

Evidence for different subgroups of difficult asthma in children

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Abstract

Background—Children with difficult asthma experience frequent symptoms despite treatment with high dose inhaled steroids. Persistent symptoms may result from persistent airway inflammation which can be monitored by measuring exhaled nitric oxide (NO). This study aimed to assess the role of airway inflammation, using NO as a surrogate, in children with difficult asthma and to investigate the response to oral prednisolone.

Methods—NO was measured in 23 children (mean age 11.7 years) with difficult asthma, before and after 2 weeks of treatment with oral prednisolone. The clinical response was assessed by spirometric tests, peak flow, bronchodilator use, and symptoms. Adherence to treatment was assessed by measuring serum prednisolone and cortisol concentrations. NO was measured in 55 healthy children to establish a normal range.

Results—NO concentrations were higher in asthmatic patients than in controls (geometric mean 11.2 v 5.3 ppb, $p < 0.01$). Using grouped data, the concentration of NO fell following prednisolone (11.2 v 7.5 ppb, $p < 0.01$) accompanied by an improvement in morning peak flow ($p < 0.05$). The baseline NO concentration was raised (> 12.5 ppb) in nine asthmatic patients and remained high after prednisolone in five. Thirteen had normal levels of NO (< 12.5 ppb) before and after prednisolone. Thirteen asthmatic patients remained symptomatic following prednisolone; NO levels were raised on both occasions in five of these and were normal in seven.

Conclusions—As a group, the asthmatic subjects demonstrated evidence of airway inflammation which responded to prednisolone. At least two subgroups of patients were identified: one with persistently raised NO levels despite treatment with oral prednisolone indicating ongoing steroid insensitive inflammation, and another with normal levels of NO. Both subgroups included patients with persistent symptoms, which suggests that different patterns of difficult asthma in children exist.

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Keywords: exhaled nitric oxide; asthma; children

The European Respiratory Society (ERS) Taskforce report on difficult/treatment resistant asthma suggests that children whose asthma remains poorly controlled despite treatment with > 800 $\mu\text{g}/\text{day}$ inhaled budesonide or equivalent should be classified as “difficult”.¹ It is unclear whether persistent symptoms are related to ongoing airway inflammation or some other mechanism, and whether increasing the corticosteroid dose well beyond that recommended by the current guidelines is beneficial.² Demonstration of persistent inflammation despite doses of corticosteroids high enough to cause side effects would suggest a degree of steroid insensitivity in the airways and the need to consider alternative anti-inflammatory treatments. Conversely, there would seem to be little logic in increasing the dose of anti-inflammatory medication if there is no evidence of ongoing inflammation.

Exhaled nitric oxide (NO) is a marker of airway inflammation which can be measured in children.^{3,4} NO is produced from the conversion of L-arginine to L-citrulline, a reaction catalysed by nitric oxide synthase (NOS). The inducible form of this enzyme, iNOS, has been identified in a range of cells including endothelial and epithelial cells, macrophages, smooth muscle cells, fibroblasts, and neutrophils.⁵ NO has been shown to be raised in asthmatic subjects not treated with steroids.⁶ Treatment of patients with mild to moderate asthma with inhaled steroids leads to a fall in NO concentrations and reducing the steroid dose results in a rise in the concentration of NO.⁷ These findings suggest that NO reflects airway inflammation in asthma and may be useful in monitoring the anti-inflammatory effect of steroids.

A study in adults with difficult asthma found raised NO levels compared with controls, with the highest levels being detected in a subgroup of patients taking oral prednisolone.⁸ This subgroup may represent patients with the most severe disease or those with relative steroid insensitivity. The aim of this study was to test the hypothesis that persistent symptoms in children on high dose inhaled steroids are caused by ongoing airway inflammation, as measured by NO, and that a clinical response

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to prednisolone would be reflected by a fall in the NO level.

Methods

SUBJECTS

Patients

Children were recruited from the paediatric asthma clinic of the Royal Brompton Hospital. Inclusion criteria were as follows: (1) clinical diagnosis of asthma (a history of at least two of the following: recurrent wheeze, chest tightness, shortness of breath, cough); (2) exclusion of other diagnoses mimicking asthma; (3) increase in forced expiratory volume in one second (FEV₁) after bronchodilator of >15% or a concentration of methacholine required to provoke a 20% fall in FEV₁ (PC₂₀) of ≤8 mg/ml; (4) symptoms requiring rescue bronchodilator on average ≥3 days/week for the past 3 months despite treatment with ≥1000 µg/day inhaled budesonide or equivalent; (5) no clinical evidence of a respiratory infection in the preceding 2 weeks.

