

The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma

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Summary

Background Assessment of eosinophilic airway inflammation may be helpful in the management of asthma. Nitric oxide (NO) has potential advantages as a tool to monitor airway inflammation although little is known about the relationship between NO and eosinophilic airway inflammation and the factors which influence it.

Methods We set out to define the relationship between exhaled NO and the sputum eosinophil count, identify the exhaled NO concentration that best identified a sputum eosinophil count >3% and investigate the impact of several potential confounding factors in 566 consecutive patients with varying severity of asthma. Finally we examined the ability of exhaled NO concentrations measured at differing exhalation flows to identify the presence of a sputum eosinophilia.

Results We found a significant positive relationship between exhaled NO and sputum eosinophil count ($R^2 = 0.26$, $P < 0.001$) which was best described using a non-linear model. There were no clinically important confounding factors to this model. In non-smokers an exhaled NO concentration of >8.3 p.p.b. at 250 mL/s gave 71% sensitivity and 72% specificity for identifying a sputum eosinophil count of >3%.

Conclusions This value of exhaled NO would seem to be the best for identifying significant eosinophilic airway inflammation. It is applicable to a wide range of non-smoking patients with asthma; exhalation flow does not alter the ability of exhaled NO concentration to detect a sputum eosinophilia.

Keywords asthma, eosinophil, nitric oxide

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Introduction

There is increasing evidence that monitoring airway inflammation is clinically helpful: a sputum eosinophilia identifies patients who respond well to corticosteroid treatment in isolated chronic cough [1], asthma [2] and COPD [3] and a management strategy directed at symptoms and maintenance of a sputum eosinophil count below 3% results in a marked reduction in asthma exacerbations in patients with moderate-to-severe asthma [4]. Assessment of exhaled nitric oxide (NO) concentration is also potentially useful for measuring lower airway inflammation [5, 6] and has the advantage of being simple, repeatable and providing an immediate result, making it particularly well suited to repeated measurement in the clinic.

There is some evidence that monitoring exhaled NO concentration might be clinically useful since Jones et al. found an exhaled NO concentration of 15 p.p.b. (at 250 mL/s) or an increase in exhaled NO concentration of 10 p.p.b. predicted failure of withdrawal of inhaled corticosteroids [7]. However, in a similar study Leuppi did not find a difference

in exhaled NO concentration between patients with asthma who failed corticosteroid withdrawal and those who did not [8]. In the absence of consistent data linking exhaled NO concentration with outcome, a reasonable basis for a study investigating whether measurement of exhaled NO is useful in the assessment and management of asthma is to determine the concentration which best identifies the presence or absence of eosinophilic airway inflammation.

A number of studies have examined the relationship between exhaled NO concentration and sputum eosinophil counts in small homogeneous populations of children [9] and adults with asthma [10]; inhaled corticosteroids [11], cigarette smoking [12], female sex hormones [13, 14] and atopy [15, 16]; have been shown to affect the concentration of exhaled NO in adults and might conceivably alter the relationship between exhaled NO and eosinophilic airway inflammation. The aim of this study was to investigate the relationship between exhaled NO concentration and induced sputum eosinophil count in a large heterogeneous population of adult subjects with asthma and to identify important factors influencing this relationship. We have used these data to determine the concentration of exhaled NO that best identified a sputum eosinophil count above 3%. This cut-off value was used to increase anti-inflammatory treatment in an earlier study

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which showed that an asthma management strategy that aimed to normalize eosinophilic airway inflammation was associated with a reduction in asthma exacerbations [4]. Finally, we have examined whether measurement of exhaled NO concentration at different flows alters the relationship between exhaled NO concentration and sputum eosinophil count.

Methods

Patients

Five hundred and sixty-six consecutive patients who were seen at the Glenfield hospital outpatients with stable asthma were recruited. All subjects had symptoms of asthma and objective evidence of airway hyper-responsiveness and/or airflow variability as demonstrated by one or more of the following: an inhaled concentration of methacholine causing a fall of more than 20% in forced expiratory volume in 1 s (FEV₁) (PC₂₀) of less than 8 mg/mL; an increase in FEV₁ of 15% or greater following inhalation of 200 µg of Salbutamol; or peak flow variability of greater than 20% recorded twice daily over a 2-week period. Atopy was defined as a weal 2 mm greater than control on skin prick testing or specific IgE (Pharma CAP test) test to one or more of dust mite, grass, tree, cat, dog or *Aspergillus* allergens. Subjects were classified as smokers (current smokers), ex-smokers (current non-smokers with >5 pack year smoking history) or non-smokers (never smokers or ex-smokers with <5 pack year history). All patients gave informed consent before entering the study and ethical approval was given by the Leicester Research and Ethics Committee. Demographic details of patients are given in Table 1.

