults (about 3 nl/kg/min^{−1}). They

concluded that the lower NO levels in preterm children are due to the smaller volume of ventilated sinuses and smaller epithelial surface at that age (43). Mammals without sinuses have no age-related increase in nasal NO (44).

Recently, Qian et al. contradicted Lundberg et al.'s conclusions. They showed that intranasal flow had a great influence on the result of NO measurement (16, 34). As there are many differences in ventilation and measurement techniques between children and adults, intranasal flow will not always be comparable. More work needs to be done to make measurements in children and adults more comparable, in order to draw conclusions about age-dependent NO differences (34).

Sex

There is no evidence that nasal NO levels are sex-related (10, 34, 39).

Menstrual cycle

Variation in nasal NO levels in relation to the menstrual cycle has not yet been studied.

Physical exercise

Several studies show that nasal NO decreases during physical exercise (6, 10, 45). Lundberg et al. (6) showed that nasal NO decreased by 47% after 1 min of physical exercise. A maximal reduction of 76% was found at the end of the exercise period; thereafter, NO levels slowly increased. They reached normal basal levels in about 15–20 min. There are several possible reasons for this decrease in nasal NO. Firstly, changes in nasal cavity volume could result in lower NO levels by dilution of nasal air (46). This possibility has been rejected by a recent finding that nasal NO is independent of nasal cavity volume (47). Secondly, NO could be destroyed by reactive agents produced in the nasal mucosa during physical exercise. Thirdly, changes in NO could be caused by a reduction of blood flow in the nasal mucosa with a concomitant decrease in substrate supply to the highly producing NOS type 2 in the paranasal sinuses (6, 46).

Influence of external factors

Smoking

Smoking control subjects have somewhat lower exhaled NO and nasal NO values than age- and sex-matched nonsmokers. The reason for this could be related to the toxic effect of inhaled smoke on the downregulation in NOS and/or the disruption of NO-producing cells (6, 10, 23).

Drugs

When evaluating the effect of drugs on nasal NO, one should be aware of interactions among drugs, patients, and diseases. It is not always easy to determine whether the changes in nasal NO are caused by the drug or by the disease itself. Topical and systemic glucocorticoids showed no effect on the nasal NO levels in healthy people (6, 8, 48, 49). Antibiotics in healthy persons do not alter nasal NO levels (6, 8).

Topical nasal decongestants, such as oxymetazoline, result in a decrease of nasal NO levels (6, 10, 40, 47, 50). The reason for this may be a reduction, caused by vasoconstriction, in substrate supply to the high-output NOS type 2 in the sinuses. Histamine seems to have no influence on nasal NO levels (51).

Pathologic variations in nasal NO

Upper respiratory tract infections (URTI)

Nasal NO levels in people suffering from an upper respiratory tract infection (URTI) do not differ from nasal NO levels in healthy people. Specifically, Ferguson & Eccles (50) and Lindberg et al. (23) found no significant differences in nasal NO levels during and after an episode of URTI. Lindberg et al. (23) found similar nasal NO levels in patients with URTI and healthy controls (23). Baraldi et al. reached the same conclusion when comparing children with and without URTI (41).

Allergic rhinitis

The effect of allergic rhinitis on nasal NO is not consistent. Some researchers report higher nasal NO levels in patients with allergic rhinitis (9, 40, 42). This may be due to an upregulation of iNOS by local infection, resulting in higher NO production (9). Kharitonov et al. found that nasal NO levels in patients suffering from allergic rhinitis and treated with topical nasal glucocorticoids are even lower than nasal NO levels in controls (9). This led to the hypothesis that iNOS in nasal epithelial cells gives rise to increased nasal NO levels in allergic rhinitis and contributes to the normal NO production in basal circumstances, since topical nasal glucocorticoids normally do not reach the sinus cavity and decrease nasal NO values in allergic rhinitis to levels lower than nasal NO levels in controls. According to this hypothesis, iNOS in the nasal cavity, as its activity is altered by glucocorticoids, must be different from iNOS found in the paranasal sinuses, which is not influenced by glucocorticoids (9, 52). Lundberg et al. (36) and Henriksen et al. (53) found no alterations in nasal NO levels in patients with allergic rhinitis.

The cause of these discrepancies is not very clear. One could speculate that the upregulation of iNOS in the nose leads to higher nasal NO levels in rhinitis, as is the case in local infections in the lower airways, such as

asthma (9, 52, 54). In contrast, swelling of the nasal mucosa in rhinitis can lead to occluded sinus ostia, which results in a reduced passage of NO from the paranasal sinuses to the nasal cavity, where it is measured (40). An interesting finding supporting this view was made by Arnal et al. (40), who found increased nasal NO levels in patients with allergic rhinitis. But patients without symptoms at the moment of the measurement had even higher nasal NO levels than patients with symptoms. One could postulate that nasal NO levels in patients with symptoms are lower because of a reduced contribution of the NO produced in the paranasal sinuses, as a result of obstructed sinus ostia. In patients without symptoms, ostial patency is mostly better preserved, leading to a higher output of NO from the paranasal sinuses into the nasal cavity (40). Thus, in certain conditions, the nasal NO level we measure may be the result of counteracting mechanisms, which, in the extreme case, may even counterbalance each other. This must be taken into consideration when a given nasal NO value is interpreted.

Asthma

Nasal NO levels seem not to be influenced by asthma (18, 22, 36, 55). One can assume that asthma affects the upper respiratory tract to a lesser extent than the lower airways, where increased NO levels are found. Inhalation of glucocorticoids can reduce NO levels by reduction of iNOS expression. NO levels are considered to be a marker of airway inflammation; thus, measurement of NO levels in the lower airways could indicate the efficacy of treatment (6, 7, 22).

