

ASTHMA

Longitudinal study of grass pollen exposure, symptoms, and exhaled nitric oxide in childhood seasonal allergic asthma

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Background: Exhaled nitric oxide (NO) has been proposed as a marker of airway eosinophilic inflammation in asthma. There is currently a paucity of longitudinal data relating it to allergen exposure and asthma symptoms.

Methods: Forty four children (6–16 years) with seasonal allergic asthma were sequentially followed before and during the grass pollen season. Asthma symptoms, lung function, NO levels, and pollen counts were recorded. The relationship between exhaled NO and both the pollen levels and asthma control were assessed longitudinally, comparing a subject's measurements with their previous ones.

Results: The median exhaled NO concentration was significantly increased during the pollen season (6.2 v 9.2 parts per billion (ppb), $p < 0.002$; median change 2.9 ppb, 95% confidence interval 1.5 to 5.4). Exhaled NO was best associated with the mean pollen count in the week before measurement. It was also significantly associated with asthma control.

Conclusions: The results suggest that, within a longitudinal model, the exhaled NO concentration is related to preceding allergen exposure and asthma control. It may be clinically more useful to compare exhaled NO values with a subject's previous values than to compare them with a population based normal range.

Asthma affects up to one fifth of children in the UK.¹ It is primarily a disorder of allergic inflammation characterised by the presence of lymphocytes, eosinophils, and mast cells.² Our ability to manage asthma and predict exacerbations is currently impeded by the lack of non-invasive markers of airway inflammation. At present we rely on symptoms and lung function measurements. These do not directly reflect airway inflammation^{3–4} and do not allow us to predict future asthma exacerbations.⁵ Considerable effort has been devoted to finding a useful inflammatory marker for asthma—for example, serum eosinophilic cationic protein has been shown to correlate with airway inflammation in cross sectional studies.^{6–7} However, many markers require a serum sample, results are not immediately available, and correlate poorly with disease severity.⁶ It is also possible to determine a child's percentage sputum eosinophilia.³ However, this is only successful in 50–75% of children, is labour intensive and, again, the results are not immediately available. For all these reasons, other surrogate measures of airway inflammation in asthma have been investigated.

Exhaled nitric oxide (NO) levels have been studied by a number of groups as a possible inflammatory marker of asthma control.^{8–10} Subjects with asthma have higher fractional exhaled NO concentrations (Fe_{NO}) as a group than non-asthmatic controls.¹¹ Fe_{NO} increases with worsening asthma control and, in general, correlates with markers of inflammation within the lungs.¹² Studies comparing Fe_{NO} between groups of treated and untreated asthmatics, while revealing clear differences, demonstrate considerable overlap of data points between the two groups.¹³ However, these studies rely on cross sectional measurements at one time point and do not reflect sequential changes in Fe_{NO} within individuals.

The aim of this study was to determine whether sequential measurements of Fe_{NO} in individual children with seasonal allergic asthma provide a clinically useful tool to monitor asthma. Children with seasonal allergic asthma develop allergic asthma with environmental exposure to grass pollen and are generally asymptomatic during the rest of the year.

This group therefore provides a unique clinical paradigm to study the sequential effects of allergen exposure on allergic asthma.

METHODS

Study participants

Subjects were recruited from the paediatric chest clinic at St Mary's Hospital. All had mild to moderate summer asthma and rhinoconjunctivitis, normal winter lung function without treatment, and sensitisation to *Phleum pratense*. The diagnosis of asthma was based on clinical history, examination, and reversibility with inhaled bronchodilators.¹⁴ Skin testing was performed with negative and positive controls, *P pratense*, three tree pollens, *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, *Alternaria*, *Cladosporium*, *Aspergillus*, cat and dog (positive ≥ 3 mm; ALK, Horsholm, Denmark).¹⁵ Asthma symptoms were treated according to the British Thoracic Society guidelines¹⁶ with inhaled steroids being commenced or increased if there were three episodes of symptoms a week.

Study design

All subjects were assessed at least once before the pollen season when they were clinically well. They were then asked to attend for a clinical assessment at regular 4 weekly intervals during the grass pollen season of 2000. Lung function and Fe_{NO} levels were measured and daily asthma symptoms were recorded on a scale of 0 (none) to 3 (severe). Bi-daily peak expiratory flows were measured using a mini-Wright meter. An exacerbation of asthma was defined as: (1) three episodes of wheeze, chest tightness or shortness of breath per week; (2) two consecutive peak flows of $< 80\%$ of the patient's maximum; or (3) an increase in daily inhaled corticosteroids (≥ 100 μ g) or systemic steroids. Families were asked to contact the clinic if an exacerbation occurred. Exacerbations were managed using the British Thoracic

Abbreviations: Fe_{NO} , fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second

Society guidelines.¹⁶ Asthma control during each week of the study was defined as: excellent (no symptoms); good (symptomatic on 1 day); acceptable (2 days), or poor (at least 3 days).

