doi: 10.1111/j.1365-2222.2010.03687.x
Clinical & Experimental Allergy, **41**, 556–564

ORIGINAL ARTICLE **Epidemiology of Allergic Disease**

Exhaled nitric oxide discriminates children with and without allergic sensitization in a population-based study

T-C Yao[1,2], L-S Ou[1,2], W-I Lee[1,2], K-W Yeh[1,2], L-C Chen[1,2] and J-L Huang[1,2,3] , for the PATCH study group*

[1]Community Medicine Research Center, Chang Gung Memorial Hospital at Keelung, Keelung, Taiwan, [2]Division of Allergy, Asthma, and Rheumatology, Department of Pediatrics, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taoyuan, Taiwan and [3]Department of Pediatrics, Chang Gung Memorial Hospital at Keelung, Keelung, Taiwan

Clinical & Experimental Allergy

Summary

Background Fraction of exhaled nitric oxide (FeNO) as a biomarker of airway inflammation in children warrants better clarification.

Objective To identify the determinants of FeNO in children and assess the validity of FeNO as a discriminative tool for asthma, rhinitis or allergic sensitization in a population setting.

Methods Children aged 5–18 years ($N = 1717$) were evaluated using online FeNO measurements, questionnaires, anthropometric measurements, pulmonary function tests and total and specific serum IgE.

Results FeNO levels were age-dependent, with an average increase of 7.4% per year of age. It decreased with increasing body mass index (BMI), estimated at 1.5% decrease per kg/m^2. Children with allergic sensitization had elevated FeNO independent of allergic symptoms. In the combined analyses of asthma, rhinitis and allergic sensitization, elevated FeNO levels were confined mainly to children having allergic sensitization. After adjusting for allergic sensitization, a significant association between rhinitis and FeNO remained, but no such association was seen with asthma. The sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) of FeNO at the optimum cut-off of 28 p.p.b. for diagnosing asthma were 64.3%, 69.9%, 8.8%, and 97.7%, respectively (area under the ROC curve [AUC] 0.67), and were slightly better for diagnosing allergic asthma: 70.0%, 70.4%, 9.0%, 98.3%, respectively (AUC 0.71). FeNO had modest accuracy in discriminating rhinitis with an AUC value of 0.70, and performed better in discriminating allergic rhinitis (AUC 0.78). FeNO was a robust discriminator of allergic sensitization independent of symptoms at a cut-off of 15.4 p.p.b. (AUC 0.80; sensitivity 72.2%; specificity 71.2%; PPV 76.9%; NPV 65.8%).

Conclusion and Clinical Relevance FeNO measurement discriminates children with and without allergic sensitization independent of allergic symptoms. On the other hand, low FeNO levels in children may help exclude allergic asthma but high levels may be caused by allergic sensitization, older age, rhinitis, and lower BMI, in addition to asthma.

Keywords allergic rhinitis, allergic sensitization, asthma, atopy, body mass index, children, exhaled nitric oxide

Submitted 6 August 2010; revised 5 November 2010; accepted 30 November 2010

Correspondence:
Dr Tsung-Chieh Yao and Jing-Long Huang, Division of Allergy, Asthma, and Rheumatology, Department of Pediatrics, Chang Gung Memorial Hospital, 5 Fu-Hsin Street, Kweishan, Taoyuan, Taiwan.
E-mails: yao@adm.cgmh.org.tw and long@adm.cgmh.org.tw
Cite this as: T-C Yao, L-S Ou, W-I Lee, K-W Yeh, L-C Chen and J-L Huang , for the PATCH study group, *Clinical & Experimental Allergy*, 2011 (41) 556–564.

Introduction

Exhaled nitric oxide has been widely reported as a marker for eosinophilic airway inflammation [1]. Recently, the fraction of exhaled nitric oxide (FeNO) has attracted much attention because it has been advocated as a valuable tool for diagnosing asthma, monitoring therapeutic response, evaluating current symptom control, and predicting exacerbations [1–5]. Evidence of its overall diagnostic usefulness for asthma in children is mixed, as early clinic-based studies depict robust results [6–13], while more recent population-based studies have disappointing

*Prediction of Allergies in Taiwanese Children (PATCH) study group. Study coordinator: J-L Huang. Principle investigators: T-C. Yao, L-S. Ou, K-W. Yeh, W-I. Lee, L-C. Chen.

outcomes [14–17]. Such inconsistencies may reflect small sample sizes or differences between studies with respect to sampling strategies, measurement techniques, and confounding factors such as age, gender, anthropometric measurements, pulmonary function, and atopy (allergic sensitization).

These conflicting findings make it difficult to interpret FeNO levels in the clinic and hamper the advance of FeNO as a research tool in epidemiology. Undoubtedly, one of the most frequent questions raised by medical professionals and epidemiologists, as well as parents of children with asthma-like symptoms, is how FeNO levels should be interpreted. In this regard, a better understanding of the determinants of FeNO levels based on current measurement standards and its diagnostic accuracy in a large population-based study will help clarify its value in clinical practice and epidemiologic research. The opportunity to address these issues stems from the Prediction of Allergies in Taiwanese Children (PATCH) study, which is a joint study initiated in 2007 to investigate the epidemiology and predictive factors of asthma and allergies in children, including subjects from a birth cohort as well as several cohorts of school and pre-school children.

The objective of the current study was to identify the determinants of FeNO levels in children according to current measurement standards in a large population sample. Of particular interest was the validity of FeNO measurement as a discriminative tool for asthma, rhinitis or allergic sensitization in children in the community.

