

ORIGINAL ARTICLE

Longitudinal measurements of exhaled nitric oxide in children—what is a significant change in FE_{NO}?

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Abstract

Background: The principle aim of this study was to describe the variability of exhaled nitric oxide (FE_{NO}) concentrations over 10 months in children with and without asthma.

Methods: FE_{NO} was measured on six occasions at 2-month intervals in a community-based cohort of children with and without asthma.

Results: There were 178 children recruited, 47 had asthma, mean age 9.6 yr. A total of 851 FE_{NO} measurements were made. The change in FE_{NO} values was positively associated with the initial FE_{NO} concentration ($p < 0.001$) and duration between paired measurements ($p = 0.016$) but not asthma diagnosis; there was an interaction between initial FE_{NO} and duration between measurements. As an approximate rule-of-thumb, a child's FE_{NO} may rise by up to 100% of their current FE_{NO} over 2 and 4 months, independent of asthma.

Conclusions: Both the baseline FE_{NO} and interval between repeated FE_{NO} measurements are relevant to FE_{NO} values, independent of asthma. These findings may be useful to clinical interpretation of FE_{NO} results in children.

Elevated exhaled nitric oxide (FE_{NO}) may be a biomarker for airway eosinophilia (1) and potentially be a useful guide for asthma treatment. In adults, there is evidence supporting the use of FE_{NO} as a guide for asthma treatment in some settings (2, 3) but not all (4). In children, FE_{NO} may be a useful biomarker for stopping inhaled corticosteroid treatment (5) and for stepping down treatment in one study (6) but not another (7). However, there is no evidence that FE_{NO} is useful in guiding day-to-day management of childhood asthma (8–10). These apparently inconsistent results may reflect differences in study design (11). One systematic review and meta-analysis (12) has concluded that FE_{NO} may not be useful in guiding asthma management. In contrast, an expert group have strongly recommend use of FE_{NO} in monitoring airway inflammation in patients with asthma (13) but acknowledge a low-quality evidence base.

Given the positive relationship between FE_{NO} and eosinophilic inflammation (14), bronchial hyperreactivity (15), asthma control (16), and stepping down treatment in children (5, 6), the negative results of paediatric clinical trials are surprising but perhaps, the wrong cut-off values were used (11). To date, paediatric studies have either derived threshold FE_{NO} values by extrapolation to elevated sputum eosinophil counts (17) or cross-sectional whole-population normative data (18). These estimates of what merits 'elevated' FE_{NO} do not give

insight into the variability of FE_{NO} value over time, and therefore, a clinically significant difference in repeated FE_{NO} measurements has been variously estimated at 4 ppb (19) or between 20% and 35% (20, 21).

Here, we describe an observational study where longitudinal measurements of FE_{NO} were made over a whole school year (i.e. 10 months) in children with and without asthma. Our study design allowed us to address the question 'What is variability in FE_{NO} measured over a school year in children?' Additionally, to make the best use of the data available, we explored the relationship between current FE_{NO} and future asthma control among the subset of children with asthma.

Methods

Study design

This was a longitudinal observational study where FE_{NO} was measured at 2-month intervals between September 2009 and August 2010. Children were recruited from local primary schools and FE_{NO} measurements were made in schools at the same time of day (± 1 h). At the start of the study, individuals were given the option of attending detailed clinical assessment at the hospital where the first FE_{NO} measurement was made and also the following were measured: height, weight, skin

prick reactivity, spirometry, and bronchodilator responsiveness. Parents completed a respiratory questionnaire previously used by our group for FE_{NO} methodology studies (22), an asthma diagnosis was defined as an affirmative response to the question 'does your child currently have asthma which has been diagnosed by a doctor?'. The presence of pets, smokers, and visible mould in the home was reported by parents. The 4–11-year-old Children's Asthma Control Test (23) was completed at each FE_{NO} measurement for children with asthma (parents were contacted by telephone to complete the relevant questions); a score of <20 was defined as loss of asthma control. Prior to each FE_{NO} measurement, children were asked whether they had had a cold, that is, nasal discharge, during the previous month. The start and end of the tree, and grass pollen seasons were determined from data provided by a sampling site in East Lothian (100 miles south of Aberdeen but representative in climate and flora). Ethical approval was provided by North of Scotland Research Ethics Committee, and written parental consent and verbal assent from the child were obtained.

Exhaled nitric oxide measurements

A MINO[®] portable NO analyser (Aerocrine, Solna, Sweden) was used for all FE_{NO} measurements in school and only one FE_{NO} measurement was taken. Ambient NO was recorded at the start of each assessment. The mean of three technically acceptable measurements from the NIOX[®] (24) was compared with a single measurement from the MINO[®] among the children who attended the phenotyping assessment. Measurements with NIOX[®] and MINO[®] were not performed in random order.