The study was approved by the research ethics committee at St Mary's Hospital. Informed consent was obtained from all the participants and their parents.

Spirometry

Pulmonary function (forced expiratory volume in 1 second, FEV₁) was measured with a paediatric Masterscreen (Jaeger, Wuertzburg, Germany). FEV₁ measurements were standardised by expressing them as a percentage of the child's best value during the preceding winter. The 95% confidence interval (CI) for the difference in percentage best FEV₁ in subjects measured by different observers in our department is $\pm 7.5\%$.

Measurement of exhaled nitric oxide (Fe_{NO}) levels

Fe_{NO} levels were measured using the single expiratory breath method with a chemiluminescence analyser (Logan Research LR2500, Rochester, UK).¹⁷ A biofeedback mechanism was used to maintain the expiratory flow rate at 250 ml/s and subjects exhaled against a resistance to prevent upper airway contamination.¹⁸ Measurements were made in a standardised manner with the subject standing without a nose clip. Fe_{NO} measurements were repeated until two consecutive results within 10% were obtained; this generally required 2–4 attempts. All Fe_{NO} measurements were undertaken before spirometric testing. The Fe_{NO} measurement was read from the plateau phase. Exhaled NO values were only discarded if the ambient level was above 100 ppb as ambient NO has been shown not to affect readings when the single breath technique is used.¹⁹ Subjects and medical staff involved in their care were blinded to Fe_{NO} results. When Fe_{NO} is measured twice by different observers in our department, the interquartile range for the relative difference is -3.3% to 12.9% .

Pollen counts

Daily atmospheric pollen counts for the North West London area were measured during the pollen season with a Burkard 7 day volumetric spore trap (National Pollen Centre, University of Worcester, UK).

Statistical analysis

Analyses were undertaken using absolute and standardised Fe_{NO} values. Standardised Fe_{NO} values were derived by dividing each Fe_{NO} value by the subject's mean result from the Fe_{NO} result from all their asymptomatic time points outside the pollen season. The relationship between Fe_{NO} and the pollen count was examined using a time series regression model.²⁰ This statistical model calculates the degree of association between Fe_{NO} and the pollen count at the level of the subject. It allows for multiple sequential observations from individual subjects and enables a subject's Fe_{NO} measurements to be compared with their previous measurements. The relationship between Fe_{NO} and pollen count on the assessment day was determined. As exposure to even small amounts of allergen over a number of days can impair lung function,²¹ we looked to see whether Fe_{NO} was related to the mean pollen count during different periods leading up to and including the assessment day. In addition, as allergens give rise to a delayed asthmatic reaction,²² the possibility of a lagged relationship between the two variables was investigated by looking at different time periods leading up to but not including one or more days immediately before the assessment. For this analysis the p values were adjusted using a Bonferroni transformation to take into account the

multiple comparisons examined. The relationship between Fe_{NO} and asthma control was assessed using a summary measure for each subject.²³ The slope of the line of best fit for the relationship between Fe_{NO} and asthma control was calculated for each subject having recoded excellent to poor asthma control as 1–4. We then tested whether the mean slope was significantly different from zero, indicating that Fe_{NO} was related to asthma control. Again this methodology enables individual subject's Fe_{NO} measurements to be compared with their previous ones. Similar comparisons were also undertaken for the percentage best FEV₁.

RESULTS

Forty four subjects (33 male) aged 6–16 years with seasonal allergic asthma were studied sequentially at regular 4 weekly intervals over the grass pollen season (table 1); 38 (86%) had required treatment with inhaled corticosteroids during the previous summer. Pollen counts were initially low (<20) in May, then high (median 58.5, range 3–178) in June and moderate (median 19, range 1–125) in July. A total of 289 weeks of symptom diary scores and Fe_{NO} measurements were obtained from these 44 subjects during the pollen season. Fe_{NO} was measured every 4 weeks, giving a median of two measurements (range 1–3) per subject during the pollen season. Twelve subjects (27%) required treatment with inhaled corticosteroids during the pollen season.

