

Heterogeneity of FeNO response to inhaled steroid in asthmatic children

F. Buchvald*, H. Eiberg† and H. Bisgaard*

*Department of Paediatrics, Rigshospitalet, National University Hospital, Copenhagen, Denmark and †Department of Human Genetics, Panum Institute, Copenhagen University, Copenhagen, Denmark

Summary

Background Nitric oxide in exhaled air is regarded as an inflammation marker, and may be used to monitor the anti-inflammatory control from inhaled corticosteroids (ICSs). However, this response to ICSs exhibits a heterogeneous pattern.

Objective The study aimed to describe the independent variables associated with the heterogeneity in the response of exhaled nitric oxide to ICSs.

Methods Exhaled nitric oxide (FeNO), lung function, bronchial hyper-responsiveness (BHR), specific IgE to common inhalant allergens, blood eosinophils, other atopic manifestations and variants in nitric oxide synthetase 1 (NOS1) gene were studied in a double-blind, placebo-controlled crossover comparison of budesonide (BUD) Turbohaler 1600 mcg daily vs. placebo in asthmatic schoolchildren.

Results Forty children were included in the study from a screening of 184 asthmatic children with moderately persistent asthma, well controlled on regular BUD 400 mcg daily: 20 children with normal FeNO and 20 with raised FeNO. FeNO, BHR and forced expiratory volume in 1 s improved significantly after BUD 1600 mcg (BUD1600). However, FeNO after ICS treatment exhibited a Gaussian distribution and FeNO was significantly raised in 15 children. Allergy and BHR, but none of the other independent variables under study were significantly related to FeNO after BUD1600.

Conclusion Exhaled nitric oxide exhibited a heterogeneous response to ICS in asthmatic schoolchildren. Allergy and BHR were driving FeNO level independently of high-dose steroid treatment. This should be considered when using FeNO for steroid dose titration and monitoring of ICS anti-inflammatory control in asthmatic children.

Keywords bronchial asthma, bronchial hyper-reactivity, child, exhaled nitric oxide, heterogeneity, inhaled corticosteroid

Submitted 20 January 2003; revised 13 August 2003; accepted 28 August 2003

Introduction

Treatment guidelines emphasize the need to minimize symptoms and optimize lung function [1]. This strategy may fail to control airway inflammation completely [2]. Nitric oxide in exhaled air (FeNO) correlates with eosinophils in bronchoalveolar lavage fluid in asthmatic children [3], and has been suggested as a more sensitive marker of sub-clinical airway inflammation and control from steroids [4]. However, the effect of inhaled corticosteroid (ICS) on FeNO has been reported to reach a plateau at a dose of 400 mcg of budesonide (BUD) [5] and remains elevated in some children despite oral steroid [6]. This may reflect heterogeneity in the sensitivity to ICS in the part of the airway inflammation reflected by FeNO, or eventually it may point toward FeNO being driven by processes only partly associated with airway inflammation and asthma severity. This study aimed to describe independent variables associated with the hetero-

geneity in the FeNO response to ICS in asthmatic children. Particularly, we studied markers of allergy, lung function, bronchial reactivity and genetic polymorphism for the NO synthase 1 (NOS1).

Patients

Screening population

FeNO was measured according to European Respiratory Society recommendations with an exhalation flow of 100 mL/s [7] in a population of asthmatic children from our outpatient clinic, 6–15 years of age, with a history of moderately persistent asthma [8]. Children eligible for the study were all well controlled according to international guidelines [1] on regular treatment with a titrated daily dose of 400 mcg inhaled BUD Turbohaler [9]. Increased level was defined as the upper 75% quartile.

Study population

Forty children from the screening population were invited for the controlled study, 20 with FeNO above the upper 75%

The study medication was sponsored by AstraZeneca®.

Correspondence: Hans Bisgaard, MD, DMSci, Department of Paediatrics, Rigshospitalet, National University Hospital, DK-2100 Copenhagen, Denmark. E-mail: Bisgaard@copsac.dk

quartile and 20 from the lower quartile. Children were included if asthma was considered to be clinically stable with unchanged controller treatment for 4 weeks (forced expiratory volume in 1 s (FEV₁) > 85% of predicted, no nocturnal awakenings and use of rescue medication less than three times per week). All children were never smokers and had no current airway infections.