Study design

The data were obtained from a single visit at which exhaled NO concentration was measured prior to measurement of

airway responsiveness, administration of Salbutamol and induced sputum collection.

Measurements

Spirometry was performed using a Vitalograph[®] spirometer (Vitalograph Ltd, Maids Moreton, UK), morning and evening peak flow was measure using Mini-Wright[®] peak flow meters (Clement-Clark International, Harlow, UK) and skin test was performed using standard techniques taking a weal of 2 mm greater than the negative control to be positive. Induced sputum was obtained as previously described [17]. Eosinophil count was expressed as a percentage of non-squamous cells based on a count of 400 inflammatory cells. Exhaled NO was measured using a chemiluminescence analyser (LR2000 Logan Research, Rochester, UK) at an exhalation flow rate of 250 mL/s; the mean of three recordings of the plateau phase of NO was taken as the expired NO concentration [18]. Sixty randomly selected patients had exhaled NO concentration measured on two chemiluminescence analysers, LR2000 (Logan-Sinclair, Rochester, UK) and NIOX (Aerocrine, Stockholm, Sweden), in random order at exhalation flows of 250 mL/s and 10, 30, 50, 100 and 200 mL/s, respectively.

Analysis

Percentage eosinophil count and exhaled NO concentrations were log normally distributed and were therefore log transformed for statistical methods which assume normal distribution. Comparisons between two groups were made using *T*-tests and between more than two groups with one-way ANOVA with Tukey's *post hoc* test for between individual group differences. Correlation coefficients were calculated using Pearson's product-moment correlation coefficient. Multiple independent regression was used to examine the relationship between exhaled NO and sputum eosinophil counts and the effect of smoking, gender, inhaled steroids, age and FEV₁ % predicted on this relationship. Maximal bronchial NO output was calculated as previously described [19]. Receiver-operating characteristic curves were constructed to determine the concentration of exhaled NO which best identified a sputum eosinophil count above 3% separately for smokers and non-smokers and at different flows. Within the non-smoking group ROC curves were subsequently generated separately for atopic and non-atopic subjects, males and females and steroid-treated and naïve subjects. Measurements of 0 were assigned the lowest measurable value for each test (0.25% for sputum eosinophil count, 0.1 p.p.b. for exhaled NO) prior to log transformation. All statistical calculations were made using SPSS 10 for Windows.

Results

Complete data were obtained from 566 (90%) patients from 632 volunteers; 58 (9%) patients were unable to complete sputum induction, three (0.4%) patients were unable to do exhaled NO measurements and five (0.8%) were not able to do either. Table 1 summarizes the baseline characteristics

Table 1. Summary of patient characteristics for cross-sectional data

Number (female)	566 (319)
Age*	49 (16–82)
Current smokers (%)	113 (22)
Ex-smokers (%)	51 (9)
Pack years history (ex- and current smokers only)‡	15 (6–80)
Currently on inhaled steroids (%)	317 (56)
Beclomethasone equivalent dose of inhaled corticosteroid (treated patients only)‡	800 (200–4000)
Currently on oral corticosteroids (%)	33 (6)
Atopic (%)	289 (51)
FEV ₁ % predicted (%)	85 (19)
FEV ₁ /FVC (%)	72.8 (11.9)
PC ₂₀ (mg/mL)†	0.32 (0.71)
Sputum eosinophil count (%)‡	2.3 (0.73)
Sputum neutrophil count (%)	60.1 (25.8)
Exhaled nitric oxide concentration at 250 mL/s (p.p.b.)†	6.7 (0.65)

*Mean (range).

†Geometric mean (log standard deviation).

‡Median (range).

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; PC₂₀, provocative concentration of methacholine causing a 20% fall in FEV₁. Data are shown as mean (standard deviation) except where marked.

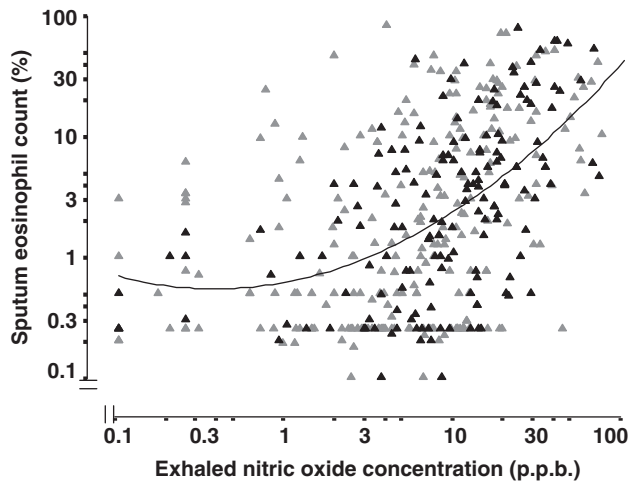


Fig. 1. Scatter plot of sputum eosinophil count vs. exhaled nitric oxide concentration in non-smokers with quadratic regression line; males are shown in black triangles and females in grey triangles.