Methods

Study population and study design

The investigation was performed in public schools in Keelung, Taiwan, from January 2008 to January 2009. Study participants were recruited from a school-based sample of 5351 children (2616 boys, 48.9%; age, 10.4±2.9 years) in an International Study of Asthma and Allergies in Childhood (ISAAC) epidemiologic survey, T. C. Yao, L. S. Ou, W. I. Lee, K. W. Yeh, L. C. Chen, J. L. Huang, unpublished data. A random sample of 1900 subjects were invited to participate, aiming to include approximately 1500 children in the study with an expected participation rate of 80%. Of these, FeNO measurements were attempted in 1717 subjects (842 boys, 49.0%; age, 10.3±2.6 years) whose parents agreed to participate, representing a participation rate of 90.4%. All subjects were born to parents who were both of Asian descent. There was no significant difference in terms of age, gender, and prevalence of asthma, allergic rhinitis, and atopic dermatitis between these 1717 subjects and the original 5351 cohort members.

Parents answered a questionnaire regarding demographic data, general health information, and additional questions on possible clinical symptoms and diagnosis of allergic diseases. FeNO measurements and pulmonary function tests were performed, and if parents agreed to blood sampling, blood was collected for total and allergen-specific serum IgE. Weights and heights were measured according to standard protocols. Body mass index (BMI) was defined as weight (kg) divided by the height squared (m^2) whereas body surface area (BSA) was defined as the square root of product of weight (kg) times height (cm) divided by 3600. Assessments were conducted in the schools. This study was conducted with the approval of the Institutional Review Board of Chang Gung Medical Foundation (96-0370B) and the parents of all subjects provided written informed consent.

Exhaled nitric oxide and pulmonary function

FeNO measurements were performed by chemiluminescence analyzer (CLD 88sp NO analyzer®, Eco Medics, Duernten, Switzerland) according to 2005 ATS/ERS recommendations for standardized online measurement [18]. The children were requested to avoid eating, drinking, and strenuous exercise 1 h before FeNO measurements, which were performed before spirometry. Ambient nitric oxide and ambient temperature at the time of each test were recorded. The subjects inhaled NO-free air to total lung capacity over a period of 2–3 s through a mouthpiece. The subjects exhaled against expiratory resistance to prevent contamination with nasal nitric oxide. Repeated exhalations (three that agree within 10% or two within 5%) were performed without a nose clip at a constant flow rate of 50 mL/s. The mean FeNO was recorded. Pulmonary function was measured using spirometry (Spirolab II®, Medical International Research, Roma, Italy) in accordance with the ATS/ERS recommendations [19]. Three technically acceptable forced expirations were performed for up to eight tests. The highest forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), and forced expiratory flow (FEF_{25-75}) were recorded and percentages of the predicted values were calculated [20].

Total and allergen-specific serum immunoglobulin E

Total serum IgE was measured by ImmunoCAP™ (Phadia, Uppsala, Sweden). Allergen-specific serum IgE was measured by ImmunoCAP™ Phadiatop® Infant (Phadia), a reliable alternative to skin prick tests for detecting allergic sensitization [21]. The following allergens were included: house dust mite, cat, dog, birch, timothy, ragweed, wall pellitory, egg white, cow's milk, peanut, and shrimp. The Phadiatop Infant results were expressed on a scale of classes 0–6.

Phenotype definitions

Current and past allergic symptoms and diagnosis of allergic diseases were assessed using a modified ISAAC

questionnaire [22]. Asthma was defined as ever having asthma and either the occurrence of wheeze in the last 12 months or current use of asthma medication. Rhinitis and eczema were defined as ever having the two diseases, respectively, and either the presence of symptoms in the last 12 months or current use of medication for the two diseases, respectively. Allergic sensitization was defined as a positive Phadiatop Infant test result ($\geqslant$0.35 PAU/L; class 1–6).

Statistical analysis

All data analyses were performed using the SPSS statistical package version 15.0 for Windows (SPSS, Chicago, IL, USA). FeNO values were logarithmically transformed for analysis and results presented as back-transformed values [i.e. geometric means with 95% confidence intervals (CI)]. Univariate analyses using simple linear regression and Student's *t*-test were carried out to assess associations between log-transformed FeNO values and the following explanatory variables: age, anthropometric measurements, total IgE, pulmonary function tests, ambient nitric oxide and temperature, gender, asthma, rhinitis, eczema, allergic sensitization, recent upper respiratory infection (URI) symptoms (in the past 2 weeks), active and passive smoking, premature birth, and eating or drinking within 1 h before measurements. For multivariate analysis, multiple linear regression used a stepwise-selection method with variables that had a *P*-value <0.1 in univariate analyses for consideration of inclusion into the model. Receiver–operator characteristic (ROC) curves were generated to assess the overall validity of FeNO for discriminating asthma, rhinitis, or allergic sensitization, respectively. In addition, relationships among allergic sensitization, rhinitis, and asthma were further investigated using a general linear model ANCOVA with log-transformed FeNO values as the outcome variable and with adjustments for age and BMI. A $P<0.05$ was considered statistically significant.

Results

Subject characteristics

Acceptable FeNO measurements were obtained in 1651 of 1717 (96.2%) study subjects. The characteristics of the 1651 subjects (807 boys; age: 10.3$\pm$2.6 years [range 5–18]) were shown in Table 1. Acceptable pulmonary function tests and total and specific IgE levels were available in 1619 (98.1%) and 1278 (77.4%) of 1651 subjects, respectively. The geometric mean FeNO level in the study population was 18.6 parts per billion (p.p.b.) (95% CI, 17.9–19.3).

