Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission

M W Pijnenburg, W Hofhuis, W C Hop, J C De Jongste

Background: Nitric oxide in exhaled air (FE\textsubscript{NO}) is a marker of eosinophilic airway inflammation. A study was undertaken to determine whether FE\textsubscript{NO} predicts asthma relapse in asymptomatic asthmatic children in whom inhaled corticosteroids are discontinued.

Methods: Forty children (21 boys) of mean age 12.2 years on a median dose of 400 \textmu g budesonide or equivalent (range 100–400) were included. FE\textsubscript{NO} was measured before and 2, 4, 12, and 24 weeks after withdrawal of steroids. A relapse was defined as more than one exacerbation per month, or need for \beta\textsubscript{2} agonist treatment on 4 days per week for at least 2 weeks, or diurnal peak flow variability of >20%. FE\textsubscript{NO} measurements were performed online with an expiratory flow of 50 ml/s.

Results: Nine patients relapsed. Two and 4 weeks after withdrawal of steroids geometric mean FE\textsubscript{NO} in children who were about to relapse was higher than in those who did not relapse: 35.3 ppb (2 weeks, ratio 2.3; 95% CI 1.2 to 4.1; \(p=0.01\)) and 40.8 ppb (4 weeks, ratio 2.6; 95% CI 1.3 to 5.1). An FE\textsubscript{NO} value of 49 ppb at 4 weeks after discontinuation of steroids had the best combination of sensitivity (71%) and specificity (93%) for asthma relapse.

Conclusion: FE\textsubscript{NO} 2 and 4 weeks after discontinuation of steroids in asymptomatic asthmatic children may be an objective predictor of asthma relapse.
children were seen and treated by their own paediatric pulmonologist, not the investigator. A relapse was defined as more than one exacerbation per month and/or exacerbations requiring oral or inhaled steroid use and/or need for rescue bronchodilators on 4 or more days per week for at least two consecutive weeks and/or mean diurnal PEF variability of >20% according to the guidelines of the Dutch Paediatric Respiratory Group. The primary end point was relapse. At the point at which relapse occurred, children dropped out of the study.

**FE\textsubscript{NO} measurements**

FE\textsubscript{NO} was measured online with an expiratory flow of 50 ml/s according to ATS and ERS guidelines. NO was continuously sampled with a sampling flow of 175 ml/min and analysed by a chemiluminescence analyser (Sievers 280 NOA, Boulder, CO, USA). The analyser was calibrated weekly using 0 and 115 ppb NO certified gases (BOC, Herenthout, Belgium).

**Lung function testing**

Flow-volume curves were obtained with a dry rolling seal spirometer (Jaeger, Würzburg, Germany) according to ATS guidelines. After maximal inspiration, three reproducible loops with a maximum variability in FVC of 10% were obtained. FVC and FE\textsubscript{V1} are expressed as percentage predicted.

**Statistical analysis**

FE\textsubscript{NO} values were logarithmically transformed before statistical analyses and the results expressed as geometric means. FE\textsubscript{NO} at \( t = 0 \) weeks was used as baseline. For each interval between two measurements we assessed whether FE\textsubscript{NO} at the beginning of the interval was predictive for the occurrence of relapse during the interval. Subsequently, the information from these four analyses were combined using conditional logistic regression which relates the probability of relapse in each period to FE\textsubscript{NO} at the start of this period. Multivariate analysis was repeated using either FE\textsubscript{NO} or the ratio of FE\textsubscript{NO} to baseline FE\textsubscript{NO} at baseline and at the various time points was compared in patients with and without clinical relapse using Mann-Whitney U tests. ROC curves for FE\textsubscript{NO} 2 and 4 weeks after discontinuation of ICS were constructed. The correlation between FE\textsubscript{NO} and clinical and lung function parameters was assessed using Spearman’s correlation coefficient.

**RESULTS**

Of the 40 children included in the study, one dropped out because of a high symptom score during the run-in period and two were lost to follow up. The remaining 37 patients (21 boys) had a mean age of 12.2 years (range 7.3–16.9). Data on the study population are shown in table 2. Twenty nine were atopic; these children did not differ from non-atopic children in age, height and weight, nor in pulmonary function tests or baseline FE\textsubscript{NO}. None of the children used long acting \( \beta \)\textsubscript{2} agonists or leukotriene antagonists.

