PEDIATRIC PULMONOLOGY

What is a clinically meaningful change in exhaled nitric oxide for children with asthma?

Journal:	*Pediatric Pulmonology*
Manuscript ID	PPUL-19-0700.R1
Wiley - Manuscript type:	Original Article: Asthma
Date Submitted by the Author:	n/a
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Keywords:	Asthma & Early Wheeze, Biomarkers, Nitric Oxide (NO)
Other Keywords:	
Abstract:	Introduction. Fractional exhaled nitric oxide (FENO) may be a useful objective measurement to guide asthma treatment. What remains uncertain is what change in FENO is clinically significant. Methods. An individual patient data analysis was performed using data from seven randomised clinical trials which used FENO to guide asthma treatment. The absolute and percentage intra-subject change in FENO measurements over "stable" and also "unstable" three-month periods were described. Results. Data were available in 1112 RCT participants and ≥1 stable period was present for 665 individuals. The interquartile range (IQR) and limits of agreement (LoA) for change in absolute FENO among individuals whose initial FENO was <50ppb were -7 to 9ppb and -43 to

	50ppb, and for those with initial FENO≥50ppb IQR was -29 to 17 ppb and LoA was -80 to 76ppb. For percentage change in FENO, the IQR and LoA for individuals whose initial FENO was <50ppb were -33 to 51% and -157 to 215%, and for those with initial FENO ≥50ppb were -33 to 35% and -159 to 192%. The variation in FENO values for a stable period was similar irrespective of whether it was followed by a stable or unstable period. Conclusions. Over a three-month period where FENO is initially <50ppb, a rise of <10ppb or of <50% (based on IQR) is unlikely to be related to asthma. When FENO is initially ≥50ppb an percentage change of <50% (based on IQR) is unlikely to be asthma-related.

What is a clinically meaningful change in exhaled nitric oxide for children with asthma?

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Keywords: Asthma, Child, Monitoring, Exhaled nitric oxide

Running head: What is a meaningful change in F_ENO in children?

ABSTRACT

Introduction. Fractional exhaled nitric oxide (F_ENO) may be a useful objective measurement to guide asthma treatment. What remains uncertain is what change in F_ENO is clinically significant.

Methods. An individual patient data analysis was performed using data from seven randomised clinical trials which used F_ENO to guide asthma treatment. The absolute and percentage intra-subject change in F_ENO measurements over "stable" and also "unstable" three-month periods were described.

Results. Data were available in 1112 RCT participants and ≥1 stable period was present for 665 individuals. The interquartile range (IQR) and limits of agreement (LoA) for change in absolute F_ENO among individuals whose initial F_ENO was <50ppb were -7 to 9ppb and -43 to 50ppb, and for those with initial F_ENO≥50ppb IQR was -29 to 17 ppb and LoA was -80 to 76ppb. For percentage change in F_ENO, the IQR and LoA for individuals whose initial F_ENO was <50ppb were -33 to 51% and -157 to 215%, and for those with initial F_ENO ≥50ppb were -33 to 35% and -159 to 192%. The variation in F_ENO values for a stable period was similar irrespective of whether it was followed by a stable or unstable period.

Conclusions. Over a three-month period where F_ENO is initially <50ppb, a rise of <10ppb or of <50% (based on IQR) is unlikely to be related to asthma. When F_ENO is initially ≥50ppb an percentage change of <50% (based on IQR) is unlikely to be asthma-related.

INTRODUCTION

Asthma is a very common condition and affects millions of children in the UK (1) and the US(2). There is no cure for asthma, but there is effective treatment to control asthma symptoms and reduce exacerbations. Guidelines recommend that asthma treatment is guided by symptoms(3-5), but symptom-reporting and interpretation is subjective and this leads to inconsistent care. There is a need for an objective biomarker to allow greater consistency in asthma treatment (6).

Fractional exhaled nitric oxide (F_ENO) is a surrogate for airway eosinophilia (7) and has potential roles in asthma including diagnosis, treatment stratification, treatment adherence and monitoring airway inflammation (7). A recent Agency for Healthcare Research and Quality comparative effectiveness review of F_ENO has summarised evidence which supports a number of potential clinical applications of F_ENO in the management of asthma(8). F_ENO has many of the characteristics required of an objective tool to monitor allergic asthma over time since it rises before symptoms occur(9-11), falls when asthma treatment is administered(12,13), can be measured with minimal discomfort to the patient and results are available within a few minutes using commercially-available Food and Drink Administration approved apparatus(14). When compared to standard treatment, asthma treatment guided by F_ENO measurements is associated with reduced asthma exacerbations, but not improved asthma control(15). There are some key questions which need to be answered before F_ENO can be incorporated into the routine longitudinal management of childhood asthma, and current asthma guidelines do not recommend F_ENO for monitoring asthma outside specialist clinics(3-5).

One key question which needs answering is “what change in F_ENO is clinically relevant in children?” The American Thoracic Society (ATS) guideline proposes that a change of 10 parts per billion at lower concentrations or 20% at higher concentrations may have clinical relevance(7), but acknowledge a low level of evidence. However, one study where children with mild asthma and without asthma had repeated measurements of F_ENO, questioned this ATS recommendation by finding that values may change by as much as 100% without any clinical change (16). A second study has reported that F_ENO values fluctuate between 6ppb and 40ppb in children on stable asthma treatment (17).

Our group has pooled the data collected in more than 1000 participants in seven RCTs where F_ENO was used to guide asthma treatment (18). This analysis found that a 50% increase in FeNO between baseline and three months was associated with 11% increase in odds for poor asthma control six months after baseline(18)but it remains unknown the degree of F_ENO variability over stable and unstable periods. Thus, we used individual patient data from the same database of seven RCTs to address the question “What is the variability in F_ENO over three months among children with stable asthma?”.