Control population

FeNO was measured in 42 healthy children (7–16 years) from public schools without a history of recent airway infection, asthma, hayfever, eczema or self-reported allergy. All were non-smokers.

Design

This was a double-blind placebo-controlled crossover study with no washout period. All children stopped their regular treatment with ICS during the study. The children were randomized by a computer-generated schedule to receive BUD 1600 mcg daily (400 mcg $\times$ 4 once in the evening) inhaled from Turbohaler (Astra Zeneca®, Lund, Sweden) (BUD1600), or matching placebo (PLAC). β_2 -agonists were used for reliever therapy, but no other anti-asthma therapy was allowed. Each treatment period was for 14–18 days. All children noted daily the use of rescue medication and study medication to enforce compliance.

FeNO, spirometry and dry-air hyperventilation challenge test was measured at the end of each treatment period, and FeNO and spirometry at baseline.

Methods

Dry-air challenge test

The child hyperventilated for 4 min from dehumidified atmospheric air +5% CO₂ from a calibrated gas bottle (AGA Gas, Stockholm, Sweden) during a mouthpiece and with a nose clip as previously described [10, 11]. Lung function was measured 3 min after the challenge test.

Spirometry

Spirometry was performed with a Master Screen unit (E:Jaeger GmbH, Würzburg; Germany) in accordance with American Thoracic Society (ATS) guidelines [12] to calculate FEV₁, forced vital capacity and forced expiratory flow between 25%–75% of forced vital capacity (FEF_{25–75}).

Exhaled NO

Single-breath on-line (SBOL) measurement of FeNO was performed with Aerocrine NO system® (Aerocrine AB, Stockholm, Sweden) in accordance with the recommendations from the ATS with an exhalation flow of 50 mL/s [13]. Short- and long-acting β_2 was not allowed for 6 and 12 h, respectively, before the study visits.

Increased FeNO level was defined as levels above 20 p.p.b. estimated as the mean +2SD of the FeNO in the healthy controls.

NOS genotype

DNA was extracted after standard methods on an ABI DNA extractor (Applied Biosystems, Foster City, CA, USA) and polymerase chain reaction with oligonucleotide primers complementary to the NOS1 alleles at the ATT repeat in intron 20 of the NOS1 gene as previously described in detail [14].

Atopic status

Blood eosinophils and specific serum IgE to the common Scandinavian allergens (Paediatric Cap Phadiatop; Pharmacia Diagnostic, Uppsala, Sweden) was measured. Skin prick test was performed to the same allergens (ALK, Hørsholm, Denmark) [15]. The current atopic history of asthmatics and healthy subjects was collected according to the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [16].

Ethics

The study was approved by the local Ethics Committee (KF 01-126/00) and Health Authorities (2612–2005). Written informed consent was obtained from the parents and verbal acceptance from the children.

Data analysis and statistics

The study was performed and data were collected according to the principles of Good Clinical Practice. Computer program packages were used for statistic calculations (SAS Version v8 and MedCalc Version 1997–2000).

Abnormal FeNO was defined from healthy children as mean FeNO + 2SD. A significant change in FeNO was defined as a change beyond the day-to-day variability measured at 5 consecutive days (15%) [17].

Data were handled according to basic statistic principles [18]. Parametric tests were used if data were normally distributed and non-parametric test for other data (numbers of positive skin prick test; Phadiatop, blood eosinophils). Normality distribution could be accepted for FeNO data (Kolmogorov–Smirnov test), but was log-transformed to improve the normality distribution and expressed as transformed estimates by geometric means with 95% confidence intervals (CIs). Paired *t*-test or ANOVA analysis was used to compare change in FeNO from treatment, whereas the unpaired *t*-test was used to compare different groups. Chi-square test was used to compare frequencies and Yates' correction for continuity was applied.