of the patients who completed both tests. Exhaled NO concentrations were higher in non-smokers than current smokers (6.1 vs. 2.8 p.p.b., mean fold difference 2.2%, 95% confidence intervals (CIs) 1.6, 3.0, $P < 0.001$), but no significant difference between ex-smokers (5.3 p.p.b.) and either of the other groups. There was no significant difference in sputum eosinophil counts between current smokers, ex-smokers and non-smokers ($P = 0.31$). Males had significantly higher exhaled NO concentrations (6.4 vs. 4.3 p.p.b., mean fold difference 1.5%, 95% CI 1.2, 1.9, $P < 0.002$) and sputum eosinophil counts (2.37% vs. 1.6%, mean fold difference 1.4%, 95% CI 1.1, 1.9, $P = 0.009$) than females.

There was a significant association between exhaled NO and sputum eosinophil count in the group as a whole ($R^2 = 0.26$, $P < 0.001$) which was best described with a non-linear (quadratic) model. The association was closer in non-smokers ($R^2 = 0.28$, $P < 0.001$; Fig. 1) than current smokers ($R^2 = 0.15$, $P < 0.001$) or ex-smokers ($R^2 = 0.08$, $P = 0.11$). In the non-smoking model FEV₁ had a significant association with sputum eosinophil count independent of exhaled NO (coefficient $-0.18\%/L$, 95% CI -0.28 , -0.08 ; $P < 0.001$) as did age (coefficient $-0.007\%/year$, 95% CI -0.01 , -0.003 ; $P < 0.001$). Gender, height, inhaled corticosteroids, oral corticosteroids and atopy did not have an association with sputum eosinophil count independent of exhaled NO. The high number of low exhaled NO concentrations in females meant that the linear but not the non-linear relationship differed in men and women.

The area under the ROC curves for smokers (AUC = 0.63%, 95% CI 0.48, 0.78; $P = 0.10$) or ex-smokers (AUC = 0.62%, 95% CI 0.47, 0.77; $P = 0.09$) was not significantly different from 0.5 for identifying a sputum eosinophil count $> 3\%$, and therefore no further analysis was performed on these groups. Within the non-smoking group the area under the ROC curve for identifying a sputum eosinophil count $> 3\%$ was 0.77 (95% CI 0.73, 0.82; $P < 0.001$). An exhaled NO concentration of 8.3 p.p.b. identified a sputum eosinophil count $> 3\%$ with 71% sensitivity and 72% specificity (Fig. 2). The results of the

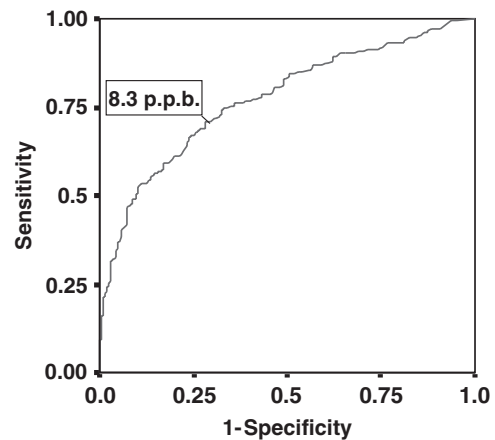


Fig. 2. Receiver operating characteristic curve for non-smokers determining an exhaled nitric oxide concentration which identifies a sputum eosinophil count $> 3\%$.

Table 2. Table of analysis of ROC curves in subgroups using an exhaled nitric oxide concentration of 8.3 p.p.b. to identify a sputum eosinophil count $> 3\%$

	Number	Sensitivity	Specificity	AUC	95% CI	Significance
Smokers	113	—	—	0.63	0.48, 0.78	0.10
Non-smokers	405	71	72	0.77	0.73, 0.82	< 0.001
Steroid naïve						
Female	100	60	82	0.69	0.54, 0.84	0.01
Male	82	73	60	0.71	0.51, 0.91	0.037
Steroid treated						
Female	122	55	68	0.72	0.64, 0.81	< 0.001
Male	101	81	76	0.84	0.76, 0.91	< 0.001
Atopic	206	83	64	0.77	0.64, 0.89	0.001
Non-atopic	199	62	64	0.66	0.54, 0.72	0.032

ROC, Receiver-operating characteristic; AUC, area under curve.

application of these concentrations to subgroups of patients are given in Table 2.