Sinusitis

Nasal NO levels seem to be decreased in patients suffering from sinusitis, but not all studies are consistent. Lindberg et al. studied patients with chronic sinusitis and found that nasal NO production was reduced by more than 50% in comparison with healthy subjects (23). In contrast, Arnal et al. found no significant differences in their study of patients with chronic sinusitis (52). Lindberg et al. found similar nasal NO levels in patients after successful chronic sinusitis surgery and healthy subjects (23). Decreased nasal NO levels were measured by Baraldi et al. in children with acute sinusitis. These decreased nasal NO levels increased again after treatment with systemic antibiotics. After successful treatment, nasal NO levels were equal to the levels of healthy age-matched children (41).

It has not yet been established whether low nasal NO levels in sinusitis result from reduced passage of NO via the sinus ostia, or whether the NO production itself is reduced in those patients (41). A low production of NO as a cause of low nasal NO levels in chronic sinusitis is supported by the study of Lindberg et al., who found nasal NO levels to be low despite large sinus ostia or

patent neo-ostia, created by sinus surgery, as confirmed by endoscopic examination (23). In contrast to Lindberg et al.'s findings, Baraldi et al. found only a markedly reduced NO level in children with a radiologic asymmetry of the acute sinus process in the air derived from the corresponding nostril (41).

Nasal polyps

The effect of nasal polyps has not been clearly determined. The relationship of nasal NO and nasal polyps has been examined in only one study. Arnal et al. (52) reported slightly increased nasal NO levels in patients with nasal polyps and allergy compared to controls, whereas patients with nasal polyps without allergy had significantly lower nasal NO levels. The nasal NO concentration in patients with untreated allergic polyposis was significantly higher than in patients with untreated nonallergic polyposis. For a similar degree of sinus alteration, nasal NO was higher in allergic than in nonallergic patients. This shows that allergy is an important factor in relation to the level of nasal NO in polyposis. In each group, the nasal NO concentration was inversely correlated with the extent of tomodesitometric alterations of the paranasal sinuses. This indicates that the obstruction of the para-nasal sinuses by the polyps statistically decreases the nasal NO concentration. Within groups with a similar degree of obstruction, broad dispersion of the nasal NO levels was observed, suggesting that sites of production other than the sinuses also contribute to the nasal NO. It has recently been shown that polyps themselves also may contribute to the NO production, as they also generate iNOS in their epithelial cells (2).

Thus, we can conclude that the degree of paranasal sinus alteration and the allergic status strongly influence the nasal NO level in nasal polyposis.

Cystic fibrosis

All studies report very low nasal NO levels in patients suffering from cystic fibrosis (12, 22, 36). This may be the result of reduced NO production by destroyed epithelial cells or reduced NOS activity. An increased mucosal barrier impeding NO diffusion into the sinus and a reduced NO passage from the sinuses to the nasal cavity may be another possible explanation (12, 22, 36).

Primary ciliary dyskinesia (PCD) and Kartagener's syndrome

Kartagener's syndrome is a triad comprising sinusitis, bronchiectasis, and situs inversus. They are part of PCD. In patients with PCD, nasal NO levels are extremely low, sometimes undetectable (8, 12, 19, 38, 52). Possible explanations are reduced NO production by a deficient NOS, reduced diffusion from the nasal and paranasal mucosa, and reduced passage of NO via the sinus ostia (8, 12, 19, 22, 52).

In our studies on PCD, we confirmed significantly lower nasal NO levels in PCD than in disease controls. Normal nasal NO values, however, do not exclude PCD. We found that the ultrastructural subtypes in PCD have no significant influence on the nasal NO level (Jorissen et al., unpublished data).

Usefulness in clinical practice

Measurement of NO can become an interesting and attractive diagnostic and therapeutic tool. However, much remains to be done in order to make it a useful aid in daily practice. This noninvasive measurement can conveniently be performed, even in young children. It could be used as an easy screening test for the diagnosis of PCD. In the future, therapeutic efficiency may become easily measurable. It is reasonable to believe that drugs will be used to facilitate or decrease NO production in such a manner that it can have a positive influence on (airway) disorder. However, there is still much to be done, especially in identifying the various physiological and pathologic factors, such as allergy, that affect nasal NO, particularly the counteracting mechanisms. Future research should concentrate on standardization and on making measurements more reliable and comparable.

Conclusion

NO is a small gaseous molecule, the significance of which in man began to be investigated in the late 1980s. During the last decade, it has attracted the attention of many scientists, who have revealed its significance in various physiological and pathologic processes. It has distinct functions in the cardiovascular system, the nervous system, and the upper and lower airways. In the airways, NO levels in the upper respiratory tract are much higher (200–2000 ppb) than those in the lower respiratory tract (4–160 ppb).

The chemiluminescence method, which is based on a reaction of NO with O₃ resulting in the emission of light, is the most widely used measurement technique for NO.

NO has a major influence on airway physiology by mediation in ciliary activity, inflammation, host defense, bronchial tone, and pulmonary vascular resistance. It is also considered to be an aerocrine messenger between the upper and lower airways.

Factors such as physical exercise, age, smoking, and some drugs influence physiological nasal NO concentrations. Pathologic conditions such as allergic rhinitis, sinusitis, nasal polyps, cystic fibrosis, and PCD lead to altered nasal NO concentrations.

Measurement of nasal NO can easily be carried out at all ages and can be used to screen for disease or to monitor the effects of treatment. However, the clinical

relevance of the measurement of nasal NO in different physiological and pathologic conditions remains to be established before it can be used as a diagnostic tool.

Today, research on the actual function of NO in health and illness is laying bare its prospects in diagnostic and therapeutic assessment of some medical problems.

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