Carry-over effect (treatment-period interaction) was analysed for FeNO, FEV₁ and bronchial hyper-responsiveness (BHR) by calculating for each subject the average ($\bar{a}_i$) of the observations in the two treatment periods and averaging these for each group. The test for interaction was performed by a two-sample *t*-test comparing $\bar{a}_1$ with $\bar{a}_2$ [18].

Linear multiple regression with backward elimination (if $P > 0.05$) was used to describe the relation between FeNO and selected variables (Phadiatop, BHR and FEV₁ and FEF_{25–75}).

The children were classified according to NOS1 ATT repeats (homozygous for 12 ATT repeats, heterozygous for 12 ATT repeats, or homozygous for <12 ATT repeats) [14].

All P -values were two-sided and a P -level <0.05 was considered to be statistically significant.

Results

One hundred and eighty-four asthmatic children on 400 mcg daily dose of BUD were screened for the study. FeNO described a Gaussian distribution after log-transformation as previously reported [9]. Forty children were included from this screening pool selecting children from the upper and lower quartiles of FeNO at BUD400.

Thirty-nine asthmatic and 42 healthy children completed the study (Table 1). FeNO of the asthmatic children was significantly increased during treatment with BUD400 as well as during treatment with BUD1600 compared with the group of healthy controls ($P < 0.0001$; $P = 0.0004$, respectively). FEV₁ (% of predicted) of the asthmatic children was 96% at baseline.

The children reported no asthma exacerbations or adverse events throughout the study.

FEV₁ (% of predicted) improved significantly after BUD1600 compared with PLAC (mean: 90% vs. 95%; $P < 0.05$), as did FEF_{25–75} (mean: 67% vs. 73%; $P < 0.05$).

BHR to dry air improved significantly after BUD1600 compared with PLAC. The mean (95% CI) decrease in FEV₁ after dry-air hyperventilation was 12% (9–16%) after PLAC compared with 8% (5–11%) after BUD1600 ($P < 0.01$).

FeNO was significantly reduced after BUD1600 geometric mean (95% CI) (16 p.p.b. [12–21]) compared with FeNO after placebo (steroid-free) (33 p.p.b. [22–40]) and BUD400 at

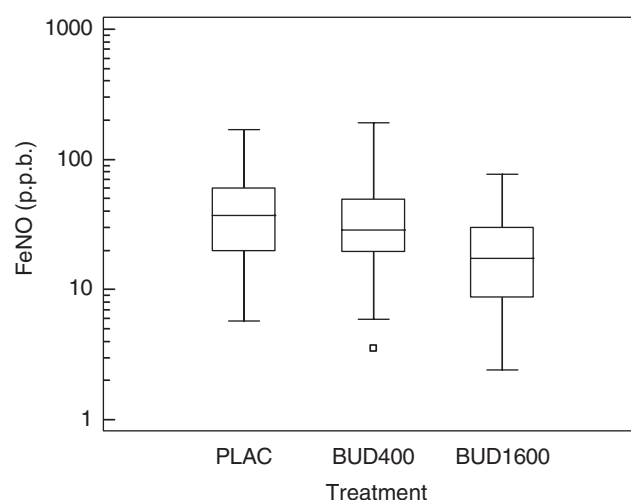
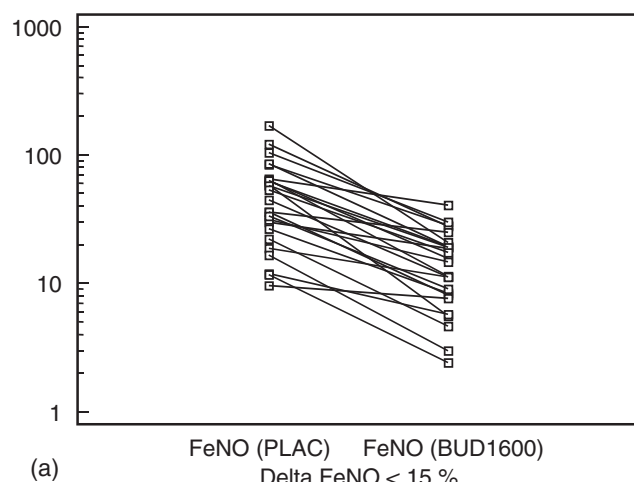
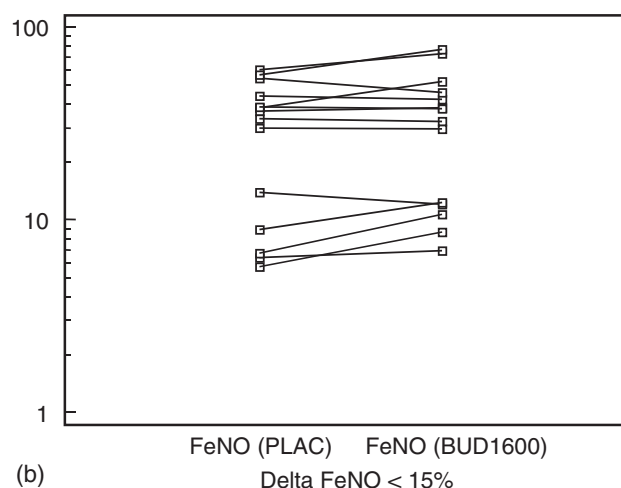


