Increase in exhaled nitric oxide levels in patients with difficult asthma and correlation with symptoms and disease severity despite treatment with oral and inhaled corticosteroids

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Abstract
Background—Patients with difficult asthma suffer chronic moderate to severe persistent asthma symptoms despite high doses of inhaled and oral corticosteroid therapy. These patients suffer a high level of treatment and disease related morbidity but little is known about the degree of airway inflammation in these patients.

Methods—Fifty two patients were examined to assess levels of exhaled nitric oxide (NO) as a surrogate marker of inflammatory activity in this condition. From this group, 26 patients were defined with severe symptoms and current physiological evidence of reversible airway obstruction requiring high dose inhaled (>2000 µg beclometasone dipropionate (BDP) equivalent) or oral steroid therapy to maintain disease control.

Results—Exhaled NO levels were higher in subjects with difficult asthma (mean 13.9 ppb, 95% CI 9.3 to 18.5) than in normal controls (7.4 ppb, 95% CI 6.9 to 7.8; p<0.002), but lower than levels in steroid naive mild asthmatics (36.9 ppb, 95% CI 34.6 to 39.3; p<0.001). Prednisolone treated patients had higher exhaled NO levels than patients only requiring inhaled corticosteroids (17.5 ppb, 95% CI 11.1 to 24.0 versus 7.2 ppb, 95% CI 4.6 to 9.8; p = 0.016), suggesting greater disease severity in this group. Non-compliance with prednisolone treatment was observed in 20% of patients but this did not explain the difference between the treatment groups. Exhaled NO levels were closely correlated with symptom frequency (p = 0.03) and with rescue β agonist use (p<0.002), but they did not correlate with lung function.

Conclusions—Exhaled NO may serve as a useful complement to lung function and symptomatology in the assessment of patients with chronic severe asthma, and in the control and rationalisation of steroid therapy in these patients.

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Keywords: exhaled nitric oxide; inflammatory markers; difficult asthma

Difficult asthma is characterised by uncontrolled symptoms and a failure to respond to usual “adequate treatment”. Patients frequently require maintenance oral corticosteroids and have inhaled corticosteroid requirements of more than 2 mg per day. Disease impact in these patients is increased by uncontrolled symptoms, a higher exacerbation rate, high dose therapy, and the adverse effects of this therapy. Although patient numbers are relatively small, the significance of this group results from their disproportionate morbidity and consumption of resources.

Monitoring asthma activity has the potential to reduce morbidity and, indeed, forms the basis for current management guidelines. Peak expiratory flow (PEF) is currently used to monitor disease control but may not reflect the degree of inflammation in the airways. Since bronchial biopsy (the gold standard for assessment of airway inflammation) is not generally feasible in severe disease, much effort is focused on less invasive measures of airway inflammation as these may provide guidance relating to both treatment and prognosis.

Biopsy specimens of the bronchial wall show increased numbers of eosinophils in patients with asthma and this is reflected in the airway lumen where eosinophils and eosinophil cationic protein (ECP) in induced sputum are increased during asthma exacerbations. Other indices of inflammation include hydrogen peroxide (H₂O₂) in exhaled breath condensate, serum levels of ECP, and urinary leukotriene E4 levels. Each of these techniques is beset by technical and handling difficulties which make them relatively impractical as routine tests for serial disease monitoring.

Measurement of exhaled nitric oxide (NO) levels provides a rapid, reproducible, and reliable test which may reflect airway inflammation in asthma. The increase in exhaled NO levels in asthma exacerbations is thought to be due chiefly to activation of airway iNOS induced by inflammatory cytokines. In support of this notion, NO is increased in acute exacerbations of asthma and is suppressed by corticosteroids. Persistent elevation of NO in treated patients may then suggest either more severe inflammation or inadequately treated inflammation.