Controls

Exhaled NO was measured on a single occasion in a group of healthy children with normal spirometric parameters and no history of acute or chronic respiratory disease.

The study was approved by the local ethics committee and informed consent was obtained from parents of all children and from the children, if appropriate.

STUDY PROTOCOL

Patients were seen twice. Before visit 1, patients completed a 2 week diary in which they recorded bronchodilator use, symptom free days, and morning and evening pre-bronchodilator peak expiratory flow (PEF) using a Wright mini peak flow meter. At visit 1 spirometric tests were performed followed by measurement of NO concentrations. Methacholine challenge was then performed and the spirometric tests were repeated after administration of salbutamol. Blood was taken for measurement of total IgE and radioallergosorbent tests (RAST) to cat, dog, house dust mite (HDM), and grasses. Patients were then treated with oral prednisolone (40 mg/day) for 2 weeks. The diary was continued and, after completion of the prednisolone course, the patients were reviewed. Spirometric tests, NO measurement, and methacholine challenge were repeated. Blood was taken for measurement of serum prednisolone and cortisol concentrations and the time of the last prednisolone dose was recorded.

SPIROMETRY AND BRONCHIAL RESPONSIVENESS

Spirometric tests were performed using a portable spirometer (Compact Vitalograph) which was calibrated before each set of measurements with a 1 litre syringe. Patients were asked to withhold long acting bronchodilator for 12 hours and short acting bronchodilator for 4 hours, if possible. Three technically acceptable manoeuvres were performed and the highest value of FEV₁ was recorded. This value did not exceed the next highest by more than 100 ml.⁹

Following spirometric tests and measurement of NO, bronchial responsiveness to methacholine was measured using a dosimeter, providing the baseline FEV₁ was >65% predicted and short acting (but not long acting) bronchodilators had been withheld for more than 4 hours. Doubling concentrations of methacholine were inhaled and the PC₂₀ was calculated by interpolation of the log dose response curve. Post-bronchodilator FEV₁ was also measured. Salbutamol was administered (5 mg via a nebuliser or 1 mg via a spacer, depending on patient preference) and spirometric tests were performed 15 minutes later. For those patients who underwent methacholine challenge, the post-bronchodilator FEV₁ was measured after the challenge test.

CLINICAL DATA

Data from the last 7 days before each visit were obtained from patient diaries. Symptom free days were calculated from the number of days on which rescue bronchodilator had not been used and expressed as a percentage of total days studied. Bronchodilator use was calculated as the mean number of puffs of bronchodilator used per day. Morning PEF was calculated as the mean of the morning pre-bronchodilator PEF values. PEF coefficient of variation was calculated from all the morning and evening PEF values. Coefficient of variation is the standard deviation of all values divided by their mean and gives a measure of peak flow variability.¹⁰

MEASUREMENT OF NO

Exhaled NO was measured using a chemiluminescence analyser (LR 2000 series; Logan Research, Rochester, UK) according to ERS guidelines.¹¹ The equipment was sensitive to NO from 0 to 5000 parts per billion (ppb) and gave continuous online recordings with a resolution of about 0.3 ppb, with a response time of <0.5 seconds. The analyser also measured CO₂ (resolution 0.1% CO₂, response time 200 ms) and exhalation pressure and volume (or flow) in real time. The analyser was calibrated weekly using certified NO mixtures (0–490 ppb) in nitrogen (BOC Special Gases, Guildford, UK).

Subjects breathed room air. Ambient NO levels were measured and did not exceed 120 ppb.¹² After maximum inspiration subjects exhaled for as long as possible (slow vital capacity manoeuvre) into a wide bore tube. A fine bore Teflon tube connected directly to the analyser continuously sampled the exhaled air adjacent to the mouthpiece at 0.25 l/min. Subjects kept the flow during expiration within a constant range by the use of auditory and visual guides (lights, the flapping ears of a toy and a tune) which were activated when exhalation was within the required range of expiratory flow (200–280 ml/s). The mouth pressure generated (5 cm H₂O) was sufficient to exclude nasal NO production by raising the soft palate and preventing nasal contamination. The NO trace was observed until a stable plateau, lasting at least 2 seconds, was reached. The plateau value was recorded. The test was