Baseline geometric mean FE\textsubscript{NO} at \( t = 0 \) was 11.2 ppb (95% CI 8.5 to 15.3). This did not differ significantly from FE\textsubscript{NO} at \( t = -2 \), the start of the run-in period (\( p = 0.67 \)). Intra-individual variability between values at \( t = -2 \) and

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>( t = -2 )</th>
<th>( t = 0 )</th>
<th>( t = 2 )</th>
<th>( t = 4 )</th>
<th>( t = 12 )</th>
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<tr>
<td>FE\textsubscript{NO}</td>
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Symptom scores were obtained during the 2 weeks before each visit. At \( t = 0 \), inhaled steroids were discontinued.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Without relapse</th>
<th>With relapse</th>
</tr>
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<tbody>
<tr>
<td>(n = 28)</td>
<td>(n = 9)</td>
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<td>Age (years)</td>
<td>12.2 (7.3–16.9)</td>
<td>12.3 (10.0–15.8)</td>
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<td>Atopy (n)</td>
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<td>8</td>
</tr>
<tr>
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<tr>
<td>FVC (% pred)</td>
<td>102 (66–126)</td>
<td>105 (87–118)</td>
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<tr>
<td>FE\textsubscript{V1} (% pred)</td>
<td>100 (73–134)</td>
<td>99 (88–109)</td>
</tr>
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<td>FE\textsubscript{NO} (ppb)</td>
<td>10.5 (7.3–14.2)</td>
<td>14.8 (8.5–25.8)</td>
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ICS, inhaled corticosteroid; FE\textsubscript{V1}, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow; FE\textsubscript{NO}, fractional nitric oxide concentration in exhaled air.

Data are given as mean (range) except for the dose of ICS which is given (budesonide equivalent).

**Table 2** Baseline anthropometric and lung function data of study population (\( n = 37 \) children, 21 boys)

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Data are given as mean (range) except for the dose of ICS which is given with 95% confidence interval. None of the items differed significantly between children with and without relapse.

*After bronchodilatation with 1000 \( \mu \)g terbutaline.

![Figure 1](http://thorax.bmj.com/) FE\textsubscript{NO} values in patients with and without an asthma relapse. For each period (0–2, 2–4, 4–12, and 12–24 weeks) patients were classified according to whether they relapsed or not in the period indicated. FE\textsubscript{NO} values were obtained at the start of each period. For patients without a relapse geometric mean FE\textsubscript{NO} and 95% confidence intervals are given. The x axis depicts number of weeks after withdrawal of ICS. One patient relapsed in the first period (0–2 weeks), one between 2 and 4 weeks, six between 4 and 12 weeks, and one after 12 weeks.
Exhaled NO predicts asthma relapse

FE\textsubscript{NO} and relapse of asthma

Nine patients (24%), one of whom was non-atopic, had a clinical relapse after a median of 36 days (range 14–141). Of these nine patients, five had two exacerbations within 1 month or a single exacerbation requiring oral or inhaled steroids and four used their bronchodilator as rescue therapy for ≥4 days a week during at least two consecutive weeks. Six children relapsed between 4 and 12 weeks after withdrawal of ICS; in the periods 0–2 weeks, 2–4 weeks, and 12–24 weeks, one patient relapsed in each period (fig 1). Children who experienced an asthma relapse did not differ in baseline demographic or pulmonary function data (table 2). There was no difference in initial steroid dose of children who did or did not relapse (Mann-Whitney U test, p = 0.28), nor was there a significant difference in baseline geometric mean FE\textsubscript{NO} between the two groups of patients (14.8 ppb v 10.5 ppb, respectively; ratio 1.4; 95% CI 0.7 to 2.8, p = 0.32). Two weeks after withdrawal of ICS the geometric mean FE\textsubscript{NO} in children who relapsed thereafter (n = 8) was significantly higher than in those who did not relapse (35.3 ppb v 15.7 ppb; ratio 2.3; 95% CI 1.2 to 4.1, p = 0.01). The same was true for FE\textsubscript{NO} after 4 weeks without steroids for the seven children who relapsed after 4 weeks (40.8 ppb and 15.9 ppb; ratio 2.6; 95% CI 1.3 to 5.1, p = 0.009; fig 1).

FE\textsubscript{NO} at 4 weeks after withdrawal of ICS predicted relapse in the forthcoming period (4–12 weeks after withdrawal, p = 0.025). Multivariate logistic regression combining results of all periods showed that FE\textsubscript{NO} was a better predictor of asthma relapse (p = 0.001) than the FE\textsubscript{NO} ratio (actual FE\textsubscript{NO} divided by baseline FE\textsubscript{NO}) (p = 0.04). For each doubling of FE\textsubscript{NO} the relapse rate increased by a factor 3.0 (95% CI 1.5 to 7.1). The results were similar when only atopic patients were analysed.

Two patients were included who later admitted to having used more than 400 µg budesonide regularly before enrolment. One of them relapsed. If both children were excluded, multivariate logistic regression still showed that FE\textsubscript{NO} predicted asthma relapse in the remainder (p = 0.003).

ROC curves indicated that a FE\textsubscript{NO} value of 49 ppb 4 weeks after stopping steroids had the best combination of sensitivity and specificity for predicting relapse (sensitivity: 71% (95% CI 29 to 96) and specificity 93% (95% CI 76 to 99); fig 2). The positive and negative predictive values of FE\textsubscript{NO} of 49 ppb were 71% and 93%, respectively.

The course of FE\textsubscript{NO} in all individuals who experienced a relapse is shown in fig 3. In patients who did not relapse there was a general trend for FE\textsubscript{NO} to rise with time from a geometric mean of 10.2 ppb at the start of the study to 22.2 ppb after 26 weeks of follow up (fig 1). FE\textsubscript{NO} showed an overall tracking pattern in those who did not relapse.