The correlation between symptomatology, lung function, and exhaled NO levels was therefore assessed in 26 patients with difficult asthma to determine its role in this condition.
Exhaled nitric oxide levels in patients with difficult asthma

Table 1 Characteristics of patients with difficult asthma

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<td>26</td>
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Sex (F/M) 19/7
Mean (SD) age (range) 38.8 (15.0) years (15–71)
Women 16.3 (15.6)
Men 41.0 (12.4)
Years symptomatic 19.2 (15.1)
Atopic status Atopic–non-atopic 20/6
No. of smokers 5

<table>
<thead>
<tr>
<th>Treatment:</th>
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<tr>
<td>Prednisolone</td>
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<tr>
<td>n = 17 (12F/5M)</td>
</tr>
<tr>
<td>ICS Fluticasone (n = 20)</td>
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<tr>
<td>2025 (1197) µg/day</td>
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<td>ICS Budesonide (n = 6)</td>
</tr>
<tr>
<td>3967 (2271) µg/day</td>
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<tr>
<td>Rescue β agonist MDI</td>
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<tr>
<td>8.1 (9.9) puffs/day</td>
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<tr>
<td>Lung function:</td>
</tr>
<tr>
<td>PEF (% predicted)</td>
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<tr>
<td>66.2 (5.3)</td>
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<tr>
<td>FEV1 (% predicted)</td>
</tr>
<tr>
<td>66.5 (20.7)</td>
</tr>
<tr>
<td>FEV1/FVC (mean)</td>
</tr>
<tr>
<td>67.1 (29.5)</td>
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<tr>
<td>β agonist response</td>
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<td>19.7 (median), 0–36 (range)</td>
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ICS = inhaled corticosteroid; MDI = multidose inhaler; PEF = peak expiratory flow; FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; PEVF = diary card peak expiratory flow variability values are mean (SD) or median (range) where indicated.

Methods

PATIENTS

We reviewed 52 consecutive patients from a tertiary asthma referral centre with the clinical problem of “difficult asthma” of which 26 met the following inclusion criteria: (1) current physiological evidence of reversible airways obstruction; (2) maintenance oral prednisolone requirement, or (3) inhaled corticosteroid (>2 mg/day BDP or equivalent); (4) moderate to severe persistent asthma symptoms; and (5) stable disease with no evidence of exacerbation or infection within the preceding two weeks.

Physiological criteria for reversible airways obstruction included a bronchodilator response to inhaled salbutamol 200 µg in baseline forced expiratory volume in one second (FEV1) of >15% predicted (n = 17), diurnal variation in peak expiratory flow (PEF) of >20% (n = 9), and concentration of methacholine required to provoke a fall in FEV1, of more than 20% (PC20) of ≤8 mg/ml (n = 1). Seventeen patients were dependent on oral steroids although not all were challenged by dose reduction. The characteristics of the patients are shown in table 1. A comparison was made with 46 normal subjects aged 32 (4) years (six atopic, FEV1 95.5 (5) % predicted, PC20 methacholine >16 mg/ml) and 30 steroid naive mild asthmatic subjects aged 28 (5) years (30 atopic, FEV1 91 (4) % predicted, PC20 <3.3 mg/ml) and these patients have been described elsewhere.19

Chronic bronchiectasis was detected in a single subject (airflow obstruction with normal gas transfer, chronic cough, and no evidence of bronchiectasis on computed tomographic (CT) scan), but no other pulmonary comorbidity was detected after clinical review, lung function testing, chest radiography, and CT scanning.

SYMPTOM SCORES AND DIARY CARDS

Subjects were provided with two week diary cards to record daily symptoms, morning (pre-bronchodilator) and afternoon (post-bronchodilator) PEF, and rescue β agonist use (puffs per day). Subjects were asked to score daily each symptom of cough, chest tightness, wheeze, sputum, breathlessness, and night time symptoms on a scale of 0–5 where 0 = no symptoms for three months; 1 = symptoms less than weekly or on exercise; 2 = symptoms more than weekly but less than daily; 3 = daily but not at night; 4 = daily and night-time symptoms less than twice a week; and 5 = daily with night-time symptoms more than twice a week.