Correlation of standardised Fe_{NO} but not spirometry with pollen count in preceding week

The median Fe_{NO} level before the pollen season was 6.2 ppb (interquartile range (IQR) 4.7–10.4; standardised Fe_{NO} 1.0, IQR 0.8–1.1). This is similar to our normal range for healthy atopic children (median 6.2 ppb, IQR 4.3–10.3, 30 subjects aged 7–16 years with allergic rhinitis, insect allergy or food allergy). Fe_{NO} levels increased significantly during the grass pollen season to 9.2 ppb (IQR 7.6–13.6; standardised Fe_{NO} 1.3, IQR 1.1–1.9; $p < 0.002$, Wilcoxon sum rank test). The median change for each subject from pre-season to the pollen season was 2.9 ppb (95% CI 1.5 to 5.4). This change was seen despite the fact that 12 subjects received inhaled corticosteroids during the pollen season. Whereas Fe_{NO} rose during the pollen season, there was no observed decrease in percentage best FEV₁.

The relationship between Fe_{NO} levels and the pollen count during the 2 weeks before the measurement was investigated

Table 1 Characteristics of study subjects

Mean (range) age (years)	11.2 (7–16)
Male:female	35:9
Mean (range) duration of asthma (years)	4.2 (1–12)
Mean (range) duration of rhinoconjunctivitis (years)	4.2 (1–12)
Sensitisation	
<i>Phleum pratense</i>	44 (100%)
Tree pollens	11 (25%)
House dust mite	14 (32%)
Mean (range) dose of inhaled steroids during previous summer (μ g beclomethasone equivalent)	325 (0–800)
No (%) requiring long acting antihistamine	41 (93%)
No (%) requiring long active topical nasal steroids	21 (48%)
Median (range) number of pre-season exhaled NO readings	5.0 (1–9)
Median (range) number of pollen season exhaled NO readings	2 (1–3)

NO, nitric oxide.

No subjects required treatment with inhaled corticosteroids before the pollen season.

for all subjects and a significant relationship was seen for both absolute and standardised Fe_{NO} levels (table 2). A significant relationship was found between standardised Fe_{NO} values and the pollen count on the day of measurement (regression coefficient 0.0089, $n = 122$, $p < 0.01$). The strength of this association improved if, instead of using the pollen count on the day of measurement, data from counts during the preceding weeks were used. The maximum association was observed using the mean pollen count in the 7 days preceding the standardised Fe_{NO} measurement (regression coefficient 0.0146, $n = 122$, $p < 0.001$). Similar results were obtained when the analysis was repeated after removing the 18 measurements taken while the subjects were receiving treatment with inhaled corticosteroids (data not shown).

We also examined the relationship between the grass pollen count and each subject's percentage best FEV_1 . No correlation between lung function and pollen count was apparent either on the day of measurement or for the mean count in the preceding 7 days (data not shown). Exclusion from the analysis of data from subjects on inhaled steroids did not reveal any relationship between pollen count and spirometric tests that could have been masked by treatment.

Correlation of standardised Fe_{NO} but not spirometry with asthma control

Analysis of asthma control data revealed 208 excellent patient weeks, 41 good weeks, 25 acceptable weeks, and 15 poor weeks. To examine the relationship between Fe_{NO} and asthma control, the slope of the relationship between them

was calculated for each subject. This methodology takes into account the multiple measures from each subject and enables each subject's Fe_{NO} measurements to be assessed in terms of their other measurements. For Fe_{NO} (logarithmically transformed) and asthma control the mean slope was 0.058 (95% CI 0.017 to 0.099), which was very similar to the standardised Fe_{NO} (logarithmically transformed) value (0.058 (95% CI 0.016 to 0.101)). The slope for both absolute and standardised Fe_{NO} is significantly greater than zero, which suggests that Fe_{NO} increases with poorer asthma control. The slope for the relationship between percentage predicted FEV_1 and asthma control was 2.70 (95% CI -0.18 to 5.57), suggesting that there is no relationship between FEV_1 and asthma control in these subjects. When measurements taken while subjects were on inhaled steroids were removed, there was no significant relationship between asthma control and absolute or standardised Fe_{NO} or FEV_1 .

A post hoc analysis of the data suggested that Fe_{NO} may rise before an exacerbation of asthma. Twenty three subjects had a total of 32 asthma exacerbations during the pollen season within 4 weeks after an Fe_{NO} reading, eight of which were observed in the first 5 days after the Fe_{NO} reading. Four subjects required treatment with oral prednisolone. The median standardised Fe_{NO} level during the 5 day period before an exacerbation was 1.93 (IQR 1.6–2.4), higher than the median standardised Fe_{NO} level for all subjects during the pollen season (see above). Unlike the standardised Fe_{NO} values, neither the absolute Fe_{NO} nor the percentage best FEV_1 changed in the week before an exacerbation.