Univariate analyses of factors associated with fraction of exhaled nitric oxide levels

Univariate analyses were used to investigate the relationship of FeNO levels to the explanatory variables listed in Table 1. There were significant differences in FeNO levels between subjects grouped by gender, allergic diseases (i.e. asthma, rhinitis, and eczema), allergic sensitization, recent URI symptoms in the past 2 weeks, or drinking within 1 h before test. There were significant correlations between FeNO levels and age, height, weight, BSA, total IgE levels, several parameters of pulmonary function, and ambient temperature.

FeNO levels significantly and positively correlated to age ($r=0.217$, $P<0.001$; Table 1). Anthropometric variables such as height, weight, and BSA all significantly positively related to FeNO, whereas BMI was marginally significant (Table 1). Boys had significantly higher FeNO levels than did the girls (geometric mean and 95% CI, 19.8 [18.7–20.9] vs. 17.5 [16.6–18.4] p.p.b.; $P=0.002$).

Asthma, rhinitis, and eczema were all associated with higher FeNO levels (Table 1; all $P<0.001$). Subjects with allergic sensitization had higher FeNO levels than those without (26.1 [24.7–27.7] vs. 11.6 [11.1–12.2] p.p.b., $P<0.001$), with a positive correlation between FeNO levels and the height of Phadiatop Infant titres ($r=0.653$, $P<0.001$). There was also a significant correlation of total IgE levels with FeNO levels in this cohort ($r=0.551$, $P<0.001$). URI symptoms within the past 2 weeks of survey were also associated with higher FeNO levels (21.2 [19.8–22.6] vs. 16.9 [16.1–17.8] p.p.b.; $P<0.001$). In addition, there was a significant correlation between FeNO levels and parameters of pulmonary function, including FVC, FEV_1, FEF_{25-75}, FVC %predicted, and FEV_1/FVC %predicted (Table 1; all $P<0.05$).

Despite recommendations to avoid drink and food, 59 children drank and 15 children ate within 1 h before testing. Drinking was significantly related to higher FeNO levels ($P<0.001$). There was a marginally significant negative association between FeNO levels and the ambient temperature ($P=0.044$) but no significant association was found between FeNO levels and active smoking, passive smoking, premature birth, and ambient nitric oxide.

Multivariate analyses of factors associated with fraction of exhaled nitric oxide levels

Multivariate analyses of FeNO levels were performed using variables that had a *P*-value <0.1 in univariate analyses. Allergic sensitization, age, rhinitis, and BMI were significantly and independently associated with FeNO levels in this large population cohort of children in a multiple linear regression model (Table 2). This regression model explained 52.2% of total variation in FeNO levels for subjects in this population. The gradual increase

Table 1. Characteristics of the study subjects and univariate analyses of factors associated with FeNO

Continuous variable	Mean±SD	*n*	*R*	*P*-value
Age (years)	10.3±2.6	1651	0.217	<0.001
Anthropometric measurement				
Height (cm)	139.1±14.7	1651	0.213	<0.001
Weight (kg)	37.4±13.3	1651	0.147	<0.001
Body mass index (kg/m^2)	18.7±3.6	1651	0.045	0.065
Body surface area (m^2)	1.19±0.26	1651	0.169	<0.001
Total IgE (kU/L)	295.0±533.8	1278	0.551	<0.001
Pulmonary function				
FVC (L)	2.10±0.66	1619	0.191	<0.001
FEV_1 (L)	1.82±0.58	1619	0.193	<0.001
FEV_1/FVC ratio (%)	86.9±6.1	1619	0.006	0.813
FEF_{25-75} (L/s)	2.20±0.78	1619	0.162	<0.001
FVC % predicted (%)	91.3±11.3	1619	−0.062	0.013
FEV_1 % predicted (%)	90.0±10.7	1619	−0.033	0.180
FEV_1/FVC % predicted (%)	97.0±6.9	1619	0.049	0.047
FEF_{25-75} % predicted (%)	89.1±19.8	1619	0.026	0.303
Ambient nitric oxide (p.p.b)	2.32±2.77	1651	0.003	0.903
Ambient temperature (°C)	22.2±5.8	1651	−0.050	0.044

			FeNO (p.p.b), Geometric mean (95% CI)		
Categorical variable	%	*n* (yes/no)	Yes	No	*P*-value
Gender (male)	48.9	807/844	19.8 (18.7–20.9)	17.5 (16.6–18.4)	0.002
Asthma	4.3	70/1548	30.2 (24.5–37.2)	18.1 (17.4–18.8)	<0.001
Rhinitis	28.4	455/1146	28.0 (25.9–30.2)	15.7 (15.1–16.4)	<0.001
Eczema	6.4	103/1495	29.4 (24.9–34.7)	17.9 (17.2–18.7)	<0.001
Allergic sensitization*	57.0	729/549	26.1 (24.7–27.7)	11.6 (11.1–12.2)	<0.001
Recent URI symptoms	41.3	653/929	21.2 (19.8–22.6)	16.9 (16.1–17.8)	<0.001
Active smoking	0.6	10/1641	18.9 (11.3–31.5)	18.6 (17.8–19.3)	0.946
Passive smoking	52.5	835/756	18.7 (17.7–19.8)	18.4 (17.4–19.5)	0.689
Premature birth	6.4	101/1481	19.4 (16.4–22.8)	18.5 (17.7–19.3)	0.591
Drinking within 1 h before testing	3.6	59/1592	27.1 (22.1–33.3)	18.3 (17.6–27.1)	<0.001
Eating within 1 h before testing	0.9	15/1636	23.0 (14.3–37.1)	18.5 (17.8–19.3)	0.303

*Allergic sensitization was defined as positive Phadiatop Infant test results (⩾0.35 PAU/L).