PULMONARY FUNCTION TESTS

Home PEF measurements were made using the Wright peak flow meter. Spirometric lung function was measured by a standard technique (Jaeger, Market Harborough, Leics, UK).20 Maximum bronchodilator reversibility (% change from baseline % predicted FEV1) was established by spirometric testing before and after staged inhalation of salbutamol 200 µg from a metered dose inhaler without spacer followed by 2.5 mg salbutamol delivered by nebuliser. Diary card PEF variability (PEFV) was calculated as maximum PEF—minimum PEF/mean PEF × 100%.

EXHALED NITRIC OXIDE MEASUREMENTS

Exhaled NO was measured using a chemiluminescence analyser (Model LR 2000, Logan Research, Rochester, Kent, UK) sensitive to 1–5000 ppb NO with a resolution of 0.3 ppb and response time of <0.5 seconds. Measurements were made following inhalation to total lung capacity with slow exhalation through a wide bore Teflon tube to residual volume. An expiratory pressure of 3 (0.4) mm Hg was maintained by a visual display of expiratory flow, thus excluding nasal NO contamination.21 NO sampling by the analyser was via a side arm with a constant flow rate of 250 ml/min. NO measurement was made at the end of exhalation when both NO and CO2 levels had achieved a stable plateau phase, representing alveolar sampling.

Measurements were made at least twice and the highest of two concordant readings (±5%) was recorded. Rescue β agonist use prior to testing was unrestricted. Subjects who smoked refrained from doing so for 24 hours prior to testing. There were five smokers (2–10 cigarettes/day), of whom three took prednisolone and had NO values of 20.5, 30.0 and 9.3 ppb, respectively, while the two on inhaled corticosteroids alone had values of 57.6 and 3.2 ppb.

PREDNISOLONE ASSAYS

Serum prednisolone levels were measured using an HPLC technique.

STATISTICAL ANALYSIS

Comparison between groups was made using Spearman rank correlation and Mann-Whitney non-parametric analysis, a p value of less than 0.05 being considered significant.
Results

Symptom Frequency
Seventeen of 26 subjects experienced daily symptoms with night-time symptoms at least once a week and eight experienced symptoms more than twice a week. One patient had had no symptoms for three months.

Lung Function
Lung function results varied with a mean FEV1 of 65.70 (21)% predicted, closely matching the average PEF % predicted and FEV1/FVC for the group (table 1). Diary peak flow variability (amplitude % mean) was 69.8%.

Exhaled Nitric Oxide
Exhaled NO levels were significantly higher in patients with difficult asthma (mean 13.9 ppb, 95% CI 9.3 to 18.5) than in normal subjects (7.4 ppb, 95% CI 6.9 to 7.8; p<0.002), and were significantly lower than levels in steroid naïve mild asthmatics (36.9 ppb, 95% CI 34.6 to 39.3; p<0.001). NO levels were higher in subjects taking oral prednisolone (17.5 ppb, 95% CI 11.1 to 24.0) than in those not taking prednisolone (7.2 ppb, 95% CI 4.6 to 9.8; p = 0.016; fig 1).

Exhaled NO levels did not correlate with inhaled corticosteroid dose (p = 0.43). There was also no difference in NO levels between those on very high dose inhaled corticosteroids (n = 7, >2 mg/day) and those on lower dose inhaled corticosteroids (n = 19, ≤2 mg/day), although two patients on very high dose inhaled corticosteroids were also taking oral prednisolone.

Correlation of Exhaled NO with Lung Function
Exhaled NO levels for the group did not correlate with degree of airway obstruction (FEV1 % predicted, p = 0.73; FEV1/FVC, p = 0.94), peak flow variability (p = 0.85), nor with β agonist reversibility (bronchodilator response in FEV1, p = 0.95). Airway obstruction (FEV1/FVC) was significantly greater in those on high dose inhaled corticosteroids than in those on low dose inhaled corticosteroids (p = 0.004). Lung function was not significantly different between those treated with oral steroids and those not on oral treatment.