Table 1 Clinical characteristics of children with difficult asthma

Number	23
Age (years)	11.7 (10.4 to 13.1)
Male:female	13:10
Inhaled steroid dose ($\mu\text{g}/\text{day}$)	
Budesonide (n=9)	2144 (1701 to 2588)
Fluticasone (n=14)	1500 (1216 to 1927)
Maintenance oral prednisolone	6
Long acting bronchodilator	17
Baseline FEV ₁ (% pred)	72 (63 to 81)
Post-bronchodilator FEV ₁ (% pred)	82 (74 to 90)
Alternative treatments	8 (theophylline n=4; theophylline and subcutaneous terbutaline n=1; cyclosporin n=1; monteleukast n=2)
Atopic	23

FEV₁ = forced expiratory volume in one second.

Data are expressed as means with 95% confidence intervals, or proportions.

repeated three times and the mean value calculated. Results were excluded if it was not possible to determine a plateau value because expiration time or control of expiratory flow was inadequate. The single determination 95% range for NO values for asthmatic subjects in our laboratory is ± 2.3 ppb.¹³

ATOPY

Total IgE and RAST to HDM, cat, dog and grasses were measured. IgE levels above the normal range for age or one or more positive RAST tests were taken as evidence of atopy.

PREDNISOLONE AND CORTISOL

Serum prednisolone and cortisol levels were measured using high performance liquid chromatography. For samples taken <24 hours after the last dose, adherence was considered satisfactory if there was detectable prednisolone and a cortisol level of <100 nM.^{14 15} For samples taken >24 hours after the last dose, adherence was considered satisfactory if the cortisol level was <100 nM (because of the probability of complete clearance of prednisolone after 24 hours).¹⁶

STATISTICAL ANALYSES

NO is log normally distributed in the population so the NO data were log transformed for statistical analysis.¹⁷ The upper 95% confidence range for NO in the control group was

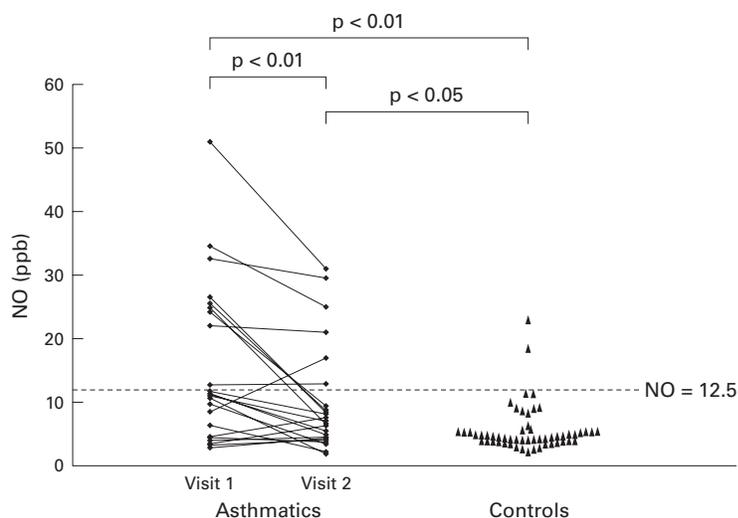


Figure 1 Exhaled nitric oxide (NO) levels in children with difficult asthma before and after prednisolone compared with healthy controls.

calculated as the antilog of (mean + two standard deviations) of the log transformed data. PC₂₀ data was also log transformed to normalise the distribution. For normally distributed data paired *t* tests were used for comparison between visits and unpaired *t* tests for comparison between groups. For non-normally distributed data Wilcoxon matched pairs tests were used for comparison between visits and Mann-Whitney U tests for comparison between groups. Correlations were measured using Pearson correlation for normally distributed data and Spearman rank correlation if one or both sets of data were non-normally distributed. Results for log transformed data are expressed as geometric means and 95% confidence intervals (95% CI). Results from other normally distributed data are expressed as means and 95% CI. Results from non-normally distributed data are given as medians and interquartile range (IQR).

Results

SUBJECTS

Twenty seven patients were recruited. Adherence was considered satisfactory in 25, and suitable NO measurements for inclusion in the analysis were obtained on both visits in 23 patients (table 1). NO concentrations in these children were compared with those measured in 55 healthy children, with an age range similar to the asthmatic group (mean 10.7 years (95% CI 9.9 to 11.6), 42% boys).