Fig. 1. FeNO (median and interquartile range) at three doses of steroid: measured at inclusion (budesonide 400 mcg (BUD400)), placebo (PLAC) and after BUD1600.



(a)



(b)

Fig. 2. (a) Responders and (b) non-responders. Individual responses in FeNO (log-scale) after placebo (PLAC) and budesonide 1600 mcg (BUD1600). The group is split based on the relative change in FeNO (>15% = responders and <15% = non-responders) from PLAC to BUD1600.

Table 1. Demographic data at randomization visit

<i>Asthmatic children, N = 39</i>	
Age mean [range]	12 years [7–16]
Gender	Females: 10 (26%) Males: 29 (74%)
Weight mean [range]	51 kg [29–117]
Height mean [range]	153 cm [131–197]
Steroid treatment	Budesonide 400 mcg
FeNO geometric mean [95% CI]	27 p.p.b. [21–36]
FEV ₁ mean [95% CI]	96 % [91–101]
Positive skin test	82%
Rhinitis	74%
Eczema	59%
Atopy in first-degree relatives	54%
Phadiatop (kU/L) median [25–75 percentile]	51 [7–129]
B-eosinophils (mill/L) median [25–75 percentile]	0.34 [0.21–0.5]
<i>Healthy children, N = 42</i>	
Age mean [95%CI]	11 years [7–16]
FeNO geometric mean [95%CI] (SD)	10 p.p.b. [8–11] (5 p.p.b.)

CI, confidence interval; FEV₁, forced expiratory volume in 1 s; B-eosinophils, blood eosinophils.

randomization (27 p.p.b. [21, 41–44, 22–32] (ANOVA; $P < 0.0001$) (Fig. 1).