Spirometry, bronchodilator response and skin prick testing

Spirometry was measured in accordance with standard guidelines using a portable spirometer (Vitalograph 21/10; Vitalograph, Maids Moreton, UK), and values were expressed as percentage of predicted according to normative data (25). The bronchodilator response was defined as the change in FEV₁ 15 min after inhalation of 400 µg salbutamol, delivered from a pressurized metered dose inhaler via large volume spacer device (Volumatic[®]; GlaxoSmithKline, Brentford, UK). The skin prick test was used to determine skin prick reactivity to the six environmental allergens to which our population is most commonly sensitized, that is, *Dermatophagoides pteronyssinus*, cat dander, dog dander, whole egg, peanut, grass (ALK, Northampton). The positive control was histamine 10 mg/ml, and the negative control was 0.9% saline. A positive skin test was defined as a weal ≥3 mm in longest diameter or, in cases of dermatographism, greater than the negative control.

Analysis

Differences between groups were compared using chi-square test, Student's t-test and Mann–Whitney U test as appropriate. Exhaled NO values were expressed as geometric mean and 95% confidence interval ($\pm 1.96 \times$ standard error of mean). Three approaches to analysis were taken. First, a Bland Altman plot was used to demonstrate absolute changes in FE_{NO} with limits of agreement. Second, a linear model was created to relate change in FE_{NO} to the initial measurement,

the interval between paired measurement and asthma diagnosis; additionally, a variable 'case number' was added to adjust for clustering of differences in FE_{NO} within an individual. The change in FE_{NO} measurements was squared and square rooted, so that all values were positive to ensure that positive and negative changes for individuals within the population did not nullify each other. Third, to determine whether individuals remain within a range of FE_{NO} values children were stratified by initial FE_{NO} [under 20 ppb, 21–30 ppb, 31–40 ppb, and >40 ppb (8)] and proportions remaining within the initial FE_{NO} range were calculated. Details of the longitudinal analyses used to study longitudinal change in FE_{NO} within an individual are presented in the Appendix S1. Standard statistical software was used (IBM SPSS 20, IBM, Armonk, NY, USA) and significance was assumed at $p < 0.05$.

Results

Study participants

There were 178 children recruited, mean age (s.d.) 9.6 (1.3) years, 88 boys and 47 with asthma. There were no correlations between ambient NO and FE_{NO}. Exhaled NO was measured on six occasions in 86 children, on five occasions in 51 children and on four occasions in 20 children, that is, a total of 851 FE_{NO} measurements. The 21 children where FE_{NO} was measured on less than four occasions were excluded from the analysis; this group was mostly representative of the whole cohort (Table 1). The 87 children who attended the clinical assessment were representative of the whole cohort (Table 1). Exhaled NO concentrations from a single MINO measurement were 2.1 ppb lower than the mean of three measurements from the NIOX (limits of agreement 7.6, –11.7 ppb), see figure on Appendix S1.

Details of children with asthma

There were 47 children with diagnosed asthma, of whom 23 attended for clinical assessment and had the usual phenotype of male sex elevated FE_{NO}, reduced spirometry and atopy (Table 2). There were 13 children with asthma who had at least one episode of poor asthma control and at recruitment, compared with children whose asthma remained well controlled throughout, these individuals had higher FE_{NO} values, lower CACT scores, were prescribed higher doses of inhaled steroids and were more likely to require long-acting beta agonist treatment (LABA), Table 2. Among the children with asthma, there were 25 receiving only as required short-acting beta agonist (SABA) treatment, 22 receiving SABA plus regular inhaled corticosteroids (ICS, median budesonide equivalent daily dose 200 µg, range 50–1000) of whom 10 were also treated with LABA (salmeterol 50 µg twice a day) and 1 with LABA plus leukotriene receptor antagonist (montelukast 5 mg once daily). The median (interquartile range) FE_{NO} among the children with asthma was 17 ppb [8.5, 44.5] and was 27 ppb [13, 46] for the subset who attended the clinical assessment (Mann–Whitney test $p = 0.244$). The median FE_{NO} at the first assessment was 13 ppb for children on SABA alone, 27 ppb for SABA plus ICS and 45 ppb for SABA, ICS plus LABA (Kruskal–Wallis $p = 0.023$).

Table 1 Comparison of the whole study population with the 87 children who attended for clinical assessment and the 157 who had at least four FE_{NO} measurements

	Whole cohort (denominator = 178 unless stated)	Individuals where at least 4 FE _{NO} measurements were obtained (denominator = 157 unless stated)	Individuals where <4 FE _{NO} measurements were obtained (denominator = 21 unless stated)	Individual who attended the clinical assessment (denominator = 87 unless stated)
Mean age (s.d.), years	9.6 (1.3)	9.6 (1.3)	9.5 (1.4)	9.5 (1.3)
% male gender (n)	49 (88)	46 (73)*	71 (15)	52 (45)
% asthmatic (n)	26 (47)	29 (46)	10 (2)	26 (23)
% wheeze in last 12 months (n)	22 (39)	24 (38) [†]	5 (1/20)	22 (19)
% prescribed inhaled corticosteroids (n)	12 (22)	14 (22)	0	13 (11)
% with at least one parent who smokes (n)	31 (55)	31 (49)	30 (6)	29 (25)
% with cat or dog at home (n)	34 (61)	35 (55)	29 (6)	38 (33)
% with damp in the house (n)	10 (18)	10 (15)	14 (3)	10 (9)
% with positive skin test (n)		41 (31/75)	67 (6/9)	43 (37/87)
Mean% FEV ₁ (s.d.)		97 (16) n = 76	97 (8) n = 9	96 (15) n = 85
Mean% FEF ₂₅₋₇₅ (s.d.)		91 (24) n = 85	78 (28) n = 9	89 (24) n = 85
Median FE _{NO} at first measurement (s.e.m.), ppb [‡]	10.5 (1.5) n = 158	10.0 (1.6) n = 145	15 (5.4) n = 13	11.0 (2.0) n = 86
Median BDR (s.e.m.), %		3.9 (1.2) n = 68	4.6 (3.7) n = 8	3.9 (1.2) n = 76

*p = 0.032 and [†]p = 0.050 compared with the whole study population and with individuals where <4 FE_{NO} measurements were obtained.