Some patients were unable to withhold bronchodilator use for the required time. Seventeen of the 23 patients were taking a regular long acting bronchodilator. On visit 1 eight of these 17 patients withheld long acting bronchodilators and 19 of the 23 patients withheld short acting bronchodilators before spirometric testing. On visit 2 the proportions were six of 17 and 17 of 23 for long and short acting bronchodilators, respectively. Methacholine challenge was performed in 10 children on visit 1 and in 15 on visit 2. Seven children underwent methacholine challenge on both visits, having either taken (n=1) or omitted (n=6) long acting bronchodilator on both occasions. Post-bronchodilator FEV₁ was measured in all patients. Completed diaries were collected from 19 of the 23 children and partially completed diaries (lacking PEF data) were collected from two.

EXHALED NO

The upper 95% confidence range for NO in the control group was 12.5 ppb. NO levels were higher in the asthmatic group than in the controls on both visit 1 (geometric mean 11.2 (95% CI 7.9 to 15.9) ppb *v* 5.3 (95% CI 4.8 to 6.0) ppb, *p*<0.01) and visit 2 (geometric mean 7.5 (95% CI 5.3 to 10.1) ppb *v* 5.3 (95% CI 4.8 to 6.0) ppb, *p*<0.05; fig 1). In the asthmatic group NO levels fell following prednisolone (11.2 (95% CI 7.9 to 15.9) ppb *v* 7.5 (95% CI 5.3 to 10.1) ppb, *p*<0.01).

CLINICAL RESPONSE TO PREDNISOLONE

Following prednisolone there was an increase in mean morning PEF from 76 (95% CI 65 to

Table 2 Nitric oxide (NO) and clinical measurements in children with difficult asthma before and after prednisolone

Measurement	Visit 1	Visit 2	p value	Mean difference before and after treatment
NO (ppb)	11.2 (7.9 to 15.9)	7.5 (5.3 to 10.1)	<0.01	1.5 (1.2 to 2.0)
FEV ₁ (% predicted)	72 (63 to 81)	78 (70 to 86)	NS	
Post bronchodilator FEV ₁ (% predicted)	82 (74 to 90)	84 (77 to 90)	NS	
PC ₂₀ (mg/ml) (n = 7)	0.92 (0.19 to 4.41)	2.12 (0.49 to 9.06)	<0.05	0.44 (0.21 to 0.91)
Symptom free days (%) (n = 21)	0 (0–0.25)	0 (0–56)	NS	
Bronchodilator use (puffs per day) (n = 21)	3 (2–3.8)	2.6 (0.8–3.1)	NS	
Mean morning PEF (% predicted) (n = 19)	76 (65 to 87)	84 (76 to 93)	<0.05	8.1 (1.7 to 14.4)
PEF coefficient of variation (n = 19)	10.8 (6.9–19.8)	9.4 (4.4–17.4)	NS	

FEV₁ = forced expiratory volume in one second; PC₂₀ = concentration of methacholine provoking a fall in FEV₁ of 20% or more; PEF = peak expiratory flow. Pre- and post-bronchodilator FEV₁ and morning PEF data are expressed as means with 95% confidence intervals. NO and PC₂₀ data are expressed as geometric means with 95% confidence intervals. Symptom free days, bronchodilator use, and PEF coefficient of variation are expressed as medians with interquartile range. For statistically significant findings an estimate of effect is given as the mean (95% confidence interval) difference before and after treatment.

87% predicted to 84 (95% CI 76 to 93)% predicted ($p < 0.05$) and in PC₂₀ from a geometric mean of 0.92 (95% CI 0.19 to 4.41) mg/ml to 2.12 (95% CI 0.49 to 9.06) mg/ml ($p < 0.05$). There was no significant change in bronchodilator use, pre- or post-bronchodilator FEV₁, PEF coefficient of variation, or symptom free days (table 2). There was a weak but significant correlation between log NO and bronchodilator use ($r = 0.5$, $p < 0.05$) on visit 1. There was no correlation between log NO and any of the other parameters on visit 2.

IDENTIFICATION OF SUBGROUPS

The identification of subgroups of patients was considered in two ways—firstly, by looking at the NO response to prednisolone and, secondly, by looking at the NO levels in patients with persistent symptoms following prednisolone. Nine patients had raised NO levels (> 12.5 ppb) on visit 1. Of these nine patients, NO levels became normal (< 12.5 ppb) in four and remained raised in five. Thirteen patients had normal NO levels on both occasions. One patient had an NO level of 8 ppb on visit 1 which rose to 17 ppb on visit 2. This was associated with a marked increase in anxiety and symptoms on the two days before visit 2, which also included a bronchoscopy.