Correlations between FE\textsubscript{NO}, clinical symptoms, and pulmonary function tests

The results of pulmonary function tests, atopic state, PEF variability, cumulative symptom score and use of rescue medication during the run-in period did not differ between children with or without a relapse. Cumulative symptom scores, spirometric data, PEF values, and PEF variability did not correlate with geometric mean FE\textsubscript{NO} at any time point.

Other parameters and relapse of asthma

Symptom scores, peak flow, diurnal variability in peak flow, or other lung function tests performed during the run-in period did not predict asthma relapse.

DISCUSSION

We found that FE\textsubscript{NO} at 2 and 4 weeks after discontinuing ICS predicted asthma relapse in asthmatic children who were taken off ICS because of clinical remission. Initial FE\textsubscript{NO} levels measured while patients were still on ICS were not predictive of relapse. An FE\textsubscript{NO} of 49 ppb or higher 4 weeks after discontinuation of ICS had the best combination of sensitivity (71%) and specificity (93%).

Few other studies have assessed FE\textsubscript{NO} longitudinally after discontinuation or reduction of ICS, and none of these included children. Our results are in agreement with the study by Jones et al\textsuperscript{11} in which adult asthmatics treated with a mean daily ICS dose of up to 1600 µg were forced off steroids and followed for loss of asthma control. In this study, in contrast to ours, discontinuing steroids was not clinically indicated, and loss of control occurred earlier, after a median of 17 days. Their FE\textsubscript{NO} values were much lower, probably due to the higher flow rate of 250 ml/s used to obtain exhaled air samples. Lim et al\textsuperscript{14} performed a similar study in adults and their preliminary report states that NO in mixed nasal/oral exhaled air did not predict asthma relapse. However, mixed expired air is contaminated by high nasal levels of NO which makes interpretation impossible. Moreover, they defined relapse as a recurrence of asthma symptoms requiring either

Figure 2  ROC curve for FE\textsubscript{NO} 4 weeks after discontinuation of inhaled corticosteroids. The optimal combination of sensitivity and specificity for identifying children with relapse was for FE\textsubscript{NO} 49 ppb (71% and 93%, respectively).
β2 agonists or ICS. This might well explain any discrepancy between their findings and ours.

Jatakanon et al19 studied several non-invasive markers of airway inflammation in asthma exacerbations induced by forced reduction of ICS doses from more than 800 μg to 200 μg budesonide in adults. FENO at baseline did not predict loss of asthma control. However, there was a rapid increase in FENO before exacerbations 2–4 weeks after decreasing inhaled steroids. Only 15 patients were included in the study, and this small number could easily lead to non-significant findings. Furthermore, ICS were not completely withdrawn, which reduces the possibility of finding a difference between the groups.

The increases in FENO over time in children who relapsed were consistent and larger than within-subject baseline fluctuations. However, the intra-individual variability in FENO measurements at the beginning and end of our 2 week run-in period was quite high. Few data are available on long term within-subject reproducibility of FENO in asthmatic children. Earlier reports have focused on short term reproducibility, which is excellent. Kharitonov et al21 found intraclass correlation coefficients better than 0.90 in adults and children with and without asthma, with 95% limits of agreement of about 4 ppb when children were tested repeatedly within 4 days. Jones et al reported a within-subject coefficient of variation of FENO measured with a 1 week interval of 10.5%.11 The variability in our study may be due to the long interval of 2 weeks between FENO assessments. We also reasoned that inclusion in the study as such might affect FENO because of better compliance with ICS during the run-in. This seems unlikely as increased compliance would lead to a reduction in FENO whereas we found no significant difference between FENO at t = −2 and t = 0, with a trend towards higher levels at t = 0. In addition, ambient NO levels can be a source of variability. However, we found no correlation between ambient NO and FENO, so we think ambient NO levels do not explain the variability in FENO.

We included both atopic and non-atopic asthmatic subjects, reflecting the asthma population in daily practice. The numbers are too small for a subgroup analysis; only one non-atopic child relapsed. The patients who did not relapse without medication showed a wide range of FENO values (Fig 1). In these children no correlation was found between FENO and symptom scores. As our follow up was 6 months, we cannot exclude that some of the children might relapse later. The possible clinical relevance of an increased FENO in asymptomatic children therefore remains unclear.

What are the implications of these results for clinical practice? Our findings in this relatively small group of asthmatic children strongly suggest that FENO measurements at 2 and 4 weeks after cessation of steroids are helpful for identifying children in whom relapse of asthma is more likely to occur and who might benefit from a close follow up. However, patient numbers in this study are small and more children who did not relapse had raised FENO levels than those who did relapse. Larger studies are needed to confirm the role of FENO in decision making on ICS in asthmatic children and to calculate more accurately the sensitivity and specificity of different cut-off levels of FENO.

In conclusion, this is the first study in children showing that FENO is an early predicting marker of relapse in asthma after cessation of ICS. Larger studies are now warranted to substantiate this finding to further define the role of FENO in this aspect of asthma management.

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