METHODS

Study design

Authors of published RCTs which used F_ENO to guide asthma treatment in children, as previously described (18), provided de-identified data for analyses in this study. All RCTs collected details of F_ENO concentrations, asthma control (using different scores) and exacerbation outcome (defined as requiring oral corticosteroid treatment) at each assessment. F_ENO was measured in all studies in accordance with the American Thoracic Society 2005 guideline(19). Percentage of predicted (%) FEV_1 was standardised to the Global Lung Initiative reference (20) with the exception of two trials (21,22) where only % FEV_1 standardised to other references was available. Covariates collected at baseline in all trials included: age, gender, height, weight, trial arm, dose of inhaled corticosteroid (ICS, as daily budesonide equivalent dose, BUD), prescribed long acting beta agonist (LABA) or not, prescribed leukotriene receptor agonist (LTRA) or not, and an asthma control score. Institutional ethical approval was provided for each trial which contributed data.

Details of each population

Fritsch et al(23). In this study, 47 children were recruited from a hospital asthma clinic in Vienna, Austria. Data were collected at six-week intervals over six months.

Peirsman et al(24). Researchers in centres across Belgium recruited 99 participants with persistent asthma attending hospital asthma clinics. Clinical assessments took place every three months over one year.

Petsky et al(25). Children in Australia and Hong Kong were recruited from hospital clinics. There were 63 patients enrolled and clinical assessment took place on eight occasions over twelve months (one, two, three, four, six, eight, ten and twelve months). The ten-month assessment was assigned the “nine month” assessment for the present analysis.

Pijnenburg et al (21). This study was carried out in the Netherlands and 86 patients were recruited and followed up at three-month periods over one year.

Pike et al (22). This was a study carried out in Southampton, UK and 90 patients were recruited and followed up at two-month intervals over twelve months. The two-month assessment was assigned the “three month” assessment for the analysis, also the ten-month was assigned the “nine month” assessment.

Szefler et al(26). Children and young adults of African or Hispanic descent and living in inner city areas of ten US cities were recruited. There were 546 participants and data were collected at the following intervals post randomisation: 6 weeks, 14 weeks (used in lieu of three months in the analysis), 22 weeks (used in lieu of six months), 30 weeks (used in lieu of nine months) and 46 weeks (used in lieu of twelve months).

Voorend-van Bergen et al(27). This RCT took place in the Netherlands and 181 participants were recruited. Data from a third arm of the RCT which delivered a web-based intervention were not included in the present analysis. Data were collected at four-month intervals over a year. The four and eight month assessments were respectively assumed to be three and six month assessment for the analysis. There was no equivalent nine-month assessment.

Analysis

Participants were identified as having a three-month period of stable asthma if they had a period between two successive assessments over three months during which: (i) asthma was controlled at the beginning and end (different RCTs used different scoring systems) (ii) the dose of inhaled corticosteroid (ICS) remained unchanged and (iii) there was no exacerbation requiring oral corticosteroid treatment. Change in F_ENO values was expressed as either an absolute difference or percentage difference over a three month period and as either the mean and 95% limits of agreement (LoA) or median and interquartile range (IQR). Change in F_ENO could not be expressed as geometric mean since values can be negative (16). Change in F_ENO was described for all individuals and also stratified for individuals with initial values of <50ppb and ≥50ppb for comparison with the guideline (7) and also for ranges <11ppb, 11-20ppb, 21-30ppb, 31-40ppb and 41-49ppb to allow comparison with data from a study by Cutts *et al* (16)which recruited children with no asthma and mild asthma(16). One author of the present paper (ST) is data custodian for the study (16) and reanalysed the data for direct comparison between studies. For patients with multiple stable periods, data from only the first stable period was included. To determine whether F_ENO values during a stable period were influenced by a subsequent unstable period we described change in F_ENO values during; (i) successive stable periods (i.e. when the child's asthma was controlled for three successive assessment, had no change in ICS dose and did not have an exacerbation) and (ii) also a stable followed by an unstable period (i.e. when the child's asthma was controlled for two successive assessments during which there was no change in ICS dose or an exacerbation but where asthma was uncontrolled on the third assessment and where ICS dose remained unchanged). Comparisons we made using Wilcoxon signed rank test.

RESULTS

Study subjects Data from 1112 participants in seven RCT were analysed (21-27). Participant details at baseline are presented in table one and have also been described elsewhere (18). The mean (SD) participant age was 12.6 (3.1) years and 58% of participants were male.

Variability of F_ENO within stable three months periods

All stable individuals. There were 251 children who had a total of 260 three-month intervals where asthma was stable. Due to missing F_ENO values in 16 individuals, change in F_ENO could be calculated in 235 participants with an overall median (IQR) absolute F_ENO

change -0.5ppb (-9, 10), and percentage F_ENO change -2% (-33%, 50%). For all stable participants combined, the mean (limits of agreement) for change in absolute F_ENO was +2 ppb (-52, +56) and for percentage change F_ENO was +26% (-157, +210).

Stable and initial F_ENO <50ppb. The 185 children with stable asthma and baseline F_ENO <50ppb had a median (IQR) absolute change in F_ENO 0 ppb (-7, +9) and the median (IQR) percentage change in F_ENO was +2% (-33, +51), table 2. The mean (LoA) change in absolute F_ENO was +3 (-43, +50) and the mean (LoA) percentage change was +29 (-157, +215), table 2.

Stable and initial F_ENO ≥50ppb. Where baseline F_ENO was ≥ 50ppb (n = 48), the median values (IQR) for absolute and percentage change in F_ENO were -7 ppb (-29, +17) and -10% (-33, +35) respectively, table 2. The mean (LoA) for change in absolute F_ENO was -2 ppb (-80, +76) and for percentage change F_ENO was +17% (-159, +192), see table 2

Stable and initial F_ENO stratified for comparison with Cutts et al (16) . Table 2 presents absolute and percentage change in F_ENO expressed as mean (LoA) and median (IQR) using data collected from children with asthma recruited to the RCTs. Data from the study of mostly healthy children by Cutts *et al (16)* are also presented for comparison.

