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Title: Exhaled nitric oxide and the management of childhood asthma - yet another promising biomarker "has been" or a misunderstood gem

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Abstract: Childhood asthma is a common chronic condition. Approximately five percent of all children in western countries are prescribed treatment with inhaled corticosteroids (ICS) to prevent asthma symptoms. Current guidelines advocate titrating ICS dose to symptoms but this approach is not without problem, e.g. how to discern asthmatic from non-asthmatic symptoms? And when to reduce ICS dose? This review describes the strengths and weaknesses of fractional exhaled nitric oxide (FENO) as an objective index for individualising asthma control in children. Epidemiological and mechanistic evidence suggest that FENO should be a promising biomarker for eosinophilic airway inflammation (a hall mark for asthma) but somewhat surprisingly, clinical trials in children have not consistently found benefit from adding FENO to a symptom-based approach to ICS treatment in children. There are a number of reasons why FENO has apparently failed to translate from promising biomarker to clinically useful tool, and one reason may be a lack of understanding of what merits a significant intrasubject change in FENO. This review describes the rise and apparent fall of FENO as biomarker for asthma and then focuses on more recent evidence which suggest that FENO may prove to have a role in the management of childhood asthma.

Conflict of Interest Statement

The author has completed three studies where consumables were provided by Aerocrine. The author has not received any consultancy fees or financial support for attending meetings from any nitric oxide analyser manufacturer.

***Cover Letter, incl. Statement on Approval by co-authors**

Dear Professor Eber,

Thank you for the opportunity of submitting a revised version of this manuscript. I would also like to thank the reviewer for their time and very helpful comments. A point-by-point response is attached as a separate folder.

I have tried to upload the documents as requested (ie cover letter, point-by-point response, manuscript, tables, figures and supplement/marked up manuscript) but despite my best efforts, the system has declined to update the file order.

Yours sincerely

Steve Turner

***Detailed Response to Reviewers**

Dear Professor Eber,

Thank you for the opportunity of submitting a revised version of this manuscript. I would also like to thank the reviewer for their time and very helpful comments. A point-by-point response is below (my comments are in capitals and page numbers refer to the marked up version of the revised manuscript).

Yours sincerely

Steve Turner

2.1 The study of De Jongste is missing (AJRCCM 2009), although in this study 'usual care'was not very 'usual'. Also, Peirsman published a study in pediatr pulmonol 2013 on FENO monitoring.
THANK YOU FOR POINTING OUT THESE PAPER WHICH HAVE BEEN OMITTED BUT NOW INCLUDED

page 8: a meta-analysis with raw data of all studies is actually missing and might be interesting, as Petsky and all did not use original data from all studies. A meta-analysis (not on original data) that is missing (although in a low impact paper) is by Mahr et al, Asthma Allergy Proc 2013.
THANK YOU FOR DRAWING MY ATTENTION TO THIS META-ANALYSIS (MAHR) WHICH IS NOW CITED

3.1 although FeNO increases with height, this is in my opinion not a major problem, as most children with asthma are seen every 3 to 6 months, a period in which you do not expect spectacular growth. This might explain an increase of 5-10 ppb max. I feel seasonal influences, viral infections (which are not mentioned here) and intraperson variability are much more of a problem in interpreting longitudinal FeNO values. Intraindividual varaibilty as described by the author may be much bigger than fluctuations due to severity or control of disease.
I HAVE AMMENDED THIS SECTION TO ACKNOWLEDGE THAT OVER THE SHORT TERM, CHANGE IN HEIGHT IS NOT LIKELY TO BE RELEVANT TO FENO MEASUREMENTS. I HAVE ALSO ADDED VIRAL INFECTION AS A TEMPORARY INFLUENCE ON FENO VALUES. INTRAINDIVIDUAL VARIABILITY IS DISCUSSED IN SECTION 3.6

3.2 As the author states, I do not think poor adherence in the dose titration studies was the case. In particular in the study by Szefler the primary outcome decrease spectacular after the run-in period, making this study even underpowered. Then even if adherence was not optimal in the referred studies, this would reflect daily practice and make the results of the studies more applicable to daily life.
I AGREE

3.3 Although I can follow the arguments of the author here, I do not think that a FENO driven treatment will be possible in an era where patient reported outcomes are becoming more and more important as primary outcomes. However, the author may be right as 'the sputum eosinophil driven treatment' by Green et al in adults, led to less (severe) exacerbations in the treatment arm where treatment was adjusted to sputum eosinophils only.
AGAIN I AGREE AND I THINK A BALANCED ARGUMENT IS PRESENTED HERE AS LATER IN THIS SECTION, THE TEXT SAYS "…THE POOR CORRELATION BETWEEN ASTHMA CONTROL AND FENO …. DOES QUESTION WHETHER ASTHMA TREATMENT CAN BE GUIDED ONLY BY FENO"