[‡]FE_{NO} was not measured at the first assessment in 20 children. There were no differences between those who attended the clinical assessment and the whole cohort.

Table 2 Comparing details of children with and without asthma

	Children without asthma	All children with asthma (n = 47)	Children with asthma which remained controlled throughout (n = 34)	Children with asthma with at least one episode of poor asthma control (n = 13)
Mean age (s.d.), years	9.4 (1.2)	9.8 (1.4)	9.7 (1.4)	9.6 (1.5)
Male gender, % (n)	45 (29)	70 (16)*	56 (19/34)	69 (9/13)
Median FE _{NO} (s.e.m.) at first assessment, ppb	10 (1.6)	27 (5.1)*	13 (6) n = 29	39 (6) n = 13 [‡]
Mean height (s.d.), cm	138 (11)	138 (9)	139 (12) n = 34	139 (12) n = 34
Mean weight (s.d.), kg	34.9 (10.4)	38.0 (10.5)	34.9 (10.7) n = 34	38.3 (13.0) n = 13
Mean% predicted FEV ₁ , (s.d.)	99 (17) n = 64	90 (9)* (n = 23)	91 (10) n = 15	89 (6) n = 8
Mean FEV ₁ /FVC ratio (s.d.)	0.90 (0.08) n = 64	0.83 (0.10)* (n = 23)	0.87 (0.10) n = 15	0.86 (0.12) n = 8
Skin prick positive, % (n)	32 (20/64)	73 (17/23)*	67 (10/15)	88 (7/8)
Median BDR (s.e.m.)	3.5 (1.4) n = 56	6.3 (2.4) (n = 20)	4.5 (2.6) n = 13	6.3 (2.7) n = 7
Median dose of inhaled steroid (microg budesonide equivalent)		200 (59) n = 22	100 (40) n = 12	450 (101) n = 10 [‡]
Median CACT [†] (range)	—	20 (7–26)	24 (20–27)	18 (7–26) [‡]
Treatment with long acting beta agonist			10% (3/34)	62% (8/13) [‡]

*p < 0.05 compared with children without asthma.

[†]CACT, Children's Asthma Control Test.

[‡]p < 0.05 compared with children with asthma which remained controlled throughout.

Change in exhaled NO within an individual over time

The absolute difference between paired FE_{NO} values remained close to zero over intervals of 2–10 months but the limits of

agreement became wider for those with higher initial FE_{NO} values and with increasing intervals between measurements (Table 3, Fig. 1). In the linear models, the difference in paired FE_{NO} measurements was positively related to the initial FE_{NO}

Table 3 Comparing the mean difference and upper limit of agreement for paired measurements of FE_{NO} made at intervals of between 2 and 10 months

Baseline FE _{NO} value (median)	Baseline to 2 months			Baseline to 4 months			Baseline to 6 months			Baseline to 8 months			Baseline to 10 months		
	Mean difference [limits of agreement]	Number of individuals (controlled)	Number of individuals (asthmatics)	Mean difference [limits of agreement]	Number of individuals (controlled)	Number of individuals (asthmatics)	Mean difference [limits of agreement]	Number of individuals (controlled)	Number of individuals (asthmatics)	Mean difference [limits of agreement]	Number of individuals (controlled)	Number of individuals (asthmatics)	Mean difference [limits of agreement]	Number of individuals (controlled)	Number of individuals (asthmatics)
0–10 ppb (7 ppb)	1 [–6, 9]	68 (8)	70 (8)	2 [–5, 9]	41 (11)	67 (9)	3 [–6, 11]	39 (11)	64 (10)	3 [–8, 14]	40 (10)	65 (10)	4 [–10, 18]	34 (9)	65 (10)
11–20 ppb (14 ppb)	2 [–18, 22]	40 (11)	41 (11)	0 [–14, 14]	10 (4)	39 (11)	–1 [–12, 10]	10 (4)	40 (10)	2 [–18, 23]	8 (3)	34 (9)	2 [–14, 19]	7 (2)	34 (9)
21–30 ppb (26 ppb)	–6 [–19, 8]	9 (3)	10 (4)	–5 [–25, 14]	9 (5)	10 (4)	–10 [–19, 0]	7 (4)	8 (3)	–7 [–22, 8]	5 (3)	7 (2)	1 [–31, 34]	6 (4)	7 (2)
31–50 ppb (44 ppb)	–3 [–44, 39]	6 (4)	9 (5)	0 [–35, 35]	*	5 (4)	–11 [–52, 30]	5 (4)	5 (3)	6 [–41, 54]	6 (5)	6 (4)	1 [–62, 64]	*	6 (4)
>50 ppb (74 ppb)	0 [–24, 24]	5 (4)		*			–2 [–24, 24]		6 (5)	1 [–20, 23]			*		