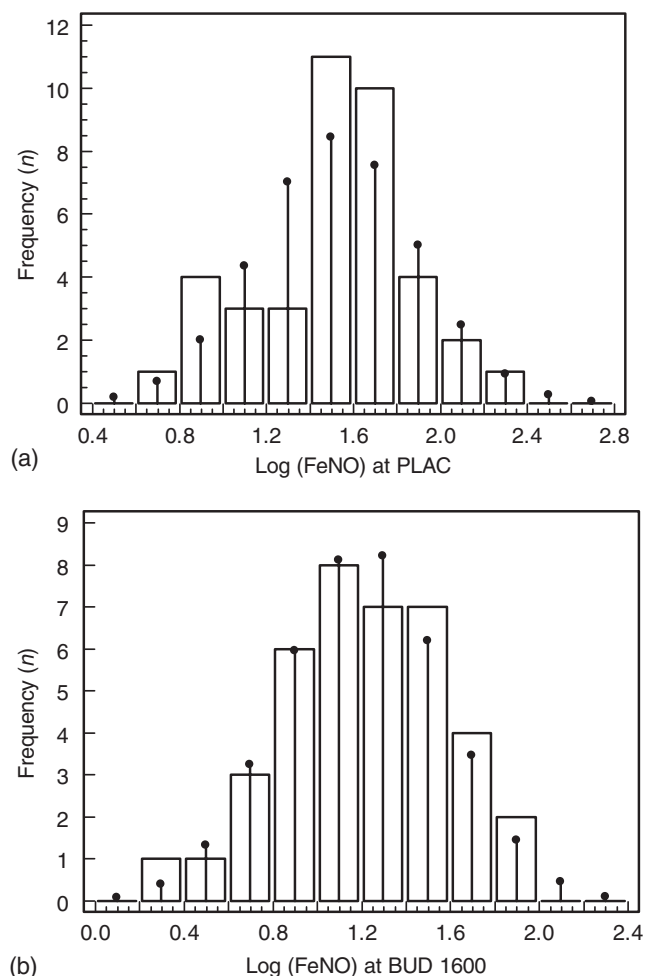


Fig. 3. Heterogeneity in FeNO (log-transformed) after placebo (PLAC) (a) and budesonide 1600 mcg (BUD1600) (b).

Table 2. Comparison in lung function, BHR, atopic markers and genetic polymorphism for NOS1 synthase between children grouped according to normalization of FeNO from high-dose ICS after BUD1600

	FeNO increased	FeNO normal	P-value
N	15	24	
FEV ₁ mean [95%CI]	94% [85–103]	95% [89–103]	NS
FEF _{25–75} mean [95%CI]	76% [62–90]	71% [63–78]	NS
BHR (Δ FEV ₁) mean [95%CI]	0.88 [0.82–0.94]	0.94 [0.90–0.97]	$P = 0.06$
Doses of β_2 during BUD ($n = 7$)	12 (3)	7 (4)	NS
Positive skin tests median [25–75 percentile]	3 [1.7–4.0]	1.5 [0.2–2.8]	$P = 0.03$
Phadiatop (kU/L) median [25–75 percentile]	99 [51–204]	21 [1–70]	$P = 0.02$
B-eosinophils (mill/L) median [25–75 percentile]	0.43 [0.26–0.50]	0.32 [0.20–0.50]	NS
Eczema	60%	58%	NS
Rhinitis	71%	72%	NS
Atopy in relatives	47%	58%	NS
Homozygous ATT repeats = 12	28%	23%	NS
Heterozygous ATT repeats = 12	36%	63%	NS
Homozygous ATT repeats < 12	36%	14%	NS

BHR, bronchial hyper-responsiveness; NOS, nitrogen synthetase; ICS, inhaled corticosteroid; BUD, budesonide; BUD1600, BUD 1600 mcg; B-eosinophils, blood eosinophils; NS, non-significant; FEV₁, forced expiratory volume in 1 s; FEF_{25–75}, forced expiratory flow between 25%–75% of forced vital capacity.

The response in FeNO was heterogeneous (Fig. 2) and FeNO (log-transformed) after both PLAC and BUD1600 exhibited a Gaussian distribution (Fig. 3). Accordingly, FeNO was within the normal range from BUD1600 in some children, but significantly raised in others. These two groups are contrasted in Table 2 and characterized by the independent variables relating to lung function at the end of the steroid treatment period, atopy and NOS1 genotype. Lung function (FEV₁ and FEF_{25–75}) and use of rescue medication were similar in the two groups, but BHR was numerically increased in children in whom FeNO did not normalize ($P = 0.059$). Indices of allergy were significantly more prevalent in children, in whom FeNO did not reach the normal level. The number of ATT_n repeats did not differ between the two groups (Chi-square: $P > 0.2$). FeNO was not different between children who have a heterogeneous or homogenous increased number of (ATT)_n repeats ($= 12$) ($P = 0.19$). A repeat analysis of the relative changes showed similar trends, with more prevalent indices of allergy and BHR in the non-responding group ($P > 0.05$).

No carry-over effects from treatment sequence could be demonstrated for either FeNO, FEV₁ or BHR ($P > 0.05$).

The multiple regression analysis of FeNO after BUD1600, including allergy indices, lung function and BHR, showed that the specific IgE level and BHR were positively related to FeNO ($P = 0.01$ and $P = 0.006$, respectively). No interaction was found between the specific IgE level and BHR ($P = 0.46$).