FeNO, fraction of exhaled nitric oxide; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; FEF, forced expiratory flow; p.p.b, parts per billion; CI, confidence intervals; URI, upper respiratory infection.

of regression coefficients with increasing Phadiatop Infant classes suggested a dose response for the effect of allergic sensitization on FeNO levels.

Validity of fraction of exhaled nitric oxide in discriminating asthma, rhinitis, or allergic sensitization

A ROC curve was generated to determine the sensitivity and specificity of FeNO for diagnosing asthma in this population (Fig. 1a). The area under the ROC curve (AUC) (95% CI) was 0.67 (0.60–0.74). The highest combination of sensitivity and specificity was observed with a cut-off level of 28 p.p.b. (64.3% and 69.9%, respectively) for predicting asthma. There were notable extremely low positive predictive value (PPV; 8.8%) and the extremely high negative predictive value (NPV; 97.7%). The discriminative accuracy of FeNO was slightly improved (AUC = 0.71) by fine-tuning the outcome variable to allergic asthma (asthma and allergic sensitization) (Fig. 1b). At a cut-off of 28 p.p.b., the sensitivity, specificity, PPV, and NPV were 70.0%, 70.4%, 9.0%, and 98.3%, respectively. It may be suggested that the combined use of both FeNO and spirometry may have better diagnostic properties for asthma. We examined this issue by applying both FeNO and pulmonary function variable (either FEV_1 < 80% predicted or FEV_1/FVC ratio < 80%) for the diagnosis of either asthma or allergic asthma. However, the addition of pulmonary function variable (FEV_1 or FEV_1/FVC) to FeNO measurement did not increase accuracy, as the combination resulted in high specificity (95.3–97.6%) but at the cost of very low sensitivity (14.5–20.4%).

Table 2. Multivariate analyses of factors associated with FeNO

Variable	Coefficient (95% CI)	*P*-value
Allergic sensitization		
Phadiatop Infant class 6*	1.449 (1.253–1.646)	<0.001
Phadiatop Infant class 5*	1.349 (1.217–1.481)	<0.001
Phadiatop Infant class 4*	1.161 (1.052–1.269)	<0.001
Phadiatop Infant class 3*	0.883 (0.783–0.983)	<0.001
Phadiatop Infant class 2*	0.296 (0.195–0.396)	<0.001
Phadiatop Infant class 1*	0.119 (0.005–0.233)	<0.001
Phadiatop Infant class 0	–	–
Age	0.071 (0.058–0.084)	<0.001
Rhinitis	0.165 (0.090–0.240)	<0.001
Body mass index	−0.015 (−0.024–−0.005)	0.002

Coefficients should be judged as the change of log FeNO when the variables change 1 unit.

*Compared with Phadiatop Infant class 0.

†Cumulative $R^2 = 0.522$.

FeNO, fraction of exhaled nitric oxide; CI, confidence intervals.

On the other hand, FeNO had modest accuracy in discriminating between children with and without rhinitis, with an AUC value of 0.70 (Fig. 1c). We also analyzed the data using allergic rhinitis (rhinitis and allergic sensitization) as the outcome variable, which improved the accuracy of FeNO (AUC = 0.78) (Fig. 1d). The sensitivity, specificity, PPV, and NPV were 70.5%, 75.8%, 47.2%, and 89.3%, respectively at a FeNO cut-off of 24.7 p.p.b.

As FeNO was closely associated with allergic sensitization, it was hypothesized that FeNO measurement might provide a simple non-invasive screening test to distinguish atopic and non-atopic children. The AUC (95% CI) was 0.80 (0.77–0.82) (Fig. 1e). The optimum cut-off level of 15.4 p.p.b. was identified as having the best combination of sensitivity and specificity (72.2% and 71.2%, respectively) for predicting allergic sensitization. The PPV and NPV were 76.9% and 65.8%, respectively. Taken together, the ability of FeNO in discriminating allergic sensitization was better than in diagnosing asthma.

Relationships between fraction of exhaled nitric oxide levels and allergic sensitization, rhinitis, and asthma

Relationships between allergic sensitization, rhinitis, and asthma were further explored using an ANCOVA model with log-transformed FeNO values as the outcome variable and with adjustments for age and BMI. In the combined analyses of allergic sensitization, rhinitis, and asthma, elevated FeNO levels were confined mainly to children having allergic sensitization (Fig. 2). Children with allergic sensitization showed significantly elevated FeNO levels when they had asthma and/or rhinitis. Notably, among children with neither asthma nor rhinitis, the geometric mean FeNO level in subjects who had allergic sensitization (21.7 p.p.b.) was 1.9 times higher than in those without allergic sensitization (11.3 p.p.b.; $P < 0.001$). In the presence of allergic sensitization, children with co-existing asthma and/or rhinitis had even higher FeNO levels. In contrast, in the absence of allergic sensitization, FeNO levels in children with asthma were not significantly different from those without asthma. Children with non-allergic rhinitis but no asthma had small but significant elevations in FeNO levels compared with asymptomatic non-sensitized subjects.

Discussion

This population-based study identifies that allergic sensitization, age, rhinitis, and BMI are independent determinants of FeNO levels in children. This is the largest study to date that measures FeNO with a single-breath online method in accordance with current standards in a population of 1717 Asian children over a wide age range in the community. The findings allow the medical community to discriminate the pros and cons of FeNO as a diagnostic tool from research to the 'real world', the community, and provide important useful information on interpreting FeNO levels in children.