Of the 21 patients who recorded symptoms and bronchodilator use, five became asymptomatic during the prednisolone course, needing rescue bronchodilator on ≤ 1 of the last 7 days. These included four patients who had normal NO levels on both occasions. Thirteen of the 21 patients continued to have daily symptoms

requiring rescue bronchodilator, and these included the five patients with raised NO levels on both visits (fig 2). Seven of the remaining eight persistently symptomatic patients had normal NO levels on both occasions. There were no significant clinical differences between these seven patients and the five with persistently raised NO concentrations.

Discussion

This study examines the role of NO in children with difficult asthma. Treatment of these patients remains unsatisfactory, with little evidence to guide the choice of therapy. Although current guidelines emphasise the use of anti-inflammatory treatment for patients with poorly controlled asthma, it is unclear what role airway inflammation plays in those patients who remain symptomatic despite such treatment.

Analysing the results of the group as a whole, NO levels were higher in children with difficult asthma than in the control group. NO fell following treatment with oral prednisolone, and this was paralleled by an improvement in some of the clinical parameters (PEF, PC₂₀). These findings are in keeping with the original hypothesis. However, there was little correlation between NO levels and the clinical parameters measured. The surprising finding was the identification of at least two different subgroups of patients, one with persistently raised NO levels, even after prednisolone, and one with normal NO levels both before and after prednisolone. Both subgroups included patients with daily symptoms despite prednisolone. This suggests a different basis for symptoms between the two subgroups, with inflammation playing a less important role in those patients with normal NO levels.

The children in the study attended a tertiary hospital asthma clinic and reported symptoms on ≥ 3 days a week despite treatment with ≥ 1000 $\mu\text{g/day}$ inhaled steroids. Asthma was confirmed on clinical and physiological grounds, other causes of symptoms mimicking asthma were excluded, and repeated attempts were made to optimise treatment adherence and inhaler technique. Despite these efforts, the patients continued to report frequent symptoms.

Previous studies have shown that NO may be raised during viral respiratory tract infections.¹⁸ Patients had to be free of a clinical upper or lower respiratory tract infection for 2 weeks

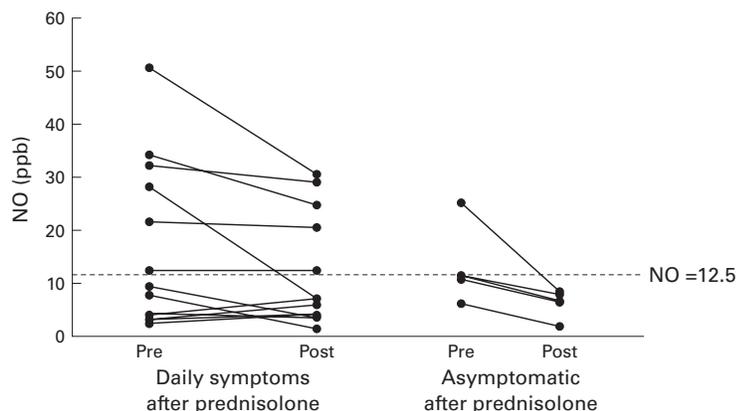


Figure 2 Exhaled nitric oxide (NO) levels in symptomatic and asymptomatic patients before and after prednisolone.

before visit 1 and during the prednisolone course. This assessment was based on reported symptoms and clinical examination. In common with most, if not all, studies of induced sputum, no objective tests of infection were performed. However, all children gave a history of ≥ 3 months of continuing poor control of their asthma, which is unlikely to be attributable to recurrent viral infections.

Demonstration of adherence to prednisolone therapy was crucial. Ways of ensuring adherence include directly observed therapy, either in hospital or at home, or the administration of an intramuscular depot injection of the corticosteroid triamcinolone.¹⁹ Financial restrictions and a limit on inpatient numbers prevented the first option, and it was felt that an injection would be unacceptable to most patients. We therefore prescribed oral prednisolone and measured serum prednisolone and cortisol levels at the end of the course. Prednisolone is detectable in the blood within 1–2 hours of administration, but is usually cleared by 24 hours.¹⁶ Measurement of serum prednisolone levels is therefore a direct measure of recent adherence. It does not, however, give information regarding how many previous doses have been taken, and we acknowledge that patients are more likely to be adherent around the time of a visit. This is a potential weakness of the study. Some measure of longer term adherence can be assessed indirectly by the suppressive effect of prednisolone on adrenal function. A single cortisol level of < 100 nM is strongly suggestive of adrenal suppression and may replace the need for more complicated tests of adrenal function.¹⁴ Measuring serum prednisolone and cortisol levels simultaneously provided a simple and practical means of assessing both short and longer term adherence.¹⁵ However, even these two measurements cannot exclude the possibility that some prednisolone doses were omitted. A few children were given their last dose > 24 hours before visit 2 and, in these cases, assessment of adherence was based only on the cortisol level.

