REVIEW

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Update on the use of nitric oxide as a non-invasive measure of airways inflammation*

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SUMMARY

Nitric oxide levels may reflect the inflammatory status of both the upper and lower airways. Measurement of exhaled bronchial nitric oxide is a useful, non-invasive tool in the diagnosis and management of eosinophilic asthma. Nasal nitric oxide may be normal, raised or lowered in disease states; however measurement may be a useful tool in the diagnosis and management of patients with chronic rhinosinusitis, nasal polyps, and cystic fibrosis, as well as in the diagnosis of primary ciliary dyskinesia. Further research is aimed at investigating the role of nitric oxide in allergic rhinitis and nasal congestion. Measuring both bronchial and nasal nitric oxide may assist the combined management of upper and lower airways.

Key words: nitric oxide, exhaled nitric oxide: eNO, nasal nitric oxide: nNO, rhinitis, rhinosinusitis, nasal polyposis, primary ciliary dyskinesia, cystic fibrosis, ostiomeatal complex, eosinophilic inflammation

INTRODUCTION

Nitric oxide (NO) is a colourless, odourless gas which is present in air exhaled through the mouth or nose. NO is produced from arginine and oxygen by nitric oxide synthase (NOS). Constitutively expressed neuronal and endothelial forms exist as well as an induced form, iNOS, which appears to be up regulated within the respiratory tract in response to pro-inflammatory signals. NO came to prominence for its role in vasodilatation [1,2] and subsequently as a neurotransmitter and inflammatory mediator [3]. The role of NO in the airways is complex, possibly including antibacterial effects, pro-inflammatory effects, and regulation of blood flow [4,5] and ciliary beat frequency [6,7]. Exhaled NO (eNO) levels are raised in eosinophilic asthma [8] and measurement of this has become a standardised, but not yet widespread, tool in diagnosis and management of asthma. It can potentially provide a rapid, low cost, objective measure of lower airway inflammation.

Far greater levels of NO are produced in the upper than in the lower respiratory tract, with contributions from the sinuses [9] and to a lesser extent from the nasal mucosa [10,11]. Measurement of nasal NO (nNO) may provide a similarly useful tool in the management of upper respiratory tract diseases although measurement procedures have yet to be fully standardised and it currently remains largely a research tool. Given the frequent co-existence of pathology affecting both upper and lower airways, measurement of both bronchial eNO and nNO may prove to be useful in the management of individual patients.

EXHALED NO

NO exhaled from the lungs of healthy individuals is usually low at < 20 ppb. Levels are increased in asthma [8], in particular in patients with eosinophilic asthma [12,13]. Exhaled NO has a relatively high sensitivity in the diagnosis of eosinophilic asthma [14]; use in combination with lung function is likely tobe superior to lung function testing alone [15]. It is potentially more convenient and safer to perform than histamine or methacholine bronchial provocation tests and can provide evidence of subclinical inflammation [16-18]. It provides rapid results, an advantage over sputum eosinophil assessment. In addition to its use in diagnosis, eNO is also valuable in facilitating optimal management of asthma. At the outset, eNO can be used to predict the response to inhaled corticosteroids and guide steroid dosing [19-22]. Levels can be used to identify the risk of exacerbations [23] and persistent eosinophilic inflammation despite treatment [24]. In stable patients eNO has been used as a guide to wean down inhaled steroid dose safely [25]. Compliance with medication and inhaler technique may also be reflected by eNO measurements. eNO is also a potentially useful tool in paediatric asthma, including diagnosis, management and assessment of compliance [26,27]. In particular, it may be useful in diagnosing the underlying cause in a child who presents with a chronic cough. Normal or low eNO measurements in a child with on-going 'asthmatic' symptoms raises the possibility of alternative diagnoses such as rhinosinusitis, CF or PCD.

Conversely, eNO has not yet been proven of significant value in preventing airway remodelling or FEV1 decline and has not

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been rigorously assessed in the context of health care costs and quality of life analyses [28]. Some investigators have suggested the cost-benefit ratio of regular testing does not favour routine use at present in the community setting [29]. Furthermore, the low baseline levels of eNO mean that background environmental NO levels can affect readings. Additional complicating factors include the undetermined significance of raised eNO levels in some atopic individuals without other clinical or spirometric features of asthma, and the inconsistent relationships between eosinophils and eNO demonstrated in some studies [29]. Numerous patient and environmental factors have also been found to affect eNO, including age in children, sex, pregnancy, menstruation, time of day, smoking, exercise, nitrate-rich foods, alcohol, viral infection and recent inhaled medications [30]. Hence standardising for these may be important particularly for measurements in one individual over time, but also if comparisons between individuals and 'normal' values are to be made. eNO is not currently a routinely available test in many centres.