*Data only available in three individuals and not presented.

measurement and the duration between measurements but not to asthma diagnosis (Table 4). When children were stratified by initial FE_{NO}, there was a median of 107 children in the ≤20 ppb group (including 20 with asthma), 11 in the 21–30 ppb group (4 with asthma), four in the 31–40 ppb group and 12 in the >40 ppb group and so the latter two groups were merged to create a group with a meaningful number of participants (including 12 with asthma); Fig. 2 shows that >75% of individuals in the ≤20 ppb group and >40 ppb remained in that group for the duration of the study and values between 21 and 30 ppb moving outside that range in 60% of individuals.

Factors associated with change in FE_{NO} values

Exhaled NO values in May–June were elevated among those with hay fever (increase in log transformed FE_{NO} 1.34 [95% CI 1.05, 1.70]) and in September–October were elevated among those with visible mould at home (increase in log transformed FE_{NO} 1.42 [1.00, 2.01], Appendix S1). Exhaled NO values were higher throughout the year among children with asthma compared to without, but this difference was greatest between November and February (increase in log transformed FE_{NO} 1.83 [1.45, 2.32]) and least during May and June (increase in log transformed FE_{NO} 1.48 [1.17, 1.88]), Appendix S1. The Appendix S1 presents complete details of intraindividual change in FE_{NO}.

Relationship between exhaled NO and asthma control

There was a weak correlation between current FE_{NO} and current CACT score and also CACT score after two and four but not 6 months (Table 5). Compared with children where good asthma control was maintained over the following 2 months (121 episodes, geometric mean FE_{NO} 20 ppb [95% CI 16–25]), values were elevated in children where control was regained (13 episodes in 11 different children, GM FE_{NO} 35 ppb [95% CI 21–49]) $p = 0.024$ (see Fig. S2, Appendix S1). The FE_{NO} for individuals where control was lost (eight episodes in eight different individuals, GM 28 ppb [95% CI 5–52]) and where control remained poor (15 episodes in 7 children, median FE_{NO} 19 ppb [95% CI 8–30]) was no different to those where good control was maintained.

Discussion

The principle finding of this study was that among children with no asthma and those with predominantly well-controlled asthma, the variability in FE_{NO} over time was greater in children with higher initial FE_{NO} values and also with an increasing interval between measurements. For paired measurements made over 2 and 4 months, the upper limit of agreement was approximately equal to the baseline value, for example, children with initial FE_{NO} of 14 ppb or 26 ppb might have measurements as high as 28 ppb or 40 ppb after 4 months (Table 3). This simple rule-of-thumb may be useful when interpreting repeated measurements of FE_{NO} in clinical practice. Due to the relatively small number of children with FE_{NO} in excess of 30 ppb in our study, we recommend further research be undertaken in this subgroup who may be more relevant to secondary care.