This study is the first to investigate the role of airway inflammation in children with difficult asthma. The higher baseline NO concentration in the asthmatic group is in keeping with the results of the adult cross sectional study by Stirling *et al.*⁸ Our study provides further information by examining the response to prednisolone. One of the parameters used to assess the clinical response to prednisolone was measurement of airway hyperresponsiveness (AHR) using methacholine challenge. A recent study has suggested that use of serial measurements of AHR to guide treatment may improve asthma control.²⁰ Unfortunately, methacholine challenge could be performed on both visits by only seven children, which suggests that it is not a useful tool in the assessment of difficult asthma in children.

The results suggest that measurement of NO can detect different patterns of difficult asthma. A subgroup of patients with evidence of airway inflammation, reflected by raised NO levels on visit 1, was identified. Some of these patients had a marked fall in NO levels after prednisolone,

whereas in others the levels continued to be raised. It is possible that these patients represent a spectrum of steroid responsiveness and that those with persistently raised NO levels might have responded to a longer course or a higher dose of prednisolone. Alternatively, these children may represent two different subgroups of difficult asthma. This study cannot distinguish between these possibilities. The most surprising pattern to emerge was in the subgroup of children who had normal NO levels both before and after prednisolone. This suggests that these children had little airway inflammation to account for their symptoms, even before the course of prednisolone. It has been suggested that higher levels of NO are found in patients with atopic asthma than in those with non-atopic asthma.^{21–22} Such a distinction does not appear to provide an explanation for the subgroups identified in this study, since all the patients were atopic.

Patients with daily symptoms after prednisolone could, with one exception, be divided into two subgroups—those with raised NO levels on both occasions, in whom symptoms are likely to be due to ongoing inflammation, and those with normal NO levels on both occasions. The basis for symptoms in this latter group is unclear. Although NO appears to reflect airway inflammation in asthma, the nature of this inflammation is still unknown and needs to be investigated further by comparison with other methods of assessing the airway. Recent work suggests that NO may reflect airway eosinophilia.^{3–4} Patients with persistent symptoms and raised NO levels may therefore be viewed as having severe classical asthma with uncontrolled eosinophilic inflammation. However, even in atopic asthma there is a poor correlation between bronchial hyperreactivity and sputum and lavage eosinophilia, implying the importance of other mechanisms^{23–24} such as non-eosinophilic inflammation and the effects of a reduction in baseline airway calibre.²⁵ Persistent symptoms with normal NO levels may reflect non-eosinophilic inflammation and this division of severe asthma based on the degree of eosinophilia has been proposed by Wenzel *et al.*²⁶ The neutrophil is an important component of the late phase response²⁷ and in endotoxin induced asthma,²⁸ and may play a role in severe asthma.^{29–30} Alternatively, normal NO levels may indicate a non-inflammatory basis for symptoms. We plan to investigate the significance of our findings through the use of more direct methods of assessing the pattern of airway inflammation in these children, such as bronchoalveolar lavage and endobronchial biopsy.

These findings are of academic and practical importance. For patients in whom steroids appear to have little impact, alternative treatments are often tried. Demonstration of persistent steroid insensitive airway inflammation would support the trial of another anti-inflammatory drug such as cyclosporin or methotrexate. This study suggests that steroid insensitive inflammation, reflected by persistently raised NO levels, was present in a few children with difficult asthma. There is also a

suggestion that there was no persistent inflammation in a proportion of children with frequent symptoms. In these children a stronger case might be made for other treatment options such as subcutaneous terbutaline. However, the main conclusion of this study is that there are different patterns of difficult asthma. Recognition of these patterns may help towards increasing our understanding of the nature of difficult asthma, as well as allowing us to make a choice of treatment on a rational rather than a haphazard basis.

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Conflict of interest: None

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