In the current study, allergic sensitization is a significant factor, although not the only one, in explaining variation of FeNO in a population setting. In the combined analyses of asthma, rhinitis, and allergic sensitization, the data here demonstrates that elevated FeNO levels are mainly confined to atopic children. The clear dose–response correlation between FeNO levels and allergic sensitization in terms of Phadiatop Infant titres suggests that the relationship between FeNO and atopy is not only qualitative but also quantitative. This is supported by the positive correlation of FeNO with the number of positive skin prick tests in other investigations [23–27]. The present results also indicate that the close relationship between allergic sensitization and elevated FeNO levels is independent of allergic symptoms, as children with allergic sensitization have elevated FeNO levels regardless of whether symptoms of asthma or allergic rhinitis are present. Thus, this study establishes FeNO as a non-invasive biomarker of atopy.

This study is the first to test the validity of FeNO measurement for discriminating children with and without allergic sensitization in a population-based survey, which establishes that FeNO is a robust discriminator of allergic sensitization. Leung et al. [28] provide support for our findings from their factor analysis which identifies that atopy-related indices and FeNO are entered into the same cluster, acting as a separate dimension that is not overlapping with airway inflammation in the assessment of childhood asthma. Of note is also the existing evidence that in atopic subjects, FeNO levels are associated with airway inflammation [29], airway hyperresponsiveness and current wheezing [30]. In addition, Malmberg et al.

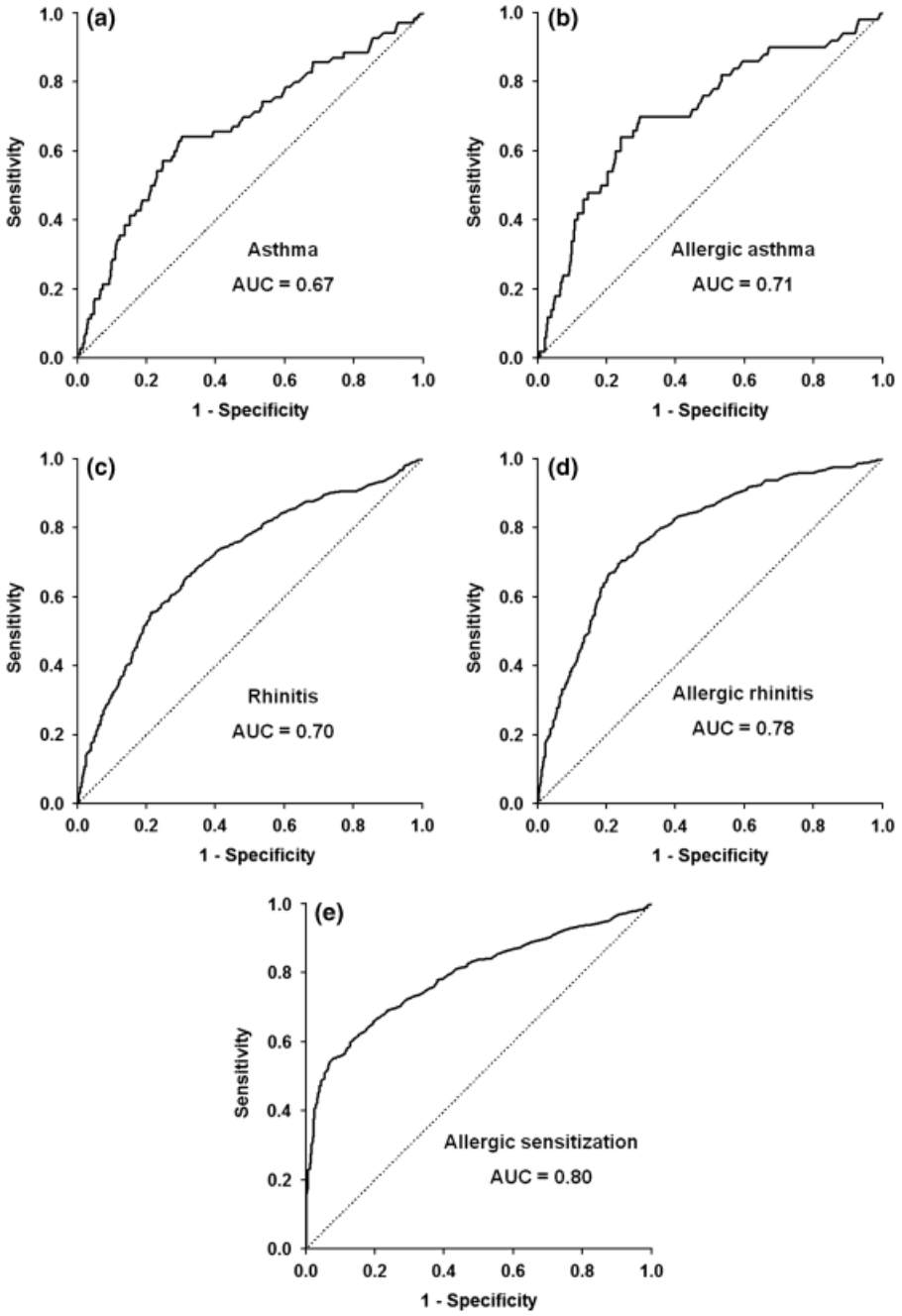

Fig. 1. Receiver–operator characteristic (ROC) curves indicating the sensitivity and specificity of fraction of exhaled nitric oxide (FeNO) measurements for predicting asthma (a, b), rhinitis (c, d), or allergic sensitization (e). (a) Asthma (area under the ROC curve [AUC] [95% CI], 0.67 [0.60–0.74]). (b) Allergic asthma (AUC, 0.71 [0.64–0.79]). (c) Rhinitis (AUC, 0.70 [0.67–0.73]). (d) Allergic rhinitis (AUC, 0.78 [0.75–0.81]). (e) Allergic sensitization (AUC, 0.80 [0.77–0.82]).