Variability during consecutive periods of stable asthma followed by stable asthma

There were 96 individuals with two consecutive periods of stable asthma. The median (IQR) F_ENO concentrations during the first and second stable periods were +1ppb (-7, +8) and +3ppb (-8, +10); corresponding percentage change F_ENO values were +8 (-29, +47) and +11 (-35, +70), see table 3. Table 3 also presents results after stratification by <50ppb and ≥50 ppb. There was no differences in change in F_ENO between the first and second stable periods.

During consecutive periods of stable asthma followed by unstable asthma

There were 48 individuals identified. The median (IQR) change in absolute F_ENO values during stable and unstable periods were -2 (-14, +14) and 3 (-13, 14) and percentage change during stable and unstable periods were -6 (-37, +25) and 13 (-33, +57), see table 4. Table 4 presents results stratified by <50ppb and ≥50 ppb. There was no differences in change in F_ENO between the stable and unstable periods.

DISCUSSION

Fractional exhaled nitric oxide has a number of potential roles in asthma and the focus of the present study was to answer the question "What is a clinically significant change in F_ENO for children with asthma?" Our results may be relevant to clinicians when interpreting repeated F_ENO measurements, e.g. when making treatment decisions in a child with already-diagnosed asthma. The premise underlying our analysis was that any change in F_ENO which occurred in children during a period of stability was not clinically relevant. Our first finding was that when applying a stringent threshold of the LoA (i.e. including 95% of all values), the variability in F_ENO during stability was extremely wide and thus likely to include clinically significant events (i.e. loss of control and/or exacerbation). In contrast when applying the more liberal threshold of IQR (i.e including 50% of all values), the upper limit of variability could be applied clinically. Indeed the upper quartile value for participants with F_ENO <50ppb was <10ppb and consistent with the ATS recommendation (7).

Our second finding was that expressing change in F_ENO as percentage change lead to a consistent IQR of between ±50% for all initial F_ENO values. The upper quartile value for % change where the initial F_ENO was >50ppb was 35%, but having a "one size fits all" recommendation (i.e. <50% is unlikely to be clinically relevant) could be more easily understood by patients and health care staff, and also would facilitate management for individuals whose F_ENO is close to 50ppb (the current dichotomy for different recommendations in the ATS guideline(7)). Our results do not support the ATS "weak" recommendation that a change of >20% may be clinically meaningful for children with F_ENO≥ 50ppb (7).

The IQR and LoA from the present study are consistent with a study where F_ENO was measured at two month intervals over a year in children with mild or no asthma, where it was found that initial F_ENO values may rise by up to 100% (16). This consistency is despite important differences between our present study and that of Cutts *et al* (16), for example our study had a relatively greater number of participants with asthma, higher median F_ENO values and F_ENO measurements were repeated over a three month period compared to two months. For everyday clinical practice it is more relevant to define clinically relevant changes in F_ENO in a population of asthmatic children who are treated with ICS as compared to healthy children or steroid naive children.

Considerable variation in F_ENO values over time has also been reported in "natural experiments" in children. For example Huss-Marp *et al*(28) observed that F_ENO values in children with mild intermittent asthma with initial F_ENO >17 ppb and attending a summer camp at altitude fell by 50% over four to six weeks independent of symptoms, and Kaminsky *et al* (29) found F_ENO values fell from 11 to 6ppb (a 45% reduction) independent of asthma control among 27 children attending a one week summer camp. Two studies of children with asthma who were sensitised to grass found the F_ENO values rose by approximately 50% during the pollen season(30,31) but asthma symptoms also rose during this time. Barreto *et al* (32) found that the coefficient of variation in paired F_ENO values taken over a one-week period was 3ppb in eight children treated with ICS. Due to the limited literature describing change in F_ENO over time, these studies provide some useful insight into the variability of F_ENO but they are limited since some of the variability will be explained by changes in the children's environment.

Within our pooled dataset we were able to explore whether variability in a stable period was different if it was followed by either stability or instability. We found no evidence that repeated measurements of F_ENO over three month intervals were influenced by subsequent control, and this is consistent with studies where daily F_ENO measurements were made and which observed rising F_ENO over only 3-7 days before an exacerbation (11,17). The typical change in F_ENO in the studies where daily measurements were obtained was <50% (11,17), and the relatively rapid and short-lived peak in F_ENO in the context of an asthma exacerbation would indicate that F_ENO was not likely to be useful in reducing exacerbation risk, but evidence from RCTs indicates that F_ENO-guided treatment more effective than standard care in reducing exacerbations (15). Despite the "noisy signal" that is evidenced by the considerable variability in both daily (11,17) and three-monthly (the present study) F_ENO measurements, there seems to be sufficient clinically relevant change in F_ENO over time for F_ENO -guided treatment to be associated with reduced exacerbations(15).

Our study has a number of limitations. First, the protocols of some of the RCTs included had intervals other than three months between repeated F_ENO measurements and this may have added greater variability into repeated measurements. Compared to the study by Cutts *et al* (16), the IQR for percentage change in the present study was relatively larger but the absolute change was relatively smaller than results so this argues against an interval of two months leading to variation which is substantially greater than for a three month interval. Second, we have no index of treatment adherence, and it is likely that some variation in repeated F_ENO measurements was due to incomplete adherence with ICS (12)and/or LTRA (33)treatment. Third the number of participants with stable followed by unstable asthma was relatively small and our analysis comparing intrasubject change in F_ENO during these two periods was probably underpowered. A further limitation of our work is that very few individuals were nonatopic and we did not stratify for variability in F_ENO by atopic status.