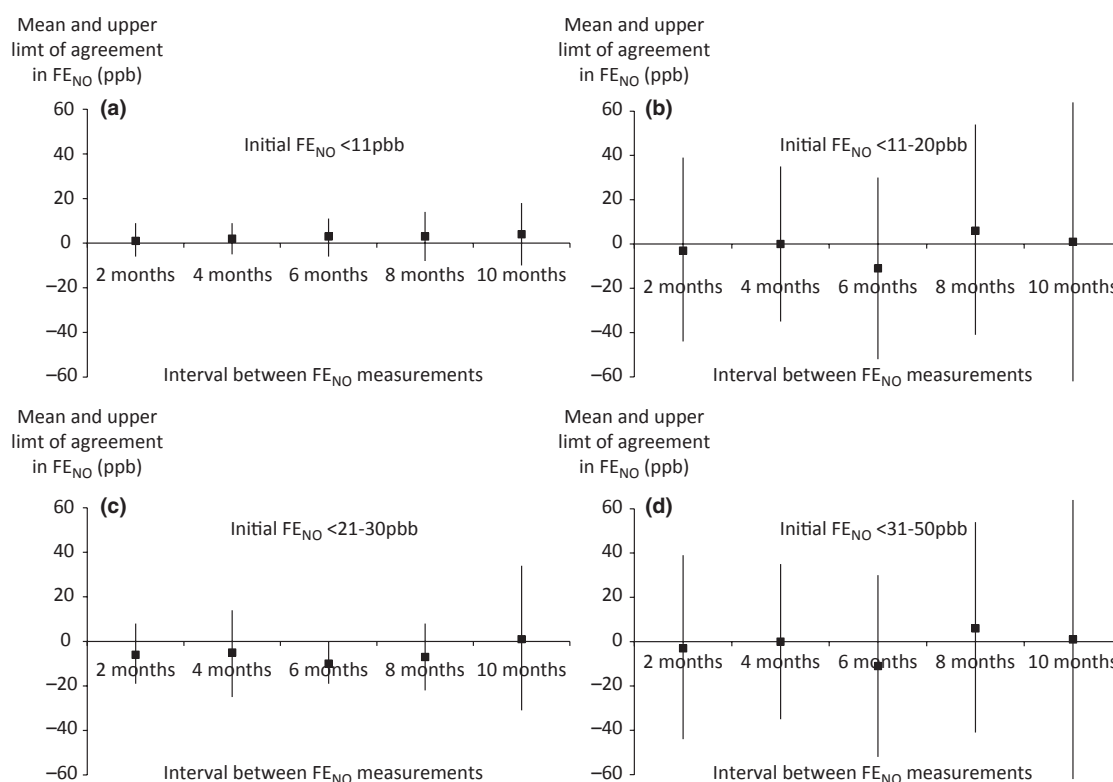


Figure 1 Mean difference and limits of agreement for paired measurements of FE_{NO} over time. Results are stratified by initial FE_{NO} value in panels A to D.

Table 4 Regression coefficients from linear models relating absolute change in FE_{NO} from baseline (positive and negative values) and relative change in FE_{NO} from baseline (positive values only). The R^2 for model including interaction term was 0.54

		Relative change in FE_{NO}	
		Regression coefficient [95% confidence interval]	p value
Duration between repeated FE_{NO} measurements	Initial FE_{NO} value	0.40 [0.36, 0.43]	<0.001
	Baseline-2 months	0	0.016
	Baseline-4 months	-0.54 [-2.27, 1.19]	
	Baseline-6 months	-0.27 [-2.02, 1.47]	
	Baseline-8 months	0.63 [-1.13, 2.39]	
Asthma diagnosis	Baseline-10 months	2.32 [0.52, 4.11]	
		-0.64 [-2.05, 0.77]	0.374
	Case number	0.002 [-0.001, 0.004]	0.194
Interaction term between initial FE_{NO} and duration between measurements			<0.001

There is consensus that the lower range of FE_{NO} values in children with asthma overlaps with the upper range of values for children without asthma (13), that is, 10–20 ppb, but interpretation of higher FE_{NO} values in children with asthma remains uncertain. By including children without asthma we were able to see variability in FE_{NO} independent of asthma. Not unexpectedly, the variability in FE_{NO} measurements

increased as values became higher and the interval between measurements was longer (particularly after an interval of 10 months, Table 3) but here, we are able to describe this variability.

Trials which have used single FE_{NO} values of >26 ppb (4), >30 ppb (9) or escalating cut-offs of >20, >30 and >40 ppb (8) to guide asthma treatment did not find superiority of FE_{NO}