NASAL NO

High levels of NO are produced constitutively in normal individuals within the paranasal sinuses by calcium-independent nitric oxide synthase, with levels measured at 20-25 ppb [9,31]. Additionally, nitric oxide is also formed in the nasal mucosa by inducible NOS (iNOS) in response to inflammation [10,11]. NO and its metabolites are toxic to microorganisms and likely form part of the innate defence mechanism of the respiratory tract [32]. NO may also stimulate cilia beat frequency within the epithelium [6,7] and regulate nasal vascular tone [4,5]. As for eNO, nNO can also be measured by chemiluminescence, using non-invasive techniques, providing immediate results. A number of different techniques have been used to ensure sampling from the upper airways only including breath-holding and breathing against resistance. Guidelines for measurement have been published [30]. In contrast to measuring eNO, high baseline levels in nNO make background environmental NO levels less of a problem. Conversely, there is a high degree of inter-individual variability amongst healthy controls. Moreover, there is also a significant degree of intra-individual variation over time, meaning that changes of 20-25% or less may be accounted for by normal variation rather than change in disease status or response to medication [33]. Additionally, the lack of universal standardisation of testing procedures means levels recorded by different study groups vary considerably even amongst equivalent patient populations [34,35]. The factors affecting eNO levels such as recent exercise or time of day, may similarly affect nNO measurements. Local factors such as nasal volume and patency may also be important. Table 1 provides a guide to the possible clinical significance of eNO and nNO values with the caveat that variability, particularly with regards to nNO, exists between measuring techniques.

Despite the above limitations nNO has a number of potentially useful clinical applications. With regards to diagnosis, nNO is useful as a screening tool for patients with possible primary ciliary dyskinesia; levels less than 100ppb should stimulate investigation of mucociliary structure and function [36]. The test is objective and may be easier to perform than a saccharine clearance test in younger children. Similarly, nNO may provide a useful tool in diagnosis of cystic fibrosis in the context of upper respiratory tract symptoms; levels significantly lower than in controls have been eported in some studies [37,38], but not others [39]. nNO has a potential role in the diagnosis and assessment of chronic rhinosinusitis, especially when associated with nasal polyps. Interestingly, despite the increased expression of iNOS in polyp epithelium [40], low nNO levels have been found in two large studies [41,42]. Moreover, nNO

Table 1. Guide to clinical correlation of measured bronchial exhaled nitric oxide (NO) and nasal nitric oxide in adults and children. Exhaled NO values represent results of standardised measurement techniques (29). Nasal NO values represent those achieved via a closed palate, breath-holding technique, with aspiration from a single naris at constant flow rate (0.25-3L/min) whilst air is entrained via the other naris (transnasal flow in series). The NO value recorded is that achieved during the plateau phase of measured NO, usually achieved after approximately 20 seconds. ppb: parts per billion of sampled air.

Exhaled NO (ppb)			Nasal NO (ppb)	
Adults	Children	Clinical relevance	Adults/Children	Clinical relevance
5-20 ppb	5-25 ppb	Normal/Absence of significant eosinophilic inflammation	<100 ppb	Very low: consider Primary Ciliary Dyskinesia or Cystic Fibrosis
25-50 ppb	20-35 ppb	Consistent with mild eosinophilic airways inflammation	<450 ppb	Low: may reflect obstruction at the sinus ostium
>50 ppb	>35 ppb	Consistent with significant eosinophilic airways inflammation	450-900 ppb	Normal range; does not exclude significant nasal disease
			>900 ppb	Consistent with nasal mucosal inflammation, sinuses probably patent

inversely correlated with endoscopic polyp size [42,43], CT scores [41,42] and clinical severity of disease [42]. Conversely, in a study involving chronic rhinosinusitis patients with and without polyps, no correlation between nNO and CT scores was found, although patients were again found to have lower baseline nNO than controls [44].