In summary, we interpret our data as showing that a percentage change in F_ENO of <50% over a three-month period is unlikely to be clinically relevant in a child with controlled asthma whatever their initial F_ENO concentration is. F_ENO is currently used in clinical practice by many subspecialists in the initial assessment of asthma, endotyping, assessment of adherence and for longitudinal monitoring. Key questions remain to be answered before F_ENO might be incorporated into clinical practice to justify a more widespread incorporation into clinical practise, key questions remain to be answered, including "should F_ENO, FEV_1 and an objective index of treatment adherence all be part of the assessment of children with asthma?" And "what specific treatment changes should be guided by changes in F_ENO?"

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Table 1. This table presents characteristics of study participants at their baseline visit for each study and also summarises characteristics across all studies combined.*Controlled status was determined from questionnaires used in each study.

		Fritsch(Fritsch et al., 2006)	Peirsman(Peirsman et al., 2014)	Petsky(Petsky, Helen L. et al., 2015)	Pijnenburg(Pijnenburg, Marielle W. et al., 2005)	Pike(Pike et al., 2013)	Szefler(Szefler et al., 2008)	Voorend-van Bergen(Voorend-van Bergen S et al., 2015)	All populations combined
% male (number/all participants		60% (28/47)	67% (66/99)	49% (31/63)	65% (56/86)	57% (51/90)	53% (288/546)	68% (123/181)	58% (643/1112)
Mean age (SD), y		11.5(3.1)	10.7 (2.1)	10.0 (3.2)	12.3 (2.8)	10.9 (2.6)	14.4 (2.1)	10.2 (3.0)	12.6 (3.1)
Median F_ENO (IQR), ppb		34 (18.6, 58.6) n=46	31 (14, 69) n=49	26 (12.2, 47.5) n=61	32 (16.6, 52.5) n=86	26 (10, 48) n=90	20 (11.2, 40.6) n=546	18 (10.2, 30.4) n=179	22 (11.6, 43.0) n=1057
Mean % predicted FEV_1 (SD)		93.5 (15.7) n=47	91.4 (15.7) n=98	90.7 (15.6) n=54	97.5 (17.5) n=86	89.2 (14.3) n=90	90.9 (16.6) n=546	93.8 (13.0) n=157	93.5 (18.1) n=1078
% with positive skin prick test		Inclusion criterion	Inclusion criterion	38% (24/63)	Inclusion criterion	76% (68/90)	88% (467/531)	Inclusion criterion	89% (972/1097)
Asthma medication	Median dose of inhaled corticosteroids (IQR)	400 (0, 800)	320 (200, 400)	400 (250, 500)	800 (400,1000)	800 (400, 1000)	1000 (400, 2000)	400 (400, 800)	400 (400, 1000)
	% prescribed LTRA	28% (13/47)	60% (59/99)	10% (6/58)	0% (0/86)	51% (46/90)	15% (80/546)	13% (23/181)	21% (227/1107)
	% prescribed LABA	38% (18/47)	32% (32/99)	67% (39/58)	38% (33/86)	76% (68/90)	66% (360/546)	46% (84/181)	57% (634/1107)
Controlled*		49% (23/47)	75% (49/65)	72% (41/57)	57% (44/77)	97% (87/90)	80% (421/528)	67% (122/181)	75% (787/1045)

SD=standard deviation, F_ENO=fractional exhaled nitric oxide, ppb=parts per billion, FEV_1= Forced exhaled volume in one second, IQR=interquartile range, LTRA=leukotriene receptor antagonist, LABA=long acting beta agonist

Table 2. Median and interquartile range (IQR) of absolute and percentage difference in two $F_{E}NO$ measurements made over an interval of three months during which asthma symptoms and inhaled corticosteroid treatment remained stable and there was no asthma exacerbation. The numbers in italics are from a reanalysis of the data in the study by Cutts *et al* (16) where data collected over a two month period are available (insufficient data were available for meaningful analysis of 31-40ppb and 41-49ppb groups.

	Initial $F_{E}NO$ measurement	N	Median (IQR) difference in $F_{E}NO$ measurements over a stable three-month period *(values in italics from Cutts et al)*	Mean difference [limits of agreement] in $F_{E}NO$ measurements over a stable three-month period *(values in italics from Cutts et al)*
Absolute $F_{E}NO$ concentration	<11ppb	51 *69*	+2 (-3, +5) *+1 (0, +3)*	+1 [-19, +21] *+1 [-6, +9]*
	11-20 ppb	59 *41*	0 (-6, +10) *-1 (-5, +4)*	+4 [-37, +45] *+2 [-18, +21]*
	21-30 ppb	41 *11*	3 (-9, +17) *-3 (-13, +5)*	+8 [-50, +65] *+3 [-41, +46]*
	31-40 ppb	18	-5 (-12, +10)	-2 [-53, +48]
	41-49 ppb	16	-7 (-19, +25)	+3 [-78, +84]
	<50 ppb	185 *129*	0 (-7, +9) *0 (-3, 4)*	+3 [-43, +50] *-15 [-77, +46]*
	>=50 ppb	48 *8*	-7 (-29, +17) *-10 (-29, +7)*	-2 [-80, +76] *-18 [-90, +52]*
Percentage change in $F_{E}NO$	<11ppb	51 *69*	+18 (-27, +59) *+11 (0, +67)*	+38 [-151, +228] *+32 [-130, +194]*
	11-20 ppb	59 *41*	+1 (-37, +64) *-7 (-36, +31)*	+30 [-170, +231] *+11 [-118, +141]*
	21-30 ppb	41	18 (-32, +49)	+27 [-155, +210]

		11	*-14 (-48, +24)*	*+10 [-158, +178]*
	31-40 ppb	18	+13 (-39, +45)	+15 [-151, +182]
	41-49 ppb	16	-182 (-47, +50)	+13 [-149, +174]
	<50 ppb	185 *129*	+2 (-33, +51) *0 (-25, +50)*	+29 [-157, +215] *-19 [-90, +52]*
	>=50 ppb	48 *8*	-10 (-33, +35) *-16 (-37, +9)*	+17 [-159, +192] *-15 [-76, +46]*

Table 3. Median and interquartile range (IQR) change in F_ENO during two consecutive three-month periods of stable asthma. Change was expressed as absolute change and percentage change and results are stratified by initial F_ENO (< or ≥50 parts per billion, ppb). Stable asthma was defined as one where symptoms were controlled at the start and the end, inhaler corticosteroid dose was unchanged and there was no asthma exacerbation.