[31] report discordant data in a clinic-based study of 132 patients with suspected asthma which shows that symptomatic steroid-naïve asthma and sputum eosinophilia are more important determinants of high levels of FeNO than allergic sensitization. It is therefore clear that FeNO is more than just a marker of atopy.

There remains considerable debate regarding the validity of FeNO measurement as a diagnostic tool for asthma from research to clinical practice. There has been evidence presented in recent years that demonstrate good sensitivity and specificity of elevated FeNO for discriminating asthmatic and non-asthmatic subjects in children and adults, mostly in small clinic-based studies [6–13]. In contrast, population surveys in children [15–17] and adults [14] report that FeNO measurement is not a useful tool in diagnosing asthma. Indeed, the current study

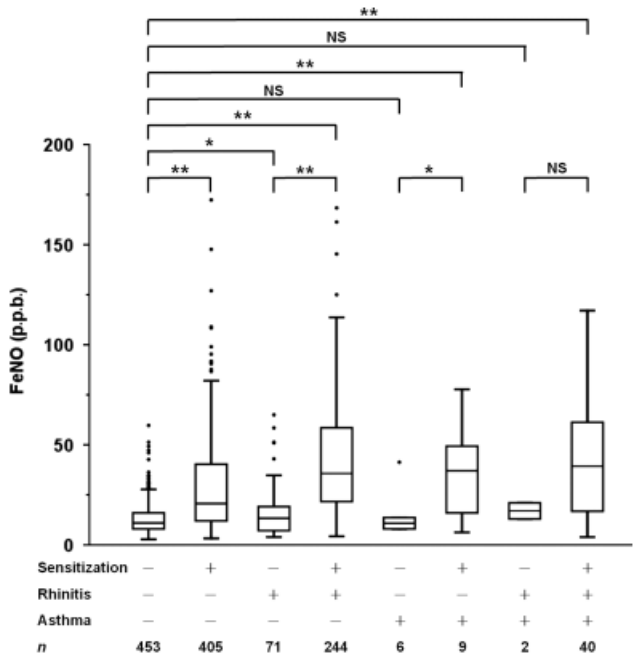

Fig. 2. Box plots showing median and interquartile ranges of Fraction of exhaled nitric oxide (FeNO) by subject group. Dots beyond the bounds of the whiskers denote outliers. *P*-values refer to the comparisons indicated by the marker with adjustments for age and body mass index using the ANCOVA model. $^{*}P<0.05$; $^{**}P<0.001$; NS, not statistically significant.

shows that asthmatic children overall have higher FeNO levels than other children. However, this association is due merely entirely to the effects of allergic sensitization, age, rhinitis, and BMI: in multivariate analyses FeNO is no longer a significant predictor of asthma. A similar result is reported by Scott et al. [23] and Jackson et al. [32]; once allergic sensitization is accounted for, the relationship of asthma and FeNO levels become insignificant in a population setting. It is somewhat disappointing that FeNO is not a reliable predictor of asthma in this population survey, as elevated levels do not distinguish between asthma and effects of other host determinants of FeNO and the low PPV will probably result in an unacceptably high false positive rate of diagnosis. Nonetheless, the almost perfect NPV (98.3%) of FeNO at a cut-off of 28 p.p.b. for allergic asthma could allow physicians to use low levels of FeNO in children with vague symptoms to exclude the diagnosis of allergic asthma. A plausible explanation for the overt discrepancies between clinic-based and population-based studies is that allergic sensitization and other important host determinants of FeNO have not been accounted for in the former studies.

It is also worth noting that, consistent with previous studies [14,24,33–36], FeNO levels are significantly increased in children with rhinitis but without asthma, independent of other confounding factors. The data in the current study also establishes an age-dependent increase of FeNO levels with an average increase of 7.4% per year of age from young children to adolescents, which has been suggested in previous smaller studies in children [37–40] and adults [41]. The mechanism for this age dependency is unclear but having the same fixed expiratory flow in all ages is a possible reason [3]. Some studies suggest the role of gender on influencing FeNO levels [42–44], while others disagree [37,41]. In the multivariate analyses here, gender is not independently associated with FeNO levels, after accounting for effects of allergic sensitization and clinical status. A plausible explanation for the gender difference of FeNO levels in univariate analysis is the higher prevalence of allergic sensitization and allergic rhinitis in boys.

What is interesting is also the previously unreported negative independent association between BMI and FeNO levels in children, although the impact is only modest (i.e. 1.5% decrease in FeNO per kg/m^2). Similarly, Linn et al. [45] note that increasing weight-for-height is associated with decreasing FeNO in children aged 7–10 years. In addition, Maniscalco et al. [46] observe a reduction in FeNO in 24 adults with severe obesity which is restored after weight loss, supporting our data. The relationship between BMI and FeNO remains to be explained. Ho et al. [47] demonstrate that FeNO levels are affected by airway calibre. It is therefore reasonable to speculate that, with increasing BMI, the airway is narrower than expected on the basis of the reduction in lung volume [48], then the consequent increase in airflow velocity when the exhalation rate at the mouth is kept constant diminishes the transit time of alveolar gas in the airway and thereby reduces the amount of nitric oxide exhaled.