FeNO at BUD400 or PLAC was significant positively linear correlated to FeNO at BUD1600 ($P = 0.002$ and $P = 0.006$, respectively). However, neither FeNO nor lung function at randomization (BUD400) or PLAC predicted the relative response (%) in FeNO to the higher steroid dose (BUD1600) (data not shown).

Discussion

FeNO was normal in the majority of children with moderate persistent asthma; all clinically well controlled from a daily

dose of 400 mcg inhaled BUD Turbohaler. However, FeNO remained elevated in a subgroup of approximately 25%, despite apparent clinical control. Certainly part of this may be attributed to poor adherence to their steroid treatment in some of the children. However, FeNO even remained increased in a defined subgroup when BUD was increased to 1600 mcg in the double-blind placebo-controlled trial.

We do not believe that non-adherence during the double-blind trial significantly flawed our results. Compliance is a random process during the trial independent of the variables (BHR and allergy) significantly separating the groups with normal and raised FeNO, i.e. there is no reason to suspect that compliance was related to allergy and BHR in the two treatment groups. Furthermore, most children improved in lung function and BHR on changing from PLAC to BUD1600. Therefore, our findings are unlikely to be caused by lack of compliance.

This is the first placebo-controlled study in children with focus on the heterogeneity in FeNO response to inhaled steroid treatment applying current recommendations for FeNO measurement. Our data are consistent with a previous report of a plateau effect of ICS with little further reduction in FeNO from doses above 400 mcg [5] and a dose relationship to ICS [19]. Although FeNO is very sensitive to steroid treatment [20, 21], variability in FeNO response may hamper its general clinical usefulness in the diagnosis and monitoring of asthma.

FeNO may be considered to be a marker of disease severity and is sensitive to steroid. A complementary interpretation is that FeNO is driven by atopy. Finally, FeNO may reflect a common genetic variant [14] and hence only partly relate to asthma control.

Conflicting results have been reported on the relation between FeNO and asthma severity, partly because of different markers of disease severity and partly from lack of a standardized method of FeNO measurements [22, 41–44]. In our study, no relation was found with markers of severity such as use of rescue medication and lung function. However, the increased FeNO was significantly related to BHR independent of indices of allergy. Therefore, FeNO may partly reflect a sub-clinical inflammation unresponsive to ICS. Heterogeneous response of FEV₁ and BHR to ICS has previously been reported [23]. Increased numbers of sputum eosinophils despite good clinical control in steroid-treated asthmatics suggested partly uncontrolled airway inflammation in asthmatics on ICS treatment [24]. Likewise, persistent BHR in long-term treatment in controlled trials of ICS suggests a chronic pathology only partly responsive to ICS [25, 26]. Previous reports have corroborated a correlation between FeNO and BHR [27–29], while some have only demonstrated such correlations for steroid-naïves [30, 31] or atopic asthmatics [32], and others no correlation at all [20, 33–36].

Allergy was more prevalent in the subgroup with raised FeNO despite high-dose ICS. Specific IgE for inhalant allergens correlated significantly and independently with FeNO in the multi-regression analysis. This suggests that part of the FeNO is driven by the allergic diathesis, only partly sensitive to steroid. This finding is consistent with previous reports on increased FeNO in atopic non-asthmatic children [37] compared with atopic patients [38]. FeNO increased during allergen exposure to grass [39], and FeNO

related more closely with allergy than with BHR and lung function in adult asthmatics [40]. Yet, others found no difference in peak exhaled NO concentration between allergic and non-allergic asthmatics [45] and between atopic and non-atopic healthy individuals [38, 46, 47].

Variability in FeNO has been related to numbers of ATT repeats in intron 20 of the NOS1 gene at chromosome 12 [14]. We found that our cohort expressed the same distribution of alleles as a sample from healthy individuals (data not shown), and as reported by Wechsler et al. [14]. Genetic variation in NOS1 (ATT)_n repeats could therefore not explain the heterogeneous response to ICS, although this does not exclude the genetic variation as a general population determinant of FeNO.