	Initial F_ENO value	N	Median (IQR) in stable 0-3mo	Median (IQR) In stable 3-6mo	p-value#	Mean (SD) difference [limits of agreement] in stable 0-3m	Mean difference [limits of agreement] in stable 3-6m
Absolute F_ENO concentration	<50ppb	73	+2 (-4, +8)	+3 (-6, +10)	0.388	+10 (34) [-56, +76]	-2 (31) [-63, +59]
	≥50 ppb	23	-12 (-44, +22)	+1 (-24, +19)	0.274	-12 (39) [-89, +66]	-1 (38) [-75, +73]
	All	96	+1 (-7, +8)	+3 (-8, +10)	0.153	+5 (36) [-66, +76]	-2 (33) [-66, +62]
Percentage change in F_ENO	<50ppb	73	+16 (-24, +67)	+13 (-36, +77)	0.555	+48 (119) [-184, +280]	+42 (99) [-153, +237]
	≥50 ppb	23	-11 (-51, +32)	+1 (-30, +25)	0.484	-7 (47) [-99, +86]	+6 (55) [-100, +114]
	All	96	+8 (-29, +47)	+11 (-35, +70)	0.365	+35 (108) [-177, +247]	+33 (92) [-147, +213]

Wilcoxon matched pairs

Table 4. Median and interquartile range (IQR) change in F_ENO during a three-month period of stable asthma followed by an unstable period. Change was expressed as absolute change and percentage change and results are stratified by initial F_ENO (< or ≥50 parts per billion, ppb). Stable asthma was defined as one where symptoms were controlled at the start and the end, inhaler corticosteroid dose was unchanged and there was no asthma exacerbation.

	Initial F_ENO value	N	Median (IQR) in stable 0-3m0	Median (IQR) unstable 3-6mo	p-value[#]	Mean difference (SD) [limits of agreement] in stable 0-3mo	Mean difference (SD) [limits of agreement] in unstable 3-6mo
Absolute F_ENO concentration	<50ppb	32	-1 (-8, +11)	+3 (-12, +13)	0.702	+6 (31) [-54, +66]	-3 (26) [-54, +48]
	≥50 ppb	16	-5 (-46, +17)	0 (-34, +18)	0.255	-22 (55) [-130, +87]	-8 (37) [-80, +64]
	All	48	-2 (14, +14)	+3 (-13, +14)	0.322	-4 (42) [-86, +79]	-4 (30) [-63, +54]
Percentage change in F_ENO	<50ppb	32	-6 (-30, +40)	+18 (-30, +65)	0.736	+38 (134) [-224, +300]	+29 (77) [-121, +179]
	≥50 ppb	16	-12 (-47, +22)	0 (-38, +31)	0.438	-14 (40) [-92, +65]	-2 (45) [-91, +87]
	All	48	-6 (-37, +25)	+13 (-33, +57)	0.467	+21 (114) [-202, +243]	+19 (69) [-116, +153]

What is a clinically meaningful change in exhaled nitric oxide for children with asthma?

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Keywords: Asthma, Child, Monitoring, Exhaled nitric oxide

Running head: What is a meaningful change in F_ENO in children?

ABSTRACT

Introduction. Fractional exhaled nitric oxide (F_ENO) may be a useful objective measurement to guide asthma treatment. What remains uncertain is what change in F_ENO is clinically significant.

Methods. An individual patient data analysis was performed using data from seven randomised clinical trials which used F_ENO to guide asthma treatment. The absolute and percentage intra-subject change in F_ENO measurements over “stable” and also “unstable” three-month periods were described.

Results. Data were available in 1112 RCT participants and ≥1 stable period was present for 665 individuals. The interquartile range (IQR) and limits of agreement (LoA) for change in absolute F_ENO among individuals whose initial F_ENO was <50ppb were -7 to 9ppb and -43 to 50ppb, and for those with initial F_ENO≥50ppb IQR was -29 to 17 ppb and LoA was -80 to 76ppb. For percentage change in F_ENO, the IQR and LoA for individuals whose initial F_ENO was <50ppb were -33 to 51% and -157 to 215%, and for those with initial F_ENO ≥50ppb were -33 to 35% and -159 to 192%. The variation in F_ENO values for a stable period was similar irrespective of whether it was followed by a stable or unstable period.

Conclusions. Over a three-month period where F_ENO is initially <50ppb, a rise of <10ppb or of <50% (based on IQR) is unlikely to be related to asthma. When F_ENO is initially ≥50ppb an percentage change of <50% (based on IQR) is unlikely to be asthma-related.

INTRODUCTION

Asthma is a very common condition and affects millions of children in the UK (1) and the US(2). There is no cure for asthma, but there is effective treatment to control asthma symptoms and reduce exacerbations. Guidelines recommend that asthma treatment is guided by symptoms(3-5), but symptom-reporting and interpretation is subjective and this leads to inconsistent care. There is a need for an objective biomarkermeasurement to allow greater consistency in asthma treatment (6).