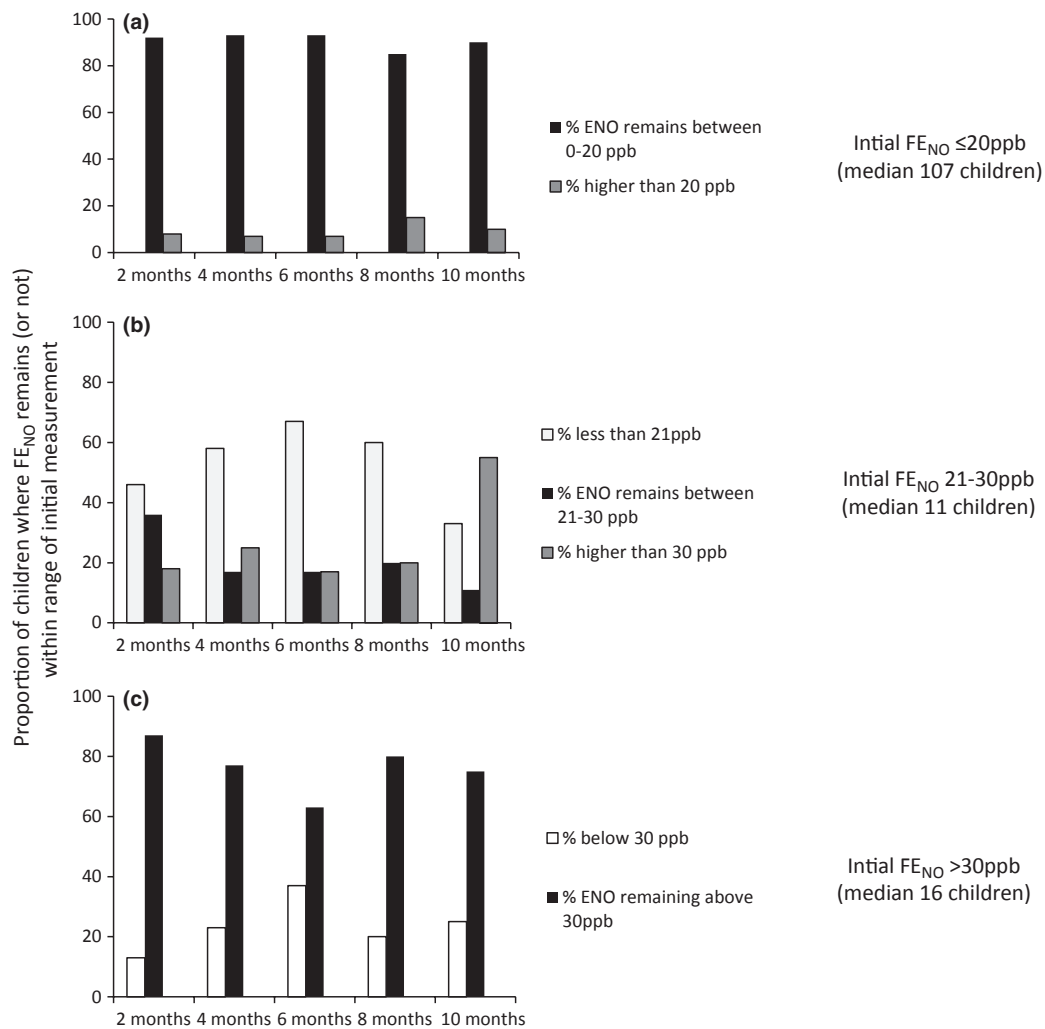


Figure 2 The proportion of children where repeated FE_{NO} values remained or did not remain within the initial range of FE_{NO} values.

Table 5 Spearman correlation coefficient for comparison between current exhaled nitric oxide (FE_{NO}) measurements and current and future asthma control

	Current CACT	CACT +2 months	CACT +4 months	CACT +6 months
Current FE _{NO}	-0.194 p = 0.005 n = 208	-0.221 p = 0.005 n = 158	-0.252 p = 0.004 n = 126	-0.143 p = 0.175 n = 91
Current CACT		0.567 p < 0.001 n = 158	0.473 p < 0.001 n = 127	0.521 p < 0.001 n = 92

CACT, Children's Asthma Control Test.

N indicates the number of comparisons made within the subset of 46 children with asthma.

guided management over standard management but did result in increased treatment with inhaled corticosteroids. Our study provides some insight into why these studies did not find

addition of FE_{NO} to usual practice useful as we found that children (predominantly with asthma) who had an initial FE_{NO} value of >30 ppb maintained these relatively high concentrations whilst remaining well controlled (Fig. 2); in this setting, increasing inhaled steroids dose when FE_{NO} exceeds 26 (4) or 30 ppb (9) might not lead to improved symptoms. More liberal FE_{NO} cut-offs where a 'high' value is approximately double the initial value could be applied in the paediatric setting.

Although not primarily designed to relate FE_{NO} to asthma control, our results are consistent with other studies. The relationship we observe between elevated FE_{NO} and low CACT score (Table 5) is consistent with some cross-sectional studies (16, 26) but not all (27). One explanation for there being a relatively poor relationship between FE_{NO} and asthma control is that FE_{NO} may be influenced by many factors independent of asthma in children, including age (18) and exposure to pollen (16), second-hand cigarette smoke (28) and certain foods (29). The relative increase in FE_{NO} between November and

February among children with asthma, compared with those without asthma, which we report here is novel and may represent respiratory virus infections during the winter months. We suggest that there are several different factors independent of asthma and atopy which each contribute a small changes in FE_{NO} concentrations over a year and these influences are generally to increase FE_{NO} , with the exception of second-hand cigarette smoke (28) exposure and corticosteroid treatment (30).

The high FE_{NO} values in children who regain asthma control may simply reflect regression to the mean of high values. Our observation that children with persistently poorly controlled asthma had relatively low FE_{NO} might indicate that the mechanism underlying their symptoms was not eosinophilic airway inflammation. In the absence of a gold standard test for asthma, some of the children with asthma may not have had asthma, and this may have implications for interpreting the results. For example, some or all of the children with persistently poorly controlled asthma may not have had asthma at all. A second example is that the change in FE_{NO} values appeared to be independent of asthma, but it is possible that by including children without asthma among the asthmatics that the influence of asthma on changing FE_{NO} values will not have been detected. The associations between FE_{NO} and asthma control in this study were based on a small number of individuals and need replication elsewhere.

We reported only one FE_{NO} measurement, in accordance with manufacturer's recommendations but not in keeping with international guidelines which recommend that at least two measurements are made (24) and this introduced some variability into measurements of FE_{NO} . We have demonstrated here that the difference in methodology was associated with a mean difference in FE_{NO} of 2 ppb and previously we have demonstrated that the mean difference in three FE_{NO} measurements from the MINO[®] and NIOX[®] was also small (4 ppb) (31). In adults, FE_{NO} values are slightly higher when concentrations from the NIOX[®] are compared with MINO[®] (32), and therefore, it is important that when comparing repeated FE_{NO} measurements, the same device is used as previously recommended in children (33). The apparent differences in FE_{NO} for the group with the lowest values may at least partly be explained by variability in the measurement rather than the child's FE_{NO} *per se*, but for children with FE_{NO} values >10 ppb, we do not believe that the MINO[®] itself has introduced significant variability into our results.