Table 2 Relationship between exhaled NO and pollen count

Time period	Exhaled NO (ppb)	Standardised exhaled NO (ppb)
Day 0		
Regression coefficient	0.0634	0.0089
95% CI	(0.0095 to 0.1173)	(0.004 to 0.013)
p value	0.210	0.01
Day 0 to -2		
Regression coefficient	0.1298	0.0129
95% CI	(0.0775 to 0.1822)	(0.0086 to 0.0173)
p value	<0.001	<0.01
Day 0 to -0.6		
Regression coefficient	0.1394	0.0132
95% CI	(0.0907 to 0.1881)	(0.0090 to 0.0174)
p value	<0.001	<0.01
Day 0 to -13		
Regression coefficient	0.1255	0.0120
95% CI	(0.0764 to 0.1747)	(0.0077 to 0.0163)
p value	<0.001	<0.01
Day -1 to -5		
Regression coefficient	0.1364	0.0146
95% CI	(0.0750 to 0.1978)	(0.0096 to 0.0196)
p value	<0.001	<0.01
Day -2 to -5		
Regression coefficient	0.1332	0.0112
95% CI	(0.0734 to 0.1930)	(0.0061 to 0.0163)
p value	<0.001	<0.01
Day -1 to -7		
Regression coefficient	0.1251	0.0144
95% CI	(0.0732 to 0.1771)	(0.0103 to 0.0186)
p value	<0.001	<0.01
Day -2 to -7		
Regression coefficient	0.0131	0.0131
95% CI	(0.0088 to 0.0173)	(0.0088 to 0.0173)
p value	<0.001	<0.01
Day -8 to -14		
Regression coefficient	0.0499	0.0081
95% CI	(0.0020 to 0.0979)	(0.0041 to 0.0121)
p value	0.410	<0.01

Data from 44 subjects assessed a total of 122 times during the pollen season. Relationship examined for the mean pollen count during different time periods from the day that exhaled NO was measured (day 0) to 14 days before (day -14). Data presented as regression coefficients (95% confidence intervals) with their associated p values for the relationship between exhaled NO levels and the pollen count were estimated using a time series regression model. Bonferroni correction for multiple comparisons was applied to p values.

DISCUSSION

The clinical usefulness of Fe_{NO} as a surrogate marker of airway inflammation in asthma is limited by a paucity of longitudinal data in individual patients. In this study we have further evaluated the clinical role of this marker by sequentially following a group of children with seasonal allergic asthma through the grass pollen season. Like other investigators,¹⁰ we found that Fe_{NO} increases with environmental allergen exposure. We have extended these observations to show that Fe_{NO} also correlates with preceding allergen load and that the timing of exposure is important. In addition, we have shown that Fe_{NO} levels increase with worsening asthma control. Our results show that spirometry does not significantly correlate with allergen load or with asthma control, even when it is standardised for each individual.

Fe_{NO} values are usually presented in absolute terms (parts per billion). When presented in this way, the range of normal values is wide.^{10–11, 24} Furthermore, there are suggestions that levels vary with many factors including age, sex, body size, and atopic status.^{12–24, 25} In this respect, Fe_{NO} behaves similarly to other lung function measures such as FEV_1 ; this may explain the considerable overlap seen between different groups of patients in cross sectional studies.¹³ To overcome this we normalised values to each subject's mean Fe_{NO} level when they were asymptomatic before the pollen season. Data from our clinic suggest that Fe_{NO} values have minimal variability in these circumstances. In a total of 148 measurements in 16 subjects the median difference from each subject's mean Fe_{NO} as a proportion of the mean Fe_{NO} was -0.037 (IQR -0.488 to 0.587).

The value of using standardised Fe_{NO} data is illustrated in a number of ways. Firstly, the mean Fe_{NO} during the grass pollen season was within our normal range. However, when intersubject variability is removed by standardising seasonal values to each subject's pre-seasonal level, exhaled NO is seen to be significantly increased during the pollen season. Secondly, using statistical techniques which, like standardised Fe_{NO} , enable an individual's observations to be compared with their previous measurements, we found relationships between Fe_{NO} and both the pollen count and asthma control. It therefore seems that levels of exhaled NO provide better clinical information when compared with a child's previous exhaled NO level than when compared with a population based normal range. This approach may not be useful for children with symptoms that persist throughout the year as it may not be possible to measure a baseline Fe_{NO} when they are symptom free. Further studies are also needed to test the general usefulness of this approach in other asthmatic populations.