Fractional exhaled nitric oxide (F_ENO) is a surrogate for airway eosinophilia (7) and has potential roles in asthma including diagnosis, treatment stratification, treatment adherence and monitoring airway inflammation (7). A recent Agency for Healthcare Research and Quality comparative effectiveness review of F_ENO has summarised evidence which supports a number of potential clinical applications of F_ENO in the management of asthma(8). F_ENO has many of the characteristics required of an objective tool to monitor allergic asthma over time since it rises before symptoms occur(9-11), falls when asthma treatment is administered(12,13), can be measured with minimal discomfort to the patient and results are available within a few minutes using commercially-available Food and Drink Administration approved apparatus(14). When compared to standard treatment, asthma treatment guided by F_ENO measurements is associated with reduced asthma exacerbations, but not improved asthma control(15). There are some key questions which need to be answered before F_ENO can be incorporated into the routine longitudinal management of childhood asthma, and current asthma guidelines do not recommend F_ENO for monitoring asthma outside specialist clinics(3-5).

One key question which needs answering is "what change in F$_{E}$NO is clinically relevant in children?" The American Thoracic Society (ATS) guideline proposes that a change of 10 parts per billion at lower concentrations or 20% at higher concentrations may have clinical relevance(7), but acknowledge a low level of evidence. However, one study where children with mild asthma and without asthma had repeated measurements of F$_{E}$NO, questioned this ATS recommendation by finding that values may change by as much as 100% without any clinical change (16). A second study has reported that F$_{E}$NO values fluctuate between 6ppb and 40ppb in children on stable asthma treatment (17).

Our group has pooled the data collected in more than 1000 participants in seven RCTs where F$_{E}$NO was used to guide asthma treatment (18). This analysis found that a 50% increase in FeNO between baseline and three months was associated with 11% increase in odds for poor asthma control six months after baseline(18)but it remains unknown the degree of F$_{E}$NO variability over stable and unstable periods. Thus, we used individual patient data from the same database of seven RCTs to address the question "What is the variability in F$_{E}$NO over three months among children with stable asthma?".

METHODS

Study design

Authors of published RCTs which used F$_{E}$NO to guide asthma treatment in children(15) were asked to provide data, as described previously described (18), provided de-identified data for analyses in this study. All RCTs collected details of F$_{E}$NO concentrations, asthma control (using different scores) and exacerbation outcome (defined as requiring oral corticosteroid treatment) at each assessment. F_ENO was measured in all studies in accordance with the American Thoracic Society 2005 guideline(19). Percentage of predicted (%) FEV_1 was standardised to the Global Lung Initiative reference (20) with the exception of two trials (21,22) where only % FEV_1 standardised to other references was available. Covariates collected at baseline in all trials included: age, gender, height, weight, trial arm, dose of inhaled corticosteroid (ICS, as daily budesonide equivalent dose, BUD), prescribed long acting beta agonist (LABA) or not, prescribed leukotriene receptor agonist (LTRA) or not, and an asthma control score. Institutional ethical approval was provided for each trial which contributed data.

Details of each population

Fritsch et al(23). In this study, 47 children were recruited from a hospital asthma clinic in Vienna, Austria. Data were collected at six-week intervals over six months.

Peirsman et al(24). Researchers in centres across Belgium recruited 99 participants with persistent asthma attending hospital asthma clinics. Clinical assessments took place every three months over one year.

Petsky et al(25). Children in Australia and Hong Kong were recruited from hospital clinics. There were 63 patients enrolled and clinical assessment took place on eight occasions over twelve months (one, two, three, four, six, eight, ten and twelve months). The ten-month assessment was assigned the "nine month" assessment for the present analysis.

Pijnenburg et al (21). This study was carried out in the Netherlands and 86 patients were recruited and followed up at three-month periods over one year.

Pike et al (22). This was a study carried out in Southampton, UK and 90 patients were recruited and followed up at two-month intervals over twelve months. The two-month assessment was assigned the "three month" assessment for the analysis, also the ten-month was assigned the "nine month" assessment.

Szefler et al(26). Children and young adults of African or Hispanic descent and living in inner city areas of ten US cities were recruited. There were 546 participants and data were collected at the following intervals post randomisation: 6 weeks, 14 weeks (used in lieu of three months in the analysis), 22 weeks (used in lieu of six months), 30 weeks (used in lieu of nine months) and 46 weeks (used in lieu of twelve months).

Voorend-van Bergen et al(27). This RCT took place in the Netherlands and 181 participants were recruited. Data from a third arm of the RCT which delivered a web-based intervention were not included in the present analysis. Data were collected at four-month intervals over a year. The four and eight month assessments were respectively assumed to be three and six month assessment for the analysis. There was no equivalent nine-month assessment.

Analysis

Participants were identified as having a three-month period of stable asthma if they had a period between two successive assessments over three months during which: (i) asthma was controlled at the beginning and end (different RCTs used different scoring systems) (ii) the dose of inhaled corticosteroid (ICS) remained unchanged and (iii) there was no exacerbation requiring oral corticosteroid treatment. Change in F_ENO values was expressed as either an absolute difference or percentage difference over a three month period and as either the mean and 95% limits of agreement (LoA) or median and interquartile range (IQR). Change in F_ENO could not be expressed as geometric mean since values can be negative (16). Change in F_ENO was described for all individuals and also stratified for individuals with initial values of <50ppb and ≥50ppb for comparison with the guideline (7) and also for ranges <11ppb, 11-20ppb, 21-30ppb, 31-40ppb and 41-49ppb to allow comparison with data from a~~t~~ study by Cutts *et al* (16)which recruited children with no asthma and mild asthma(16). One author of the present paper (ST) is data custodian for the study (16) and reanalysed the data for direct comparison between studies. For patients with multiple stable periods, data from only the first stable period was included. To determine whether F_ENO values during a stable period were influenced by a subsequent unstable period we described change in F_ENO values during; (i) successive stable periods (i.e. when the child's asthma was controlled for three successive assessment, had no change in ICS dose and did not have an exacerbation) and (ii) also a stable followed by an unstable period (i.e. when the child's asthma was controlled for two successive assessments during which there was no change in ICS dose or an exacerbation but where asthma was uncontrolled on the third assessment ~~. An unstable period was defined as one where asthma control was present but became lost and~~ and where ICS dose remained unchanged. Comparisons we made using Wilcoxon signed rank test.