There are a number of strengths and limitations to this study. Strengths include a clinically relevant follow-up period, good retention during the period of follow-up and detailed phenotyping of a representative subset of the cohort. Our study design addressed the potential confounding influence of diurnal variation (34) and included children with asthma who were and were not prescribed inhaled corticosteroids (ICS), treatment which reduces FE_{NO} (30). Whilst it is possible that ICS treatment may have affected the FE_{NO} results we do not

believe that this has substantially affected the results because more than half of the children with asthma were not prescribed ICS and the children with episodes of poorly controlled asthma had substantially higher FE_{NO} values and also higher ICS doses compared with children whose asthma remained well controlled (Table 2). One limitation was the relatively small number of children with asthma, but we have demonstrated that the difference in FE_{NO} over time is independent of asthma (Table 4). A second limitation is the mild nature of asthma among the participants, the results may not be relevant to secondary care and there were relatively few episodes where asthma control was lost or gained. Additionally, although our assessment was relatively comprehensive, we were not able to validate reported exposures such as pollen, cigarette smoke, nor did we consider the influence of ambient air pollution although we did demonstrate the absence of correlation between FE_{NO} and ambient NO concentrations. The Children's Asthma Control Test is not validated for use over the telephone, but despite this limitation, we were able to observe correlations between C-ACT and FE_{NO} and this suggests that the C-ACT scores were representative of current asthma control. A final limitation is that we may not have been notified of changes in inhaled steroid treatment and compliance with inhaled corticosteroids may also change over time.

In summary, interpretation of FE_{NO} measurements should take into consideration the initial value and the interval between measurements. Low FE_{NO} values among children with well-controlled mild asthma were associated with ongoing good control and in a child with ongoing asthma-like symptoms should raise the possibility of a non-asthmatic cause for symptoms.

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Authorship statement

ST conceived the idea, designed the study, analysed the data and wrote the initial manuscript draft. RC collected the data. Both authors made meaningful contributions to the final manuscript.

Conflicts of interest statement

Neither author has a conflict to declare. The MINO and consumables used in the study were provided by Aerocrine. Aerocrine had no role in the study design, analysis of data nor writing of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Bland Altman plot comparing the mean and the difference in paired exhaled nitric oxide (FENO) concentrations (ppb, parts per billion) from the NIOX[®] and MINO[®] analyser.

Figure S2. Geometric mean (solid box) and 95% confidence intervals (lines) for exhaled nitric oxide (FE_{NO}) measurements (parts per billion, ppb) in groups categorised by change in asthma control over 2 months.

Table 1. Relationship between exhaled nitric oxide (FE_{NO}) and hayfever, mould exposure and age over a year.

Appendix S1. Result.

Measurements of exhaled nitric oxide measurements taken over
twelve months in children with and without asthma

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Online supplement

Statistical methods

The linear regression method described by Altman and Bland²⁸ was used to study longitudinal changes in FE_{NO} within the individual and recent URTI, ambient NO and pollen season; here regression models were created which included a variable for the individual to adjust for clustering of FE_{NO} values within an individual. Repeated measures analysis of variance was used to study change in FE_{NO} over time in children with and without asthma and hayfever who were and were not exposed to smoking, cat or dog and mould at home; in this model, missing values were replaced with the mean value where >3 FE_{NO} measurements were obtained.

RESULTS

Comparison of FE_{NO} values between MINO© and NIOX©

In 85 children, FE_{NO} measurements from a single MINO© measurement were on average 2.1ppb lower than the mean of three measurements from the NIOX© and the limits of agreement were +7.6 and -11.7ppb, figure 1

The relationship between changes in FE_{NO} over the year, mould exposure and hayfever

Beech, elm, hazel and oak pollen counts were detectable in March and April, ash pollen between March and June while grass and nettle pollen counts were present between June and October. These results are presented in table 2.

Factors associated with changes in exhaled NO measurements independent of asthma

In the repeated measures analysis of variance (see online supplement for regression coefficients and figures), independent of asthma, children where mould was reported in the house had an increase in FE_{NO} in September-October compared with those where mould was not visible (mean increase in log transformed FE_{NO} 0.42 [0.10, 1.0] p=0.048). Children with hayfever had increased FE_{NO} in May-June compared with children without hayfever (mean increase in log transformed FE_{NO} 0.34 [95% CI 0.05, 0.70], p=0.020). Age and asthma were associated with elevated FE_{NO} concentrations in this model. There was no association between changing FE_{NO} values and exposures to tobacco smoke or cat

and/or dog. In the longitudinal regression models, there was no difference between FE_{NO} concentrations were not related to pollen seasons, ambient NO or recent URTI.

	Asthma		Hayfever		Mould exposure		Age in September	
	Mean increase in log transformed FE _{NO} compared to no asthma	95% CI	mean increase in log transformed FE _{NO} compared to no hayfever	95% CI	mean increase in log transformed FE _{NO} compared to no mould exposure	95% CI	mean increase in log transformed FE _{NO} per year	95% CI
Sept-Oct	1.79*	1.42, 2.26	1.10	0.87, 1.39	1.42*	1.00, 2.01	1.15*	1.06 1.24
Nov-Dec	1.83*	1.45, 2.32	1.03	0.81, 1.30	1.26	0.88, 1.79	1.16*	1.07 1.26
Jan-Feb	1.82*	1.45, 2.29	1.13	0.89, 1.42	1.26	0.90, 0.78	1.11*	1.02 1.20
Mar-Apr	1.65*	1.34, 2.03	1.04	0.85, 1.28	1.25	0.92, 1.71	1.08*	1.01 1.16
May-Jun	1.48*	1.17, 1.88	1.34*	1.05, 1.70	1.13	0.79, 1.61	1.09*	1.00 1.18
Jul-Aug	1.60*	1.27, 2.02	1.15	0.99, 1.45	1.29	0.91, 1.83	1.08	1.00 1.17