In a group of 21 children with seasonal allergic asthma Baraldi *et al*¹⁰ showed that Fe_{NO} increases during the grass pollen season. We have extended these observations to look at the temporal relationship between Fe_{NO} and allergen exposure and found that Fe_{NO} correlates best with the pollen count in the previous 7 days (table 2). This may be explained by the dual response following allergen exposure consisting of a transient early phase and a more prolonged late phase inflammatory response 4–8 hours later. Fe_{NO} has been found to be increased only during the late phase asthmatic reaction.²⁶ With Fe_{NO} increasing during the late phase response, it is not surprising that it correlates better with the mean pollen count in the days before its measurement than the count on the day of measurement.

Within this study we have restricted our experimental model to focus on the relationship between Fe_{NO} , pollen exposure, and asthma control. In doing this we have used pollen counts from only one site in North West London although the residences of the subjects were spread across

this area of London. While the trends of exposure for all subjects will be similar within this small geographical area, these values may not accurately reflect personal pollen exposure. Furthermore, we have not taken into account the many other factors that influence the allergenic potential of grass pollen including the use of inhaled corticosteroids and meteorological parameters such as rainfall, humidity, and thunderstorms. These meteorological factors seem to exert their influence by inducing the release of submicron allergenic particles from the large 15–40 μm pollen grains, thereby allowing them to penetrate further into the lower airways.²⁷ Despite these factors, we have still been able to demonstrate relationships between exhaled NO and both pollen exposure and asthma control indicating that, within a longitudinal model, these relationships are very robust.

The effects of inhaled or oral corticosteroids on Fe_{NO} measurements are unclear. Many paediatric and adult studies have clearly shown high Fe_{NO} levels in untreated asthma that decline with steroid therapy.^{8, 28} Conversely, other studies have found increased levels in association with asthma symptoms despite corticosteroid therapy.²⁹ The data presented here suggest that Fe_{NO} levels are related to allergen exposure, whether or not subjects are taking inhaled corticosteroids. It should be remembered, however, that only a quarter of the subjects required treatment with inhaled corticosteroids during the study and then only for a relatively short period of time.

This study has a number of limitations. Firstly, the method for measuring Fe_{NO} differs from the current American Thoracic Society recommendations.¹² An exhalation flow of 250 ml/s was used as originally recommended by the only guidelines available when the study commenced.¹⁷ Using a lower flow rate is unlikely to have altered the pattern of the results, but it would have increased the measured Fe_{NO} values increasing the differences seen with changing allergen exposure or asthma control.³⁰ Secondly, we have chosen children with grass pollen induced asthma as a clinical model. While there is no reason to believe that relationships seen in this group will not be replicated in children with perennial allergic asthma, this work needs to be validated in this important group of patients, particularly where viral infections are the key trigger for exacerbations. It is possible that, in this group, baseline Fe_{NO} values would need to be measured when they were asymptomatic after treatment but this awaits further study. In addition, our study group was relatively small and contained a predominance of boys. Although there is no reason to believe that there are sex differences in Fe_{NO} levels, it is important that this study is replicated in a larger group with more girls. Lastly, the potential relationship that we highlight between an exacerbation and raised Fe_{NO} measurement in the preceding week was part of an exploratory analysis of the data. Given the small number of standardised Fe_{NO} values within 5 days of an asthma exacerbation and the fact that it was a post hoc analysis of the data, we have not presented a formal statistical analysis. The presence of such a relationship is, however, supported by the results of Jones *et al*³¹ who found that Fe_{NO} measurements predicted exacerbations of asthma in adults from whom inhaled corticosteroids had been withdrawn. The value of a raised standardised Fe_{NO} as a predictor of future exacerbations should be tested in further studies.

We have presented data which show that Fe_{NO} levels are related to prior environmental allergen exposure and asthma symptoms and potentially predict future exacerbations. In contrast, lung function in this study does not reflect either allergen load or asthma severity. Sequential Fe_{NO} measurements may therefore be a more useful clinical tool than spirometry to monitor asthma control. This study provides

preliminary evidence that sequential standardised Fe_{NO} measurements may be useful for monitoring children with asthma in a clinical setting. Access to a sequential, non-invasive, rapidly available measure of airway inflammation may allow us to reduce treatment to the minimum required to suppress inflammation and, furthermore, to respond to pre-symptomatic increases in inflammation. The advantage of using a measure of airway inflammation in directing asthma management has recently been demonstrated in a randomised controlled adult study using sputum eosinophilia in which the intervention group had better asthma control without increasing inhaled corticosteroid usage.³² More frequent sequential assessment of Fe_{NO} in children will allow us to determine whether such measurements will translate into improved patient outcome in the clinical setting and whether a rise in standardised Fe_{NO} can successfully predict an asthma exacerbation.

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