RESULTS

Study subjects

Data from 1112 participants in seven RCT were analysed (21-27). Participant details at baseline are presented in table one and have also been described elsewhere (18). The mean (SD) participant age was 12.6 (3.1) years and 58% of participants were male.

Variability of F_ENO within stable three months periods

All stable individuals. There were 251 children who had a total of 260 three-month intervals where asthma was stable. Due to missing F_ENO values in 16 individuals, change in F_ENO could be calculated in 235 participants with an overall median (IQR) absolute F_ENO change -0.5ppb (-9, 10), and percentage F_ENO change -2% (-33%, 50%). For all stable participants combined, the mean (limits of agreement) for change in absolute F_ENO was +2 ppb (-52, +56) and for percentage change F_ENO was +26% (-157, +210).

Stable and initial F_ENO <50ppb. The 185 children with stable asthma and baseline F_ENO <50ppb had a median (IQR) absolute change in F_ENO 0 ppb (-7, +9) and the median (IQR) percentage change in F_ENO was +2% (-33, +51), table 2. The mean (LoA) change in absolute F_ENO was +3 (-43, +50) and the mean (LoA) percentage change was +29 (-157, +215), table 2.

Stable and initial F_ENO ≥50ppb. Where baseline F_ENO was ≥ 50ppb (n = 48), the median values (IQR) for absolute and percentage change in F_ENO were -7 ppb (-29, +17) and -10% (-33, +35) respectively, table 2. The mean (LoA) for change in absolute F_ENO was -2 ppb (-80, +76) and for percentage change F_ENO was +17% (-159, +192), see table 2

Stable and initial F_ENO stratified for comparison with Cutts et al (16). Table 2 presents absolute and percentage change in F_ENO expressed as mean (LoA) and median (IQR) using data collected from children with asthma recruited to the RCTs. Data from the study of mostly healthy children by Cutts *et al (16)* are also presented for comparison.

Variability during consecutive periods of stable asthma followed by stable asthma

There were 96 individuals with two consecutive periods of stable asthma. The median (IQR) F_ENO concentrations during the first and second stable periods were +1ppb (-7, +8) and +3ppb (-8, +10); corresponding percentage change F_ENO values were +8 (-29, +47) and +11 (-35, +70), see table 3. Table 3 also presents results after stratification by <50ppb and ≥50 ppb. There was no differences in change in F_ENO between the first and second stable periods.

During consecutive periods of stable asthma followed by unstable asthma

There were 48 individuals identified. The median (IQR) change in absolute F_ENO values during stable and unstable periods were -2 (-14, +14) and 3 (-13, 14) and percentage change during stable and unstable periods were -6 (-37, +25) and 13 (-33, +57), see table 4. Table 4 presents results stratified by <50ppb and ≥50 ppb. There was no differences in change in F_ENO between the stable and unstable periods.

DISCUSSION

Fractional exhaled nitric oxide has a number of potential roles in asthma and the focus of the present study ~~This analysis aim~~ was to answer the question "What is a clinically significant change in F_ENO for children with asthma?" Our results may be relevant to clinicians when interpreting repeated F_ENO measurements, e.g. when making treatment decisions in a child with already-diagnosed asthma. The premise underlying our analysis was that any change in F_ENO which occurred in children during a period of stability was not clinically relevant. Our first finding was that when applying a stringent threshold of the LoA (i.e. including 95% of all values), the variability in F_ENO during stability was extremely wide and thus likely to include clinically significant events (i.e. loss of control and/or exacerbation). In contrast when applying the more liberal threshold of IQR (i.e including 50% of all values), the upper limit of variability could be applied clinically. Indeed the upper quartile value for participants with F_ENO <50ppb was <10ppb and consistent with the ATS recommendation (7).

Our second finding was that expressing change in F_ENO as percentage change lead to a consistent IQR of between ±50% for all initial F_ENO values. The upper quartile value for % change where the initial F_ENO was >50ppb was 35%, but having a "one size fits all" recommendation (i.e. <50% is unlikely to be clinically relevant) could be more easily understood by patients and health care staff, and also would facilitate management for individuals whose F_ENO is close to 50ppb (the current dichotomy for different recommendations in the ATS guideline(7)). Our results do not support the ATS "weak" recommendation that a change of >20% may be clinically meaningful for children with $F_ENO \geq$ 50ppb (7).

The IQR and LoA from the present study are consistent with a study where F_ENO was measured at two month intervals over a year in children with mild or no asthma, where it was found that initial F_ENO values may rise by up to 100% (16). This consistency is despite important differences between our present study and that of Cutts *et al* (16), for example our study had a relatively greater number of participants with asthma, higher median F_ENO values and F_ENO measurements were repeated over a three month period compared to two months. For everyday clinical practice it is more relevant to define clinically relevant changes in F_ENO in a population of asthmatic children who are treated with ICS as compared to healthy children or steroid naive children.