3.5 Except for the discussion of cut offs, the 'reference values' could be debated. Maybe one should use 'reference values' obtained from data in an asthmatic population with well-controlled asthma instead of a healthy population. This was nicely summarized by Peter Gibson in Clin Exp Allergy 2009: 'The algorithm decision points should be based on outcomes in the population of interest rather than the range of values in healthy people, and the algorithm used needs to provide a sufficiently different result to clinical decision making in order for there to be any discernible benefit.' I would certainly cite this paper, as this very nicely summarizes how to design exhaled NO studies. However, the problem may be that the range of what is normal in well-controlled asthmatics is too broad.
THE PAPER BY PETER GIBSON IS CITED IN THIS SECTION (REF 62). I HAVE POINTED THE READER IN THE DIRECTION OF THIS PAPER AND CLARIFIED THE DIFFERENCE BETWEEN KNOWING WHAT A "HIGH" ONE-OFF

MEASUREMENT IS AND A HIGH MEASUREMENT RELATIVE TO PREVIOUS VALUES.

A two weeks course of prednisone will lower FENO more than the optimal dose of inhaled corticosteroids and should not be the target in my opinion (Smith JACI 2009). On the other hand, FENO immediately after prednisone may not be the optimal value that can be obtained, as was shown for FEV1 (Lex, Pediatr Pulmonol 2005).
I HAVE INCLUDED THIS GOOD POINT, IE THAT ORAL STEROIDS MAY YIELD AN UNACHIEVEABLE FENO VALUE.

Bullet 5 (page 16) Another reason why some studies did not show an effect of FENO monitoring and adjusting treatment on FENO was the fact that studies did not allow for step down if patients were symptomatic while having low FENO levels. Therefore, I would plea for stepping down if FENO is low despite symptoms.
THANKS FOR THIS HELPFUL POINT WHICH I HAVE ADDED AS AN ADDITIONAL BULLET POINT

An argument that is missing is that FENO driven treatment may be useless in children with concordant phenotypes (e.g. low FENO, low symptoms, normal FEV1 or high FENO, high symptoms and low FEV1), however, if there is discordancy between symptoms, FEV1 and FENO there might be a benefit of including FENO in treatment algorithms.
I HAVE ADDED TEXT AT THE START OF SECTION 3.1 TO ADDRESS THIS POINT.

Page 17: I suggest to do a meta-analysis with all original data.
I HAVE DONE THIS

Figure 1: I do not feel this adds much to the paper.
I HAVE REMOVED THIS FROM THE MANUSCRIPT

Figure 2 is not complete in my opinion. I would suggest to add poor inhaler technique and ongoing allergen exposure to the left upper part. Viral infections to the right upper part. Left lower quadrant: well controlled asthma? Right lower quadrant: coffee intake, after exercise, after flow-volume curves…
I HAVE ADDED POOR INHALER TECHNIQUE, EXERCISE, SPIROMETRY AND VIRAL INFECTIONS AS SUGGESTED. I HAVE CHANGED EXPOSURE TO POLLEN AND POOR AIR QUALITY TO “ONGOING EXPOSURE TO INHALED ALLERGENS AND POOR AIR QUALITY” (TOP RIGHT). CAFFEINE INTAKE INCREASES FENO IN CHILDREN.

Table 2: References 68-72 are missing. Correlations with FEV1 are missing. There are many more papers on the correlations between asthma control (as assessed with ACT for example) and FENO, FEV1, PAQLQ etc.
REFERENCES 68-72 (NOW REFERENCES 44-48) WERE CITED IN TABLE 2 BUT ARE NOW ALSO CITED IN THE TEXT (SECTION 3.1). I HAVE ADDED REFERENCES RELATING ENO TO FEV1. THE REFERENCES USED WERE NOT INTENDED TO BE EXHAUSTIVE BUT TO ILLUSTRATE THE PRESENCE AND ABSENCE OF ASSOCIATIONS SO I HAVE NOT ADDED ANY FURTHER STUDIES TO THE REVIEW BUT AGREE THAT THERE ARE MANY MORE WHICH I COULD CITE.

Table 3: add studies of De Jongste and Peirsman. One additional study was presented as an abstract at the ERS congress in 2013 by Voorend-van Bergen.
THESE TWO PUBLISHED STUDIES HAVE BEEN INCLUDED IN THE TABLE. GIVEN THE LACK OF DATA FROM THE ABSTRACT, I HAVE MENTIONED THE UNPUBLISHED STUDY IN THE TEXT AT THE END OF SECTION 2.1 BUT NOT INCLUDED THIS IN THE TABLE

Table 4: I would not say that asthma exacerbation is 'independent of asthma'.
I HAVE DELETED THIS ROW FROM THE TABLE.