The representative sampling of children in the community, large sample size, wide age range, incorporation of spirometric testing and objective markers of atopy (Phadiatop® Infant and total IgE levels), and use of current standards for single-breath online measurement all add strength to these results. Furthermore, ethnic differences of FeNO levels between Caucasians and Asians are noted in some reports [37,42,45,49]. The reasons for these ethnic differences are poorly understood. However, the small samples size of Asian subgroup in previous studies makes it difficult to investigate the determinants of FeNO levels in Asian children, which is now addressed by the current study. Although it may be argued that the low accuracy of FeNO in discriminating asthma could be attributed to the low prevalence of asthma in this cohort, it is indeed a general principle that ROC curves are theoretically independent of disease prevalence [50]. The population-based design of this study does not allow preclusion on the contribution of asthma in raising FeNO levels in children because asthmatic children recruited in a population setting tend to have milder disease and/or are receiving anti-inflammatory treatment which could affect FeNO levels. Moreover, this cross-sectional study

can not address the role of FeNO measurement as a clinical guide for asthma management which has been well documented [1–5]. Instead, it provides important practical information regarding the interpretation of FeNO levels in children. Here, we report solid evidence that elevated FeNO levels in children can be due to other host factors, including allergic sensitization, older age, rhinitis, and lower BMI rather than asthma per se, which must not be disregarded when considering the application of FeNO measurement in the diagnosis and management of asthma.

In conclusion, this large population-based study identifies that allergic sensitization is probably the most important factor affecting FeNO levels within the whole population. FeNO measurement discriminates children with and without allergic sensitization independent of allergic symptoms. We present clear evidence that while it is possible to use low FeNO levels to exclude allergic asthma, high FeNO levels may be caused by other important host factors including allergic sensitization, age, rhinitis, and BMI, in addition to asthma.

Clinical Relevance

This study offers insights into role of FeNO in the population setting. FeNO is not a reliable diagnostic biomarker of asthma, but is more robust in discriminating allergic sensitization independent of allergic symptoms. Another important message is that low FeNO levels in children may help exclude allergic asthma but high levels may be caused by allergic sensitization, older age, rhinitis, and lower BMI, in addition to asthma.

Acknowledgements

We thank the study subjects, their parents and the school teachers for active participation in the study, as well as the involved schools. We thank the Department of Education, Keelung City Government for administrative support for the study. This study was funded by Chang Gung Memorial Hospital, Taiwan (Grant CMRPG260291, CMRPG260292, CMRPG260293, and CMRPG260261).

Conflict of interest: All authors declared no conflict of interest.

References

1 Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC. Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 2006; **61**:817–27.
2 Lim KG, Mottram C. The use of fraction of exhaled nitric oxide in pulmonary practice. *Chest* 2008; **133**:1232–42.
3 Bates CA, Silkoff PE. Exhaled nitric oxide in asthma: from bench to bedside. *J Allergy Clin Immunol* 2003; **111**:256–62.
4 Smith AD, Taylor DR. Is exhaled nitric oxide measurement a useful clinical test in asthma? *Curr Opin Allergy Clin Immunol* 2005; **5**:49–56.
5 Taylor DR. Nitric oxide as a clinical guide for asthma management. *J Allergy Clin Immunol* 2006; **117**:259–62.
6 Smith AD, Cowan JO, Filsell S *et al.* Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests. *Am J Respir Crit Care Med* 2004; **169**:473–8.
7 Deykin A, Massaro AF, Drazen JM, Israel E. Exhaled nitric oxide as a diagnostic test for asthma: online versus offline techniques and effect of flow rate. *Am J Respir Crit Care Med* 2002; **165**: 1597–601.
8 Avital A, Uwyyed K, Berkman N, Godfrey S, Bar-Yishay E, Springer C. Exhaled nitric oxide and asthma in young children. *Pediatr Pulmonol* 2001; **32**: 308–13.
9 Dupont LJ, Demedts MG, Verleden GM. Prospective evaluation of the validity of exhaled nitric oxide for the diagnosis of asthma. *Chest* 2003; **123**:751–6.
10 Malmberg LP, Pelkonen AS, Haahtela T, Turpeinen M. Exhaled nitric oxide rather than lung function distinguishes preschool children with probable asthma. *Thorax* 2003; **58**:494–9.
11 Berkman N, Avital A, Breuer R, Bardach E, Springer C, Godfrey S. Exhaled nitric oxide in the diagnosis of asthma: comparison with bronchial provocation tests. *Thorax* 2005; **60**:383–8.
12 Fortuna AM, Feixas T, González M, Casan P. Diagnostic utility of inflammatory biomarkers in asthma: exhaled nitric oxide and induced sputum eosinophil count. *Respir Med* 2007; **101**:2416–21.
13 Sivan Y, Gadish T, Fireman E, Soferman R. The use of exhaled nitric oxide in the diagnosis of asthma in school children. *J Pediatr* 2009; **155**:211–6.
14 Travers J, Marsh S, Aldington S *et al.* Reference ranges for exhaled nitric oxide derived from a random community survey of adults. *Am J Respir Crit Care Med* 2007; **176**:238–42.
15 Prasad A, Langford B, Stradling JR, Ho LP. Exhaled nitric oxide as a screening tool for asthma in school children. *Respir Med* 2006; **100**:167–73.
16 Brussee JE, Smit HA, Kerkhof M *et al.* Exhaled nitric oxide in 4-year-old children: relationship with asthma and atopy. *Eur Respir J* 2005; **25**:455–61.
17 Henriksen AH, Lingaas-Holmen T, Sue-Chu M, Bjermer L. Combined use of exhaled nitric oxide and airway hyperresponsiveness in characterizing asthma in a large population survey. *Eur Respir J* 2000; **15**:849–55.
18 American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005; **171**:912–30.
19 Miller MR, Hankinson J, Brusasco V *et al.* Standardisation of spirometry. *Eur Respir J* 2005; **26**:319–38.
20 Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal expiratory flow–volume curve with growth and aging. *Am Rev Respir Dis* 1983; **127**:725–34.
21 Ballardini N, Nilsson C, Nilsson M, Lilja G. ImmunoCAP Phadiatop Infant – a new blood test for detecting IgE sensitization in children at 2 years of age. *Allergy* 2006; **61**:337–43.
22 Asher MI, Keil U, Anderson HR *et al.* International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; **8**:483–91.
23 Scott M, Raza A, Karmaus W *et al.* Influence of atopy and asthma on ex-