Low nNO levels in chronic rhinosinusitis are thought to reflect obstruction at the sinus ostium and impairment of gas transfer out from the sinuses. This is supported by the finding of raised nNO following medical [41,42] and surgical [41] treatment of rhinosinusitis with or without polyps. These increases correlated with subjective and objective tests of clinical improvement, including endoscopic scores and polyp grading. Conversely, no subsequent increase in nNO was detected 3 months post endoscopic sinus surgery compared to baseline in a recent study [44]; although no other recognised subjective or objective measures regarding the success of surgery were made at follow up and it is not described whether or not patients received on-going medical treatment as post surgery patients had in the study by Ragab et al. [41]. A study of the effects of nasal lavage with sodium hypochlorite solution over a period of 3 months resulted in improvements in endoscopic appearances and decreased nasal airways resistance measured by rhinomanometry, but no change in nNO scores [45]. However, no specific assessment was made of sinus ostium patency. It is possible that nNO is simply not a sensitive enough tool to pick up changes in the inflammatory status of the nasal mucosa alone; instead its use may be limited to assessment of changes in sinus ostial patency. This may be a very useful measure during the management of chronic rhinosinusitis, and nasal polyposis in particular, as patency of the sinus ostium cannot invariably be seen on endoscopy and does not justify the use of recurrent CT scanning.

nNO has been studied in rhinitis and allergic rhinitis in particular [46]. Patients with allergic rhinitis and asthma were found to have raised nNO which subsequently decreased following treatment with intranasal steroids [34]. Other groups also found elevated levels of nNO in allergic rhinitics [47]. More recently, nNO levels were found to be higher in a cohort of individuals with various atopic diseases, only a minority of whom had a diagnosis of allergic rhinitis, compared to controls [48]. Whether elevated nNO may identify subclinical rhinitis or even increased risk of development of rhinitis is unclear. Researchers have detected increased iNOS expression in the nasal mucosa of allergic rhinitics compared to controls [11], with levels greatest in those with associated asthma [49]. However, several recent studies have shown no differences in nNO between seasonal allergic rhinitics and controls either during the relevant pollen season [35] or in correlation with changes in symptom scores [50]. A trial of montelukast for the treatment of seasonal allergic rhinitis in 7-14 year old children achieved reduced symptom scores and peripheral blood eosinophil counts but no changes in nNO [51]. Variable baseline levels of nNO and the modest changes which may occur in allergic rhinitis or following treatment make nNO measurement of little value in the diagnosis and management of uncomplicated rhinitis at present.

The potential value of nNO as an investigative tool has also been examined in the context of the response to nasal allergen challenge. Nasal xylometazoline, a topical decongestant, was applied to 20 allergic rhinitics, resulting in a mean fall in nNO of 24% [52]. Subjects then received a nasal challenge with either allergen or placebo, and a series of nNO recordings made. At 20 minutes post challenge a significant fall in nNO was seen with allergen compared to placebo. By 7 hours there was a non-significant increase and by 24 hours nNO levels were significantly higher in the allergen- than the placebo-challenged group. This reflects a similar pattern in eNO following bronchial provocation with allergen [53]. Ex vivo studies of rat nasal epithelium suggested allergen challenge caused venous dilatation, maximal at 55 minutes, which could be inhibited by the NOS inhibitor L-NAME [4]. This appears paradoxical given the apparent fall in nNO during the early phase response to nasal allergen challenge in humans [52]. However, this may be explained by transient blockage of the sinus ostium following allergen challenge, reducing the component of nNO supplied by the sinuses. This highlights the complexity of using nNO as a biomarker even in the clinical trial setting.

Recent work suggests that NO itself may be directly responsible for some upper respiratory tract symptoms, particularly nasal congestion. As mentioned above, Chiba et al. [4] suggest in a rat model of allergic rhinitis, that blocking either cysteinyl leukotrienes or NOS could inhibit nasal venous dilatation in response to allergen challenge. They postulate that cysteinyl leukotrienes up regulate iNOS with the subsequently produced NO causing venodilatation resulting in nasal congestion. In similar experiments in guinea pigs, L-NAME also relieved leukotriene D4-induced nasal venodilatation, but not dilatation induced by a thromboxane A2 analogue [5]. Support for a role for NO in nasal congestion in humans includes the documented fall in nNO post topical vasoconstrictor application [52], as well as the observation that sildenafil, a potentiator of NO-induced vasodilatation, causes nasal obstruction as assessed by symptom scores and rhinomanometry [54]. Arai et al. [55] used cultured human nasal microvascular endothelial cells from the inferior turbinate to demonstrate increased iNOS expression and apoptosis in response to a combination of LPS and TNF-α. This implies that bacterial infection of the mucosa would be expected to increase nNO. Maxillary sinus lavage performed during functional endoscopic sinus surgery showed larger amounts of the NO metabolites nitrite and nitrate to be present in individuals having surgery for nasal polyposis or chronic rhinosinusitis compared to those having surgery for concha bullosa or paradoxical middle turbinate [56]. The study

authors suggest that these metabolites are pro-inflammatory and pathogenic, potentially damaging the sinus and nasal epithelium.