Compliance and Prednisolone Assays
Prednisolone assays were obtained two hours after planned dose administration in 15 patients on oral prednisolone. In three assays (20%) prednisolone levels were below detectable limits. Mean (SD) serum prednisolone levels were 344 (285) nmol/l. Prednisolone levels did not correlate with prednisolone dose (p = 0.6) nor with exhaled NO levels (p = 0.37). Patients with undetectable prednisolone levels were considered non-compliant but, when these three patients were excluded, NO
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levels were still significantly higher in prednisolone treated patients than in non-prednisolone treated patients (p = 0.03).

Discussion

Patients with difficult asthma have moderate to severe persistent symptoms despite high dose anti-inflammatory treatment. This group had increased NO levels as a whole in spite of treatment. Furthermore, prednisolone treated patients had significantly higher NO levels than those not requiring oral corticosteroid treatment. A likely explanation for this finding is that this group has more severe disease and consequently attracts higher intensity treatment. A further explanation, however, is that these patients have NO levels that are unaffected or only minimally influenced by steroid therapy, thus possibly identifying subjects with relative steroid insensitivity. We believe that compliance as a confounding factor is effectively excluded by the high level of compliance as detected by serum prednisolone assays.

Symptom scores and symptom frequency in this group did not correlate with PEF variability or airway obstruction. Symptom scores were significantly increased in those with the highest symptom frequency. NO levels were closely correlated with rescue β agonist use (p=0.002), but not significantly with average symptom score (p = 0.11). β agonist use has not previously been associated with raised NO levels and, when taken in high doses, has no effect on exhaled NO and is thus unlikely to be causal in this relationship.

These findings suggest that, in patients with difficult asthma, there may be persistent airway inflammation despite treatment with oral corticosteroids. How these results relate to bronchial mucosal biopsy findings and other measures of airway inflammation, however, requires further study. Since NO concentrations correlated with symptom scores, symptoms in severe asthma may therefore be a marker of airway inflammation and this relationship also remains unclear.

A consideration of inflammatory markers in mild to moderate asthma is valuable. Laitinen et al have shown an increase in airway mucosal eosinophil count associated with increased symptoms and bronchial hyperreactivity during exacerbations of asthma, all of which fall with inhaled corticosteroid treatment. Both oral and inhaled corticosteroids reduce asthma symptoms, rescue β agonist use, and improve FEV1, while leading to significant reductions in epithelial and submucosal eosinophil counts. Similar correlations in changes are seen when serum ECP, T cell activation markers (CD4, CD25) in bronchoalveolar lavage fluid, and urinary leukotriene E4 levels are considered as surrogate markers of inflammation.

In mild to moderate disease there would thus appear to be a concordant change in symptoms, lung function, and inflammatory markers during exacerbations; however, in chronic severe disease a tendency to fixed airflow limitation due to changes in extracellular matrix and basement membrane thickening may cause symptoms, lung function, and inflammatory markers to vary discordantly. Small studies have shown that apparent control of airway inflammation does not necessarily control airway hyperresponsiveness or vice versa. We were unable to show a correlation between FEV1/FVC, PEF variation, and symptom scores in this study and, indeed, a number of studies have reported a tendency to fixed airflow limitation in patients with chronic asthma.

The relationship of symptoms to inflammatory activity in severe and difficult asthma is unclear, but its importance lies in the choice of treatment response to symptoms. Exhaled NO combined with symptom score may be a useful tool for monitoring asthma control in patients with difficult/severe asthma where changes in lung function may have limited sensitivity. Furthermore, the identification of subjects in whom exhaled NO levels remain markedly increased in spite of high dose corticosteroid therapy may prompt consideration of relative steroid insensitivity or resistance, which may influence steroid therapy.

The identification of a surrogate marker of airway inflammation that may be conveniently used in the outpatient setting has the potential to serve as an indicator of the adequacy of anti-inflammatory treatment. Such markers may help to rationalise steroid therapy in those already treated with high dose oral and inhaled corticosteroids, and complement symptomatology and lung function as measures of control in chronic severe or difficult asthma.