haled nitric oxide in an unselected birth cohort study. *Thorax* 2010; **65**:258–62.
24 Jouaville LF, Annesi-Maesano I, Nguyen LT, Bocage AS, Bedu M, Caillaud D. Interrelationships among asthma, atopy, rhinitis and exhaled nitric oxide in a population-based sample of children. *Clin Exp Allergy* 2003; **33**:1506–11.
25 van Amsterdam JG, Janssen NA, de Meer G *et al*. The relationship between exhaled nitric oxide and allergic sensitization in a random sample of school children. *Clin Exp Allergy* 2003; **33**:187–91.
26 Strunk RC, Szefler SJ, Phillips BR *et al*. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol* 2003; **112**:883–92.
27 Craig TJ, King TS, Lemanske RF Jr *et al*. Aeroallergen sensitization correlates with PC(20) and exhaled nitric oxide in subjects with mild-to-moderate asthma. *J Allergy Clin Immunol* 2008; **121**:671–7.
28 Leung TF, Wong GW, Ko FW, Lam CW, Fok TF. Clinical and atopic parameters and airway inflammatory markers in childhood asthma: a factor analysis. *Thorax* 2005; **60**:822–6.
29 Olin AC, Alving K, Torén K. Exhaled nitric oxide: relation to sensitization and respiratory symptoms. *Clin Exp Allergy* 2004; **34**:221–6.
30 Leuppi JD, Downs SH, Downie SR, Marks GB, Salome CM. Exhaled nitric oxide levels in atopic children: relation to specific allergic sensitisation, AHR, and respiratory symptoms. *Thorax* 2002; **57**:518–23.
31 Malmberg LP, Turpeinen H, Rytilä P, Sarna S, Haahtela T. Determinants of increased exhaled nitric oxide in patients with suspected asthma. *Allergy* 2005; **60**:464–8.
32 Jackson DJ, Virnig CM, Gangnon RE *et al*. Fractional exhaled nitric oxide measurements are most closely associated with allergic sensitization in school-age children. *J Allergy Clin Immunol* 2009; **124**:949–53.
33 van Asch CJ, Balemans WA, Rovers MM, Schilder AG, van der Ent CK. Atopic disease and exhaled nitric oxide in an unselected population of young adults. *Ann Allergy Asthma Immunol* 2008; **100**:59–65.
34 Rolla G, Guida G, Heffler E *et al*. Diagnostic classification of persistent rhinitis and its relationship to exhaled nitric oxide and asthma: a clinical study of a consecutive series of patients. *Chest* 2007; **131**:1345–52.
35 Henriksen AH, Sue-Chu M, Holmen TL, Langhammer A, Bjermer L. Exhaled and nasal NO levels in allergic rhinitis: relation to sensitization, pollen season and bronchial hyperresponsiveness. *Eur Respir J* 1999; **13**:301–6.
36 Nordvall SL, Janson C, Kalm-Stephens P, Foucard T, Torén K, Alving K. Exhaled nitric oxide in a population-based study of asthma and allergy in schoolchildren. *Allergy* 2005; **60**:469–75.
37 Buchvald F, Baraldi E, Carraro S *et al*. Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. *J Allergy Clin Immunol* 2005; **115**:1130–6.
38 Franklin PJ, Taplin R, Stick SM. A community study of exhaled nitric oxide in healthy children. *Am J Respir Crit Care Med* 1999; **159**:69–73.
39 Latzin P, Beck J, Griese M. Exhaled nitric oxide in healthy children: variability and a lack of correlation with atopy. *Pediatr Allergy Immunol* 2002; **13**: 37–46.
40 Kissoon N, Duckworth LJ, Blake KV *et al*. Exhaled nitric oxide concentrations: online versus offline values in healthy children. *Pediatr Pulmonol* 2002; **33**: 283–92.
41 Olin AC, Rosengren A, Thelle DS, Lissner L, Bake B, Torén K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. *Chest* 2006; **130**:1319–25.
42 Wong GW, Liu EK, Leung TF *et al*. High levels and gender difference of exhaled nitric oxide in Chinese schoolchildren. *Clin Exp Allergy* 2005; **35**:889–93.
43 Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. *Eur Respir J* 2003; **21**:433–8.
44 van der Lee I, van den Bosch JM, Zanen P. Reduction of variability of exhaled nitric oxide in healthy volunteers. *Respir Med* 2002; **96**:1014–20.
45 Linn WS, Rappaport EB, Berhane KT, Bastain TM, Avol EL, Gilliland FD. Exhaled nitric oxide in a population-based study of southern California schoolchildren. *Respir Res* 2009; **10**:28.
46 Maniscalco M, de Laurentiis G, Zedda A *et al*. Exhaled nitric oxide in severe obesity: effect of weight loss. *Respir Physiol Neurobiol* 2007; **156**:370–3.
47 Ho LP, Wood FT, Robson A, Innes JA, Greening AP. The current single exhalation method of measuring exhales nitric oxide is affected by airway calibre. *Eur Respir J* 2000; **15**:1009–13.
48 King GG, Brown NJ, Diba C *et al*. The effects of body weight on airway calibre. *Eur Respir J* 2005; **25**:896–901.
49 Kovesi T, Kulka R, Dales R. Exhaled nitric oxide concentration is affected by age, height, and race in healthy 9- to 12-year-old children. *Chest* 2008; **133**: 169–75.
50 Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993; **39**:561–77.