In conclusion, our study suggests that FeNO is within the normal range in most asthmatic children on a moderate dose of ICS. However, a remarkable heterogeneity in FeNO is demonstrated and FeNO remains increased despite high-dose ICS therapy in a defined subgroup of clinically well-controlled asthmatic children. This probably reflects that part of FeNO is driven by increased BHR and the allergic diathesis, partly unchecked by ICS. Alternatively, it may rely on NO production from the NO synthases (NOS1) and NOS3, which are not sensitive to steroid [48]. The results point to the diversity of determinants of FeNO. This should be considered when using FeNO for steroid dose titration and monitoring of asthma disease control in children.

References

- 1 New NHLBI guidelines for the diagnosis and management of asthma. National Heart, Lung and Blood Institute. Lippincott Health Promot Lett 1997 Aug; 2(7):8–9.
- 2 Sont JK, Han J, van Krieken JM et al. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. *Thorax* 1996; 51:496–502.
- 3 Warke TJ, Fitch PS, Brown V et al. Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. *Thorax* 2002; 57:383–7.
- 4 Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose–response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. *Chest* 2001; 119:1322–8.
- 5 Jatakanon A, Kharitonov S, Lim S, Barnes PJ. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. *Thorax* 1999; 54:108–14.
- 6 Payne DN, Wilson NM, James A, Hablas H, Agrafioti C, Bush A. Evidence for different subgroups of difficult asthma in children. *Thorax* 2001; 56:345–50.
- 7 Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. The European Respiratory Society Task Force. *Eur Respir J* 1997; 10:1683–93.
- 8 Global strategy for asthma treatment and prevention. NHLBI/WHO workshop report. National Institute of Health, National Heart, Lung and Blood Institute, London, 2002.
- 9 Buchvald FF, Bisgaard H. Comparisons of the complementary effect on exhaled nitric oxide of salmeterol vs montelukast in asthmatic children on regular inhaled budesonide. *Ann Allergy Asthma Immunol* 2003; 91:309–13.
- 10 Eliasson AH, Phillips YY, Rajagopal KR, Howard RS. Sensitivity and specificity of bronchial provocation testing. An evaluation of