Considerable variation in F_ENO values over time has also been reported in "natural experiments" in children. For example Huss-Marp *et al*(28) observed that F_ENO values in children with mild intermittent asthma with initial F_ENO >17 ppb and attending a summer camp at altitude fell by 50% over four to six weeks independent of symptoms, and Kaminsky *et al* (29) found F_ENO values fell from 11 to 6ppb (a 45% reduction) independent of asthma control among 27 children attending a one week summer camp. Two studies of children with asthma who were sensitised to grass found the F_ENO values rose by approximately 50% during the pollen season(30,31) but asthma symptoms also rose during this time. Barreto *et al* (32) found that the coefficient of variation in paired F_ENO values taken over a one-week period was 3ppb in eight children treated with ICS. Due to the limited literature describing change in F_ENO over time, these studies provide some useful insight into the variability of F_ENO but they are limited since some of the variability will be explained by changes in the children's environment.

Within our pooled dataset we were able to explore whether variability in a stable period was different if it was followed by either stability or instability. We found no evidence that repeated measurements of F_ENO over three month intervals were influenced by subsequent control, and this is consistent with studies where daily F_ENO measurements were made and which observed rising F_ENO over only 3-7 days before an exacerbation (11,17). The typical change in F_ENO in the studies where daily measurements were obtained was <50% (11,17), and the relatively rapid and short-lived peak in F_ENO in the context of an asthma exacerbation would indicate that F_ENO was not likely to be useful in reducing exacerbation risk, but evidence from RCTs indicates that F_ENO-guided treatment more effective than standard care in reducing exacerbations (15). Despite the "noisy signal" that is evidenced by the considerable variability in both daily (11,17) and three-monthly (the present study) F_ENO measurements, there seems to be sufficient clinically relevant change in F_ENO over time for F_ENO -guided treatment to be associated with reduced exacerbations(15).

Our study has a number of limitations. First, the protocols of some of the RCTs included had intervals other than three months between repeated F_ENO measurements and this may have added greater variability into repeated measurements. Compared to the study by Cutts *et al* (16), the IQR for percentage change in the present study was relatively larger but the absolute change was relatively smaller than results so this argues against an interval of two months leading to variation which is substantially greater than for a three month interval. Second, we have no index of treatment adherence, and it is likely that some variation in repeated F_ENO measurements was due to incomplete adherence with ICS (12)and/or LTRA (33)treatment. Third the number of participants with stable followed by unstable asthma was relatively small and our analysis comparing intrasubject change in F_ENO during these two periods was probably underpowered. A further limitation of our work is that very few individuals were non-atopic and we did not stratify for variability in F_ENO by atopic status.

In summary, we interpret our data as showing that a percentage change in F_ENO of <50% over a three-month period is unlikely to be clinically relevant in a child with controlled asthma whatever their initial F_ENO concentration is. F_ENO is currently used in clinical practice by many subspecialists in the initial assessment of asthma, endotyping, assessment of adherence and for -longitudinal monitoring. Key questions remain to be answered before F_ENO might be incorporated into clinical practice to justify a more widespread incorporation into clinical practise, key questions remain to be answered, including "should F_ENO, FEV_1 and an objective index of treatment adherence all be part of the assessment of children with asthma?" And "what specific treatment changes should be guided by changes in F_ENO?"

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Table 1. This table presents c~~C~~haracteristics of study participants at their baseline visit for ~~in~~ each study and also summarises characteristics across all studies combined.*Controlled status was determined from questionnaires used in each study.

		Fritsch(Fritsch et al., 2006)	Peirsman(Peirsman et al., 2014)	Petsky(Petsky, Helen L. et al., 2015)	Pijnenburg(Pijnenburg, Marielle W. et al., 2005)	Pike(Pike et al., 2013)	Szefler(Szefler et al., 2008)	Voorend-van Bergen(Voorend-van Bergen S et al., 2015)	All populations combined
% male (number/all participants		60% (28/47)	67% (66/99)	49% (31/63)	65% (56/86)	57% (51/90)	53% (288/546)	68% (123/181)	58% (643/1112)
Mean age (SD), y		11.5(3.1)	10.7 (2.1)	10.0 (3.2)	12.3 (2.8)	10.9 (2.6)	14.4 (2.1)	10.2 (3.0)	12.6 (3.1)
Median F_ENO (IQR), ppb		34 (18.6, 58.6) n=46	31 (14, 69) n=49	26 (12.2, 47.5) n=61	32 (16.6, 52.5) n=86	26 (10, 48) n=90	20 (11.2, 40.6) n=546	18 (10.2, 30.4) n=179	22 (11.6, 43.0) n=1057
Mean % predicted FEV_1 (SD)		93.5 (15.7) n=47	91.4 (15.7) n=98	90.7 (15.6) n=54	97.5 (17.5) n=86	89.2 (14.3) n=90	90.9 (16.6) n=546	93.8 (13.0) n=157	93.5 (18.1) n=1078
% with positive skin prick test		Inclusion criterion	Inclusion criterion	38% (24/63)	Inclusion criterion	76% (68/90)	88% (467/531)	Inclusion criterion	89% (972/1097)
Asthma medication	Median dose of inhaled corticosteroids (IQR)	400 (0, 800)	320 (200, 400)	400 (250, 500)	800 (400,1000)	800 (400, 1000)	1000 (400, 2000)	400 (400, 800)	400 (400, 1000)
	% prescribed LTRA	28% (13/47)	60% (59/99)	10% (6/58)	0% (0/86)	51% (46/90)	15% (80/546)	13% (23/181)	21% (227/1107)
	% prescribed LABA	38% (18/47)	32% (32/99)	67% (39/58)	38% (33/86)	76% (68/90)	66% (360/546)	46% (84/181)	57% (634/1107)
Controlled*		49% (23/47)	75% (49/65)	72% (41/57)	57% (44/77)	97% (87/90)	80% (421/528)	67% (122/181)	75% (787/1045)

SD=standard deviation, F_ENO=fractional exhaled nitric oxide, ppb=parts per billion, FEV_1= Forced exhaled volume in one second, IQR=interquartile range, LTRA=leukotriene receptor antagonist, LABA=long acting beta agonist