Figure

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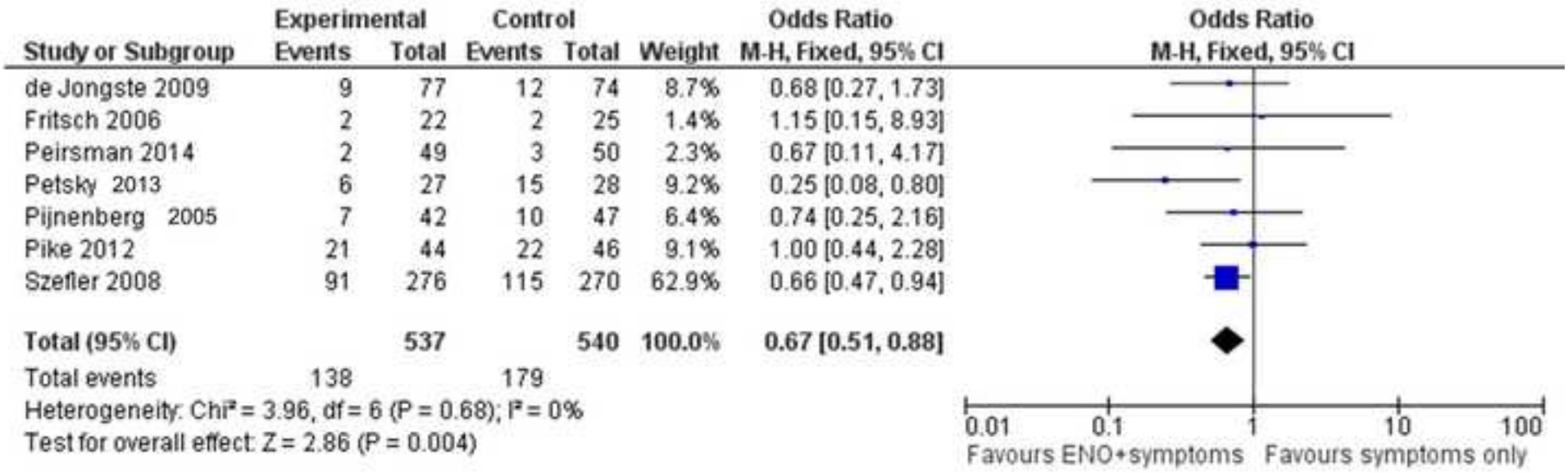

Figure 2

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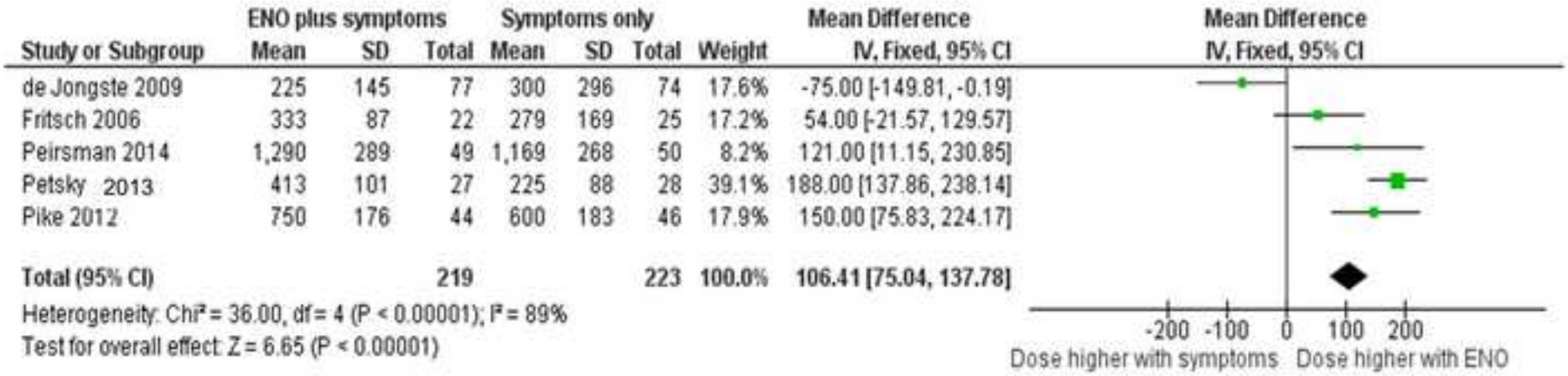

Figure 3
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