- four techniques in exercise-induced bronchospasm. *Chest* 1992; 102:347–55.
- 11 Nielsen KG, Bisgaard H. Lung function response to cold air challenge in asthmatic and healthy children of 2–5 years of age. *Am J Respir Crit Care Med* 2000; 161:1805–9.
- 12 Standardization of spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995; 152:1107–36.
- 13 Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children – 1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 1999; 160:2104–17.
- 14 Wechsler ME, Grasemann H, Deykin A et al. Exhaled nitric oxide in patients with asthma. Association with *nos1* genotype. *Am J Respir Crit Care Med* 2000; 162:2043–7.
- 15 Dresborg S, Frew AJ. Allergen standardization and skin tests. Position Paper, EAACI subcommittee. *Allergy* 1993; (Suppl.): 48–82.
- 16 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.
- 17 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.
- 18 Altman DG. Practical statistics for medical research, 1st Edn. London: Chapman & Hall/CRC, 1999.
- 19 Kharitonov SA, Donnelly LE, Montuschi P, Corradi M, Collins JV, Barnes PJ. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. *Thorax* 2002; 57:889–96.
- 20 Silkoff PE, McClean PA, Slutsky AS et al. Exhaled nitric oxide and bronchial reactivity during and after inhaled beclomethasone in mild asthma. *J Asthma* 1998; 35:473–9.
- 21 Kharitonov SA, Barnes PJ. Clinical aspects of exhaled nitric oxide. *Eur Respir J* 2000; 16:781–92.
- 22 Griesse M, Koch M, Latzin P, Beck J. Asthma severity, recommended changes of inhaled therapy and exhaled nitric oxide in children: a prospective, blinded trial. *Eur J Med Res* 2000; 5:334–40.
- 23 Szefer SJ, Martin RJ, King TS et al. Significant variability in response to inhaled corticosteroids for persistent asthma. *J Allergy Clin Immunol* 2002; 109:410–8.
- 24 Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med* 2000; 161:9–16.
- 25 Lundgren R, Soderberg M, Horstedt P, Stenling R. Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids. *Eur Respir J* 1988; 1:883–9.
- 26 Essen-Zandvliet EE, Hughes MD, Waalkens HJ, Duiverman EJ, Kerrebijn KF. Remission of childhood asthma after long-term treatment with an inhaled corticosteroid (budesonide): can it be achieved? Dutch CNSLD Study Group. *Eur Respir J* 1994; 7:63–8.
- 27 Salome CM, Roberts AM, Brown NJ, Dermand J, Marks GB, Woolcock AJ. Exhaled nitric oxide measurements in a population sample of young adults. *Am J Respir Crit Care Med* 1999; 159:911–6.
- 28 Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998; 53:91–5.
- 29 al Ali MK, Eames C, Howarth PH. Exhaled nitric oxide; relationship to clinicophysiological markers of asthma severity. *Respir Med* 1998; 92:908–13.
- 30 Dupont LJ, Rochette F, Demedts MG, Verleden GM. Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naïve patients with mild asthma. *Am J Respir Crit Care Med* 1998; 157:894–8.
- 31 Lim S, Jatakanon A, John M et al. Effect of inhaled budesonide on lung function and airway inflammation. Assessment by various inflammatory markers in mild asthma. *Am J Respir Crit Care Med* 1999; 159:22–30.
- 32 Ludviksdottir D, Janson C, Hogman M, Hedenstrom H, Bjornsson E, Boman G. Exhaled nitric oxide and its relationship to airway responsiveness and atopy in asthma. BHR-Study Group. *Respir Med* 1999; 93:552–6.
- 33 Silvestri M, Spallarossa D, Battistini E, Brusasco V, Rossi GA. Dissociation between exhaled nitric oxide and hyperresponsiveness in children with mild intermittent asthma. *Thorax* 2000; 55:484–8.
- 34 Ichinose M, Takahashi T, Sugiura H et al. Baseline airway hyperresponsiveness and its reversible component: role of airway inflammation and airway calibre. *Eur Respir J* 2000; 15:248–53.
- 35 Leuppi JD, Salome CM, Jenkins CR et al. Markers of airway inflammation and airway hyperresponsiveness in patients with well-controlled asthma. *Eur Respir J* 2001; 18:444–50.
- 36 Latzin P, Beck J, Griesse M. Exhaled nitric oxide in healthy children: variability and a lack of correlation with atopy. *Pediatr Allergy Immunol* 2002; 13:37–46.
- 37 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.
- 38 Gratziau C, Lignos M, Dassiou M, Roussos C. Influence of atopy on exhaled nitric oxide in patients with stable asthma and rhinitis. *Eur Respir J* 1999; 14:897–901.
- 39 Baraldi E, Carra S, Dario C et al. Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children. *Am J Respir Crit Care Med* 1999; 159:262–6.
- 40 Ho LP, Wood FT, Robson A, Innes JA, Greening AP. Atopy influences exhaled nitric oxide levels in adult asthmatics. *Chest* 2000; 118:1327–31.
- 41 Stirling RG, Kharitonov SA, Campbell D et al. 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. *Asthma and Allergy Group. Thorax* 1998; 53:1030–4.
- 42 Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. *Am J Respir Crit Care Med* 1999; 160:1532–9.
- 43 Artlich A, Busch T, Lewandowski K, Jonas S, Gortner L, Falke KJ. Childhood asthma: exhaled nitric oxide in relation to clinical symptoms. *Eur Respir J* 1999; 13:1396–401.
- 44 Sippel JM, Holden WE, Tilles SA et al. Exhaled nitric oxide levels correlate with measures of disease control in asthma. *J Allergy Clin Immunol* 2000; 106:645–50.
- 45 Persson MG, Zetterstrom O, Agrenius V, Ihre E, Gustafsson LE. Single-breath nitric oxide measurements in asthmatic patients and smokers. *Lancet* 1994; 343:146–7.
- 46 Frank TL, Adisesh A, Pickering AC et al. Relationship between exhaled nitric oxide and childhood asthma. *Am J Respir Crit Care Med* 1998; 158:1032–6.
- 47 Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. *J Allergy Clin Immunol* 2000; 106:638–44.
- 48 Radomski MW, Palmer RM, Moncada. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci USA* 1990; 87:10043–7.