Table 1. Relationship between exhaled nitric oxide (FE_{NO}) and hayfever, mould exposure and age over a year. Results are from repeated measures analysis of variance model which also included gender (n=157). *p<0.05

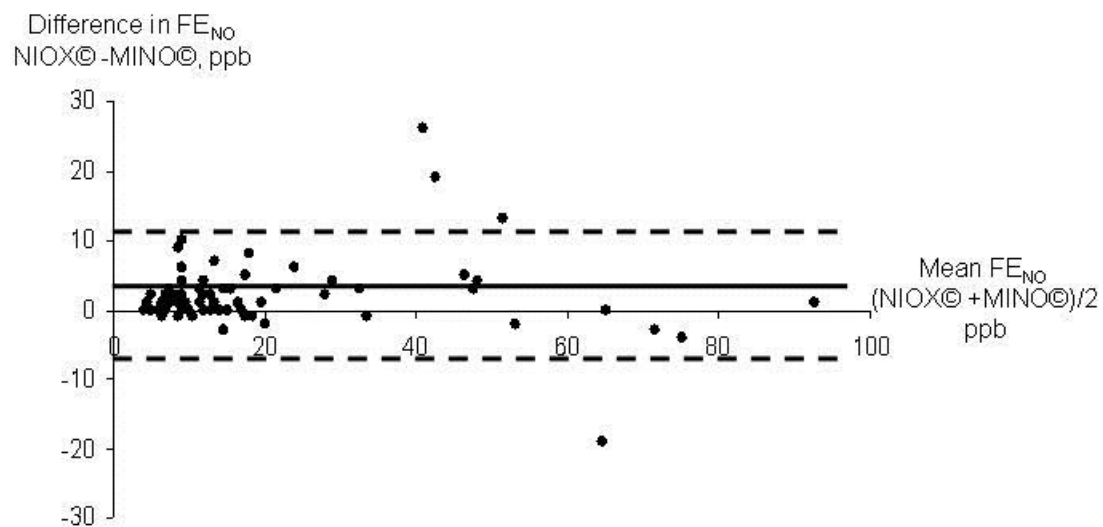


Figure 1. Bland Altman plot comparing the mean and the difference in paired exhaled nitric oxide (FE_{NO}) concentrations (ppb, parts per billion) from the NIOX© and MINO© analyser. The solid horizontal line corresponds with the mean difference and the broken lines with the limits of agreement.

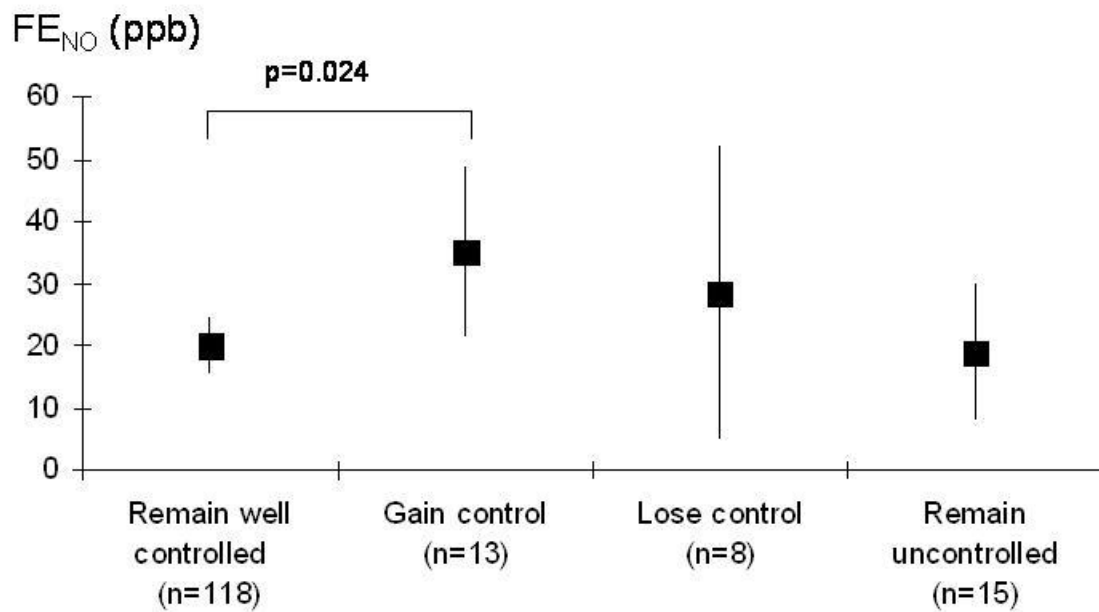


Figure 2. Geometric mean (solid box) and 95% confidence intervals (lines) for exhaled nitric oxide (FE_{NO}) measurements (parts per billion, ppb) in groups categorised by change in asthma control over two months. The p value is from a multivariate model which included age, gender and the case number; the latter allowed clustering of FE_{NO} values within an individual to be considered.