UNITED AIRWAYS

Concurrent sinus and bronchial pathology is now well recognised and highlighted by recent guidelines [57,58]. Rhinitis and rhinosinusitis are risk factors for asthma and the majority of asthmatics have associated nose or sinus pathology. Recent studies of eNO and nNO highlight this. In a prospective study, Rolla et al. [59] classified rhinitics without a previous diagnosis of asthma into 3 groups; allergic rhinitis, non-allergic rhinitis, and chronic rhinosinusitis. They investigated the lower airways by symptom histories, bronchodilator response and metacholine PC20, and subsequently diagnosed a number of patients as having concurrent asthma. New asthma diagnoses were more common amongst allergic rhinitics and chronic rhinosinusitis patients than non-allergic rhinitics, with 42% of chronic rhinosinusitis and 33% of allergic rhinitis patients having previously undiagnosed asthma. These findings correlated with higher lung eNO levels in these two groups. Higher eNO levels in allergic rhinitics than non-atopic controls despite no difference in nNO values have been reported [33,60]. Profita et al. [48] reported raised nNO in both allergic rhinitics and asthmatics without diagnosed rhinitis versus controls. Another group found that nasal allergen challenge followed 24 hours later by LPS led to increases in both nNO and eNO at 24 hours [61]. Ragab et al. [62] showed that both medical and surgical treatment of chronic rhinosinusitis significantly decreased eNO compared to pre-treatment scores. These results suggest access to eNO measurements in the ENT clinic may be advantageous in assessing undiagnosed lower airways inflammation. Conversely, nNO measurements in chest clinics could serve to highlight concurrent upper respiratory tract disease in asthmatics, particularly individuals with polyps or other causes of sinus ostium obstruction. This could speed up referral for specific management of the upper respiratory tract.

FUTURE DEVELOPMENTS

A number of recent studies have focussed on the possible use of humming to improve the sensitivity of nNO measurements. Weitzberg and Lundberg [63] found that humming induced a large increase in nNO and proposed that this reflected rapid gas exchange between the nose and paranasal sinuses. They subsequently found that these increases were not detected in patients with nasal polyps and sinus ostium obstruction [64]. Furthermore, they suggest that absence of a normal response to humming during nNO measurement could be used to identify allergic rhinitics with sinus ostium obstruction [65]. Whether this adds significant value to standard testing has yet to be fully appreciated. One study revealed no benefit over standard nNO measurement in assessment of patients with primary ciliary dyskinesia [66]. Struben et al. [37] however demonstrated improved sensitivity and specificity in differentiating cystic fibrosis patients from controls with humming nNO compared to standard nNO measurement.

Smaller, more convenient devices are also becoming available for nNO measurement. Maniscalco et al. [67] recently undertook a study validating the use of a hand held device in allergic rhinitics and controls and found similar reproducibility to that of the current gold standard stationary chemiluminescence analyser. Gupta et al. [68] also suggested a reproducible method for measuring nNO during tidal breathing in children less than 5 years; potentially easier than breath holding or breathing against resistance for young children.

CONCLUSIONS

Bronchial eNO is a useful tool in the diagnosis and management of asthma. nNO can currently be recommended in only a limited number of settings; in screening for primary ciliary dyskinesia, and possibly also cystic fibrosis; and perhaps also as a marker of sinus ostium obstruction in chronic rhinosinusitis and nasal polyposis. In the latter case it may provide an excellent means of disease monitoring, potentially obviating the need for repeat CT scanning and giving less operator-dependent, subjective results than endoscopy. Given the considerable inter- and intra-individual variability over time and the inconclusive data regarding a modest elevation of nNO in allergic rhinitics, measurement is not clinically useful in the diagnosis or management of isolated rhinitis. Humming nNO may increase the sensitivity and specificity in disease diagnosis. Access to eNO and nNO may improve the combined management of upper and lower respiratory tract disease. Further research is required regarding the physiological and pathological roles of NO in airways disease as well as standardisation of test procedures, particularly for nNO.

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