With the exception of 10 mL/s, exhalation flow did not greatly alter the ability of exhaled NO concentration to identify the presence of a sputum eosinophilia, and correlation coefficients for the relationship between exhaled NO and sputum eosinophil counts were broadly similar at all flows (Table 3).

Discussion

This observational study is the first to systematically investigate the relationship between exhaled NO concentration and sputum eosinophil count in a large heterogeneous population of adults with asthma. The study has shown that there is a significant positive association between exhaled NO and induced sputum eosinophil count, a finding that is consistent with findings in smaller, more homogeneous populations [20–22]. These data are consistent with the view that in non-smokers exhaled NO and sputum eosinophil counts identify a common airway abnormality.

We found that the relationship between exhaled NO and sputum eosinophil count in the population as a whole was best described using a non-linear model. This may be an artefact related to measurement of exhaled NO concentration

Table 3. Table of analysis of ROC curves of exhaled nitric oxide (NO) concentration at different flows to determine a sputum eosinophil count of >3% and correlation co-efficients for the association between exhaled NO concentration and sputum eosinophil counts at different flows

Flow (mL/s)	Number	AUC	95% CI	P	Value (ppb)	Sensitivity	Specificity	Correlations	
								Coefficient	P
10	60	0.68	0.54, 0.83	0.02	112	70	70	0.32	0.013
30	60	0.75	0.61, 0.88	0.002	53	74	72	0.37	0.004
50	60	0.77	0.63, 0.9	0.001	36	78	72	0.39	0.002
100	60	0.76	0.63, 0.89	0.001	22	78	72	0.39	0.002
200	60	0.73	0.59, 0.87	0.004	13	74	72	0.30	0.018
250	405	0.77	0.73, 0.82	<0.001	8.3	71	72	0.47	<0.001
CbrMax*	60	0.76	0.63, 0.89	0.001	87 [†]	83	69	0.43	0.001

*nL/min.

[†]Maximal bronchial NO output.

ROC, receiver-operating characteristic.

at a relatively high flow rate resulting in concentrations that were near to the detection level in a significant number of patients. Using a non-linear model we found relatively few factors which significantly confounded the relationship between exhaled NO concentration and sputum eosinophil counts. Furthermore, the exhaled NO concentrations that best identified the presence of eosinophilic airway inflammation were similar in different categories of patients. This is important in that it suggests that a management strategy incorporating this value would be applicable across a wide range of patients. An important caveat is that exhaled NO concentration did not relate closely to sputum eosinophil count in current smokers or ex-smokers and did not predict the presence of a sputum eosinophilia in these patients. In the small number of patients who were taking regular oral corticosteroids, the relationship between sputum eosinophil count and exhaled NO concentration was similar, although we acknowledge that the power to detect a difference in this group is limited.

There are a number of methodological issues with our study that require further discussion. There was a closer relationship between exhaled NO concentration and sputum differential cell count, than total cell count so the analysis was limited to differential cell counts. The underlying assumption that sputum eosinophil differential cell counts or exhaled NO concentration reflect eosinophilic airway inflammation measured in other ways, such as mucosal biopsy cell counts, may not be correct [23]. However, our justification for regarding sputum eosinophil count as the gold standard comparator with exhaled NO concentration is that, unlike these other measures, sputum eosinophil count has been clearly associated with outcome, specifically corticosteroid responsiveness [2] and exacerbation frequency [4]. Furthermore, a management strategy that aims to normalize eosinophilic airway inflammation as well as symptoms is associated with a reduction in asthma exacerbations [4]. We have used values of >3% sputum eosinophil count for indicating the presence or absence of eosinophilic airway inflammation, because this was the value used in this study. Response to inhaled corticosteroids is also most clearly seen in patients with sputum eosinophil counts at this level [24]. Our study was started before the publication of American and European Thoracic Society guidelines and we have consequently used a higher than recommended exhalation flow [18, 25]. However, we measured

exhaled NO concentration at a number of different flows and this did not alter the tests ability to detect a sputum eosinophilia.

There is currently a great deal of interest in measuring airway inflammation in clinical practice. As well as being associated with improved outcome in difficult asthma [4], measurement of airway inflammation may facilitate the identification of unusual disease phenotypes such as non-eosinophilic corticosteroid resistant asthma [24] and eosinophilic bronchitis [26]. However, collection and analysis of induced sputum is more technically demanding and time consuming than measurement of exhaled NO concentration, which can be carried out quickly and can provide an immediate result. Our data can be used to determine appropriate concentrations for exhaled NO to identify the presence of a sputum eosinophilia in studies designed to determine the role of measuring exhaled NO concentration in the clinical management of asthma.

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