Exhaled nitric oxide as a screening tool for asthma in school children

Anjani Prasad\textsuperscript{a}, Beverly Langford\textsuperscript{a}, John R. Stradling\textsuperscript{a}, Ling-Pei Ho\textsuperscript{a,b,*}

\textsuperscript{a}Oxford Centre For Respiratory Medicine, Churchill Hospital, Headington, Oxford OX3 7LJ, UK
\textsuperscript{b}Weatherall Institute of Molecular Medicine, The John Radcliffe, Headington, Oxford OX3 9DS, UK

Received 15 November 2004; accepted 15 March 2005

Summary It is now widely accepted that augmented levels of fractional exhaled nitric oxide (FeNO) reflect airway inflammation and the methodology has been optimised for potential clinical use. We were interested in investigating whether this measurement can be used as a tool to screen and identify school children with asthma. To do this, FeNO was measured using an on-line single exhalation analyser in 368 children aged 8–10 years in six Oxfordshire primary schools, by two investigators blinded to the disease status of the children. The children were then categorised into ‘normal’, ‘atopic asthma’, ‘non-atopic asthma’ and ‘atopy only’ groups, according to their responses to the ISAAC questionnaire and perusal of the children’s medical records kept by their family practitioners. Increased levels of FeNO were found in ‘atopic asthmatic’, ‘non-atopic asthmatics’ and ‘atopic only’ groups (median values of 24.4, 7.8 and 15.3 ppb, respectively, compared to normal controls’ of 6.9 ppb). Levels were increased in atopic children regardless of whether they had asthma and were significantly higher than non-atopic asthmatics. We conclude that FeNO measurement is not a useful tool for identifying children with asthma in the community, as increased levels did not discriminate between those with asthmatic and atopic symptoms.

\&\textcopyright\textsuperscript{2005} Elsevier Ltd. All rights reserved.

Introduction

The prevalence of asthma in children has increased dramatically in the last three decades.\textsuperscript{1} Environmental influences such as an increase in air pollution, dietary changes, and decreased exposure to immune stimulating diseases in childhood (the ‘hygiene hypothesis’) are among several hypotheses put forward to explain this trend.\textsuperscript{2} In parallel with this there is a growing need for a simple and reliable method to identify children with, or those who are at risk of developing, asthma. Due to its many phenotypic expressions through the course of childhood and often non-specific clinical
manifestations, diagnosing asthma in childhood remains a clinical challenge. In the last decade a few non-invasive methods of measuring airway inflammation have been proposed as possible methods to aid the clinical diagnosis of this condition. One of them, fractional exhaled nitric oxide (FeNO), has been the subject of particular investigations. Nitric oxide is synthesised by inflamed airway epithelial cells (e.g. in asthma) and can be measured in orally exhaled air. Since it was first used, numerous modifications have been made to improve the measurement of FeNO as a potential clinical tool. There is now sufficient evidence that increased FeNO can be used as a measure of response to corticosteroid treatment, that it correlates with eosinophilic inflammation in adults and children, and bears a positive relationship with airway hyperresponsiveness and response to bronchodilators. However, its role as a diagnostic tool is less well established and it is unclear if it can be used independently to identify children with asthma or those who might benefit from fuller investigation for this diagnosis. Some studies suggest this might be possible. We propose that if this were the case, then one potential use would be in the screening of school children in the community for early diagnosis of asthma. We have investigated if this is feasible by measuring FeNO in a large cohort of children from six different primary schools representing a cross section of social classes, and then assessing if high levels of FeNO identified children with asthma.

Methods

Study population and design

This was a cross-sectional prospective study. School children aged 8–10 years were recruited from six primary schools in an Oxfordshire town in England (Abingdon). Two studies were conducted. In the first study, 47 children aged 7–9 years had FeNO levels measured in the same month (June) on two consecutive years with the aim of: (1) examining the feasibility of measuring FeNO in the community and in a large cohort of children and (2) to provide data to examine the long term stability (biological variability) of FeNO measurements. We have already established the technical reproducibility in previous studies. Having determined that the study was feasible and FeNO measurements were biologically stable, we proceeded to the second study. Here FeNO levels were measured in 378 children aged 8–10 years by two investigators who were blinded to the children’s disease status. The schools were chosen to represent the spread of economic and social background of this town. All school children aged 8–10 years in each school were invited to participate in the study. Children with signs or symptoms of a respiratory tract infection within 4 weeks of the study were excluded.

The diagnoses of asthma and/or atopy were made by interpreting the ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire in conjunction with the child’s medical records. This questionnaire was distributed to the parents of the children a week before exhaled NO measurement and collected after all the FeNO levels had been tabulated. The responses (see below) were used to categorise the children to four groups—‘normal’, ‘asthmatic, no atopy’, ‘asthmatic, atopy’ and ‘atopy, no asthma’. In addition, the medical notes kept by the family practitioners of these children were perused and the diagnosis of ‘asthma’, ‘hay fever’ and ‘eczema’ were noted, together with the use of inhaled or oral corticosteroids.

The parents of every child provided informed assent for their child. The study was approved by the Oxford Research Ethics Committee.

Fractional exhaled nitric oxide measurement

FeNO was measured using a single exhalation method with the Logan LR2000 analyser (Logan Research, Bromley, Kent, UK). The child was coached to exhale slowly from total lung capacity (TLC), without prior breath holding while observing the screen to maintain a steady expiratory flow. Mouth pressure and flows were kept at 10 cm H2O and 50 ml/s, respectively. FeNO values were recorded from the best-fit plateau during steady exhalation. An average of three recordings were made and the mean FeNO of these recordings was taken as the representative level.

Height, FEV1 and FVC were also recorded. Spirometry was measured with a hand held spirometer (MicroSpirometer MS01, MicroMedical Ltd, Rochester, Kent, UK) after the FeNO measurement, as FEV1 manoeuvre has been shown to affect subsequent FeNO levels.

Diagnosis of asthma and atopy

From the ISAAC questionnaire, a diagnosis of current or active asthma was made if there was a positive response to the question of whether there was wheezing or whistling in the chest or being wheezy during or after exercise. Having a dry cough
at night without any other symptoms was not interpreted as asthma.

For atopy the questions were divided into those for hay fever (allergic rhino-conjunctivitis) and those for atopic eczema. To diagnose hay fever the child must have had problems with sneezing or a runny or blocked nose accompanied by itchy and watery eyes mainly in the summer months. For eczema, the child should have had a recurrent itchy rash affecting one of the characteristic sites. Having either hay fever or atopic eczema counted the child as being atopic. We took the child’s lifetime prevalence of atopic symptoms to determine if the child was atopic or not.

Case notes for each child, kept by the family practitioner, were perused by a medically qualified investigator for the diagnosis of asthma, hay fever and eczema. Inhaled medications were noted. Where there were discrepancies between the questionnaire and the family physician’s notes, the diagnosis from the latter was used.

Statistical analysis

Normality of distribution was determined using the Kolmogorov-Smirnov test. As FeNO levels showed a non-parametric distribution, they were reported as medians with inter-quartile ranges. Statistical differences between asthmatics, atopics and normals were analysed using Kruskal-Wallis ANOVA on ranks test, assessing the group as a whole. Having determined that there was a statistical difference in median values between the groups using this method, groups that differ from others were isolated using a multiple comparison method (Dunn’s Method). Correlations between FeNO and FEV₁, FVC and height were assessed using the Spearman Rank Order correlation test and between FeNO and gender with Mann Whitney Rank Sum Test. Reproducibility of FeNO from one year to the next was analysed using Spearman Rank Order correlation test and calculation of threshold changes from one year to the next (illustrated in Fig. 2).

Results

Characteristics of study population and distribution of FeNO

In study 1, all 47 recruited children performed the procedures optimally and there were no practical or logistic problems with managing the study. FeNO levels were distributed non-parametrically in this cohort as a whole.

In study 2, of the 378 children that were recruited, one child was unable to perform the FeNO manoeuvre. Questionnaires from nine children were not returned. These subjects were excluded from analysis. The study population therefore comprised 368 children, 177 females and 191 males. The range of FeNO in the whole population was 1.7–90.5 ppb. The greatest spread of values was observed in the atopic group and least in the normal group (Table 1).

The age range was 8.1–10.8 years with a mean of 9.1 years. There was no statistically significant difference between these age groups. FeNO values were non-parametrically distributed in the whole population (Fig. 1) and also within each group of normal, atopy, asthma and atopic asthma.

From the distribution in the normal (asymptomatic) children, we determined the normal range to be the 95% confidence level of the median. This was similar (as expected) to a separate assessment where the values from the normal children were log₁₀ transformed, and then taking the mean of this ± 2 SD as the normal range. From these analyses, the normal range was set as 4.2–11.2 ppb. We considered values greater than 11.2 ppb as raised. Using this criteria, 43 (21%) of asymptomatic children had raised exhaled NO levels.

Biological variability of FeNO

From study 1, we observed no significant difference between FeNO levels in the first year and in the second year (median of FeNO year 1: 10.2 ppb, I.Q

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Range of FeNO levels for each group of children.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Normal</td>
<td>209</td>
</tr>
<tr>
<td>Non-atopic asthma</td>
<td>40</td>
</tr>
<tr>
<td>Atopic asthma</td>
<td>45</td>
</tr>
<tr>
<td>Atopy alone</td>
<td>74</td>
</tr>
</tbody>
</table>

FeNO levels were significantly higher in asthmatics, atopic asthmatics and atopics compared to normal. FeNO levels were also significantly different between each other expect between ‘atopy alone’ and ‘atopic asthma’ (P < 0.001 by Kruskal-Wallis ANOVA on ranks and P < 0.05 by Dunn’s method of multiple comparison).
range 5.5–15.2 vs. FeNO year 2: 11.4 ppb, I.Q range 5.7–25.1; Mann Whitney Rank Sum test, \( P = 0.13 \)). Correlation co-efficient between the two years’ readings showed a statistically significant positive correlation \( (r = 0.7; P < 0.001) \) (Fig. 2). Therefore, those children with high FeNO in year 1 also had high FeNO in year 2. We also divided the graph into four ‘quadrants’ or boxes, the lines of division being the median value for that year (see Fig. 2). From this the upper right and upper left boxes represent ‘high’ values for year 2, here assigned as values greater than the median; and the upper right and lower right boxes represent ‘high’ values for year 1. From this analysis, it is seen that only 7 of 47 subjects crossed from ‘low’ to ‘high’ or vice versa between the 2 years. It should be noted that this is a relatively stringent assessment of ‘high’ and ‘low’ FeNO threshold, which ignored the normal range around the median that we used as our biological range (see paragraph above).

**Correlation with lung function, height and sex**

As FeNO may correlate with the NO producing surface area in the airways which is related to FVC and height; and with \( FEV_1 \) itself, \(^{17} \) we assessed the relationship between these parameters and FeNO in normal children.

\( FEV_1 \) and FVC ranged from 1.0 to 2.6 l/min and 1.4–3.3 l, respectively, in the normal group. Analysis using the Spearman Rank Order Correlation Coefficient showed no correlation between FeNO levels and \( FEV_1 \) and FVC (correlation coefficients: 0.08 and 0.04, \( P = 0.315 \) and 0.51, respectively, respectively).

**FeNO in different ‘diagnostic’ groups**

209/368 (57%) children were categorised as normal, 40/368 (11%) as non-atopic asthmatics, 45 (12%) as atopic asthma and 74/368 (20%) as atopic alone (Fig. 4). There were only eight discrepancies in diagnosis of asthma between the physicians’ case records and the ISAAC questionnaire (more in the former; and these were taken as the ‘correct’ diagnosis). FeNO was significantly higher in atopic children regardless of whether they had asthma compared to normals (Table 1). Post-hoc assessment (Dunn’s method) after Kruskal-Wallis analysis of the four groups showed significant differences between all groups except ‘atopic asthmatic’ and ‘atopic only’ groups (Table 1).
The asthmatic group was further divided according to whether the child was taking an inhaled or oral corticosteroid. No child in the 'atopic only' or 'normal' groups was on inhaled corticosteroids; and none at all were on oral corticosteroids. 15/40 (37.5%) non-atopic asthmatics and 23/45 (51%) atopic asthmatics were on an inhaled corticosteroid. Excluding children on corticosteroids, all the above findings still held true. Median FeNO levels in asthmatic children on inhaled corticosteroids (n = 47) were 14.2 ppb and in those not on inhaled corticosteroids (n = 38) were 21.7 ppb. These were not statistically different (P = 0.4); but the lower median values in those children on inhaled corticosteroids is in keeping with data from other workers.

The asthmatic group was further divided according to whether the child was taking an inhaled or oral corticosteroid. No child in the 'atopic only' or 'normal' groups was on inhaled corticosteroids; and none at all were on oral corticosteroids. 15/40 (37.5%) non-atopic asthmatics and 23/45 (51%) atopic asthmatics were on an inhaled corticosteroid. Excluding children on corticosteroids, all the above findings still held true. Median FeNO levels in asthmatic children on inhaled corticosteroids (n = 47) were 14.2 ppb and in those not on inhaled corticosteroids (n = 38) were 21.7 ppb. These were not statistically different (P = 0.4); but the lower median values in those children on inhaled corticosteroids is in keeping with data from other workers.

Discussion

In recent years a few studies have begun examining the potential use of exhaled NO as a diagnostic or screening tool for disease (asthma or primary ciliary dyskinesia) in children. Most studies have recruited children from paediatric specialist clinics and examined the correlation of FeNO and disease within an experimental setting. While FeNO is increased in asthmatic children, their diagnostic utility is unclear. Narang and colleagues found FeNO measurements not to be useful in distinguishing between cystic fibrosis and bronchiectasis, but agreed that asthmatic children had higher levels of FeNO. Malmberg et al. recruited children from their paediatric physiology clinic and compared whether exhaled NO levels or respiratory function assessments corresponded more sensitively with the final clinical diagnosis of asthma in a group of asthmatic pre-school children. They observed superiority in exhaled NO measurements in this regard. Our study is the first to report use of this method prospectively as a screening tool in children and within a potentially 'real', non-laboratory setting in the community. Here, we found that although higher exhaled NO levels were seen in children with asthmatic symptoms, this was also the case for individuals with atopy. Therefore, it did not discriminate between children with asthma and those with atopic symptoms alone.
This formed the major hurdle in the use of this method for identifying asthma. There was also no statistically significant difference in the degree of elevation in exhaled NO comparing children in the atopic only and atopic-asthmatic groups, although the median is higher in the atopic-asthmatic groups. This is not entirely surprising as we and others have already shown that exhaled NO is increased in atopic adults regardless of whether they had a formal diagnosis of asthma.19–21 Our findings also concur with a similar study on Norwegian adolescents.22

The exact mechanism for the association of increased FeNO and atopy is unclear; but it is becoming evident that NO production correlates more specifically with airway inflammation where eosinophilia and clinical atopic features dominate. Recent studies examining bronchoalveolar lavage (BAL) fluid from children may provide a unifying explanation. Eosinophilic cationic proteins are significantly higher in BAL fluid collected from atopic asthmatic children during asymptomatic periods compared to controls23 suggesting that eosinophils are active in these children even in the absence of ongoing symptoms.24 Does this suggest that atopics with increased FeNO levels could have a higher risk of developing asthma? It is apparent from our study that not all children with atopic symptoms have increased FeNO levels. From our calculated normal range, a greater number of atopic asthmatics had 'high' levels of FeNO (71% with FeNO levels greater than 11.9 compared 65% in atopic alone group). Atopy in children is the only robust factor associated with increased risk of persistence and severity of asthma in later childhood and adulthood.25,26 Patients with history of asthma or wheezy bronchitis by age of 16 years were twice as likely to report episodes of wheezing in the past year (at the age of 33 years) if they had also experience hay fever, allergic rhinitis and eczema as a child.25 It is possible to hypothesise that a combination of atopy and increased exhaled NO levels may pick out those children that may be at greater risk of having persistent asthmatic symptoms in adulthood. This suggestion can only be explored in a longitudinal study.

The spread of exhaled NO levels in children without asthmatic or atopic symptoms was interesting. This showed that normal healthy children had levels ranging from 1.7 to 64.2 ppb; with a clear tail of 'high' NO producers in the distribution profile. It is not possible to tell whether these asymptomatic high producers fall into a group that may be at an increased risk of developing of asthma in adulthood, whether this is a precursor to atopy or if these are normal individuals who simply have high levels of NO with no clinical sequelae. We had excluded upper respiratory tract infections from these children and our pilot reproducibility work demonstrated that those patients with high exhaled NO levels remained high the next year and vice versa. Again, only a longitudinal study would provide answers to these questions. The spread aside, the mean and median levels were comparable to other studies using similar flow rates and type of analyser.27

In terms of methodology, we did not encounter any major problems with measuring FeNO on a large scale in the community. Most children found the experience easy, required little coaxing to complete it successfully and the procedure was performed within 5 minutes per child. The only unexpected event was the build up of water vapour in the first part of the collection tube attached to the analyser. This, we detected during our first study and we subsequently ensured that this was eradicated every few children by removing the collection tube and flushing it with air pump. Our use of 10 cm H2O for mouth pressure and 50 ml/s flow were determined in pilot studies. We chose a mouth pressure that did not allow nasal escape and one which 6–9 year olds could tolerate with ease. This was also the flow rate validated independently by other workers.28,29

The combined use of the ISAAC questionnaire and family physician’s diagnosis for categorisation to asthma and atopy provided a more secure method of diagnosing the condition than the use of the questionnaire alone. The ISAAC questionnaire was used in our study as it provided the best validated questionnaire method.14 Several methods exist for identifying asthma in the community. Most workers acknowledge that current methods only identify asthmatic or atopic symptoms and an accurate diagnosis is necessarily a clinical one involving full individual assessment of the child. Surprisingly, there was a slight ‘over-call’ of asthma from the physician’s case records. This could be explained by a closer relationship between the family practitioners and their patients in this close knit town, than would be seen in bigger cities with higher turnover of patients.

In summary, our study shows that FeNO measurement is not a useful tool for identifying children with asthma in the community, as increased levels did not discriminate between those with asthmatic and atopic symptoms. However, increased exhaled NO levels were seen in a greater proportion of atopic asthmatic than atopic only children, and some children with no symptoms (normal) were identified as having high FeNO levels. This raises the possibility that increased NO levels may yet
identify children who are at risk of developing asthma.

Acknowledgements

The authors would like to thank Dr. David Otterburn and his colleagues at the Malthouse GP Practice, Abingdon’s participating GP practices; and staff, parents and pupils at Dunmore, Caldecott, St. Nicholas, Carswell, St. Edmund’s and Thameside Primary/Junior Schools for their participation and support in the study. The study was funded by the Oxfordshire Health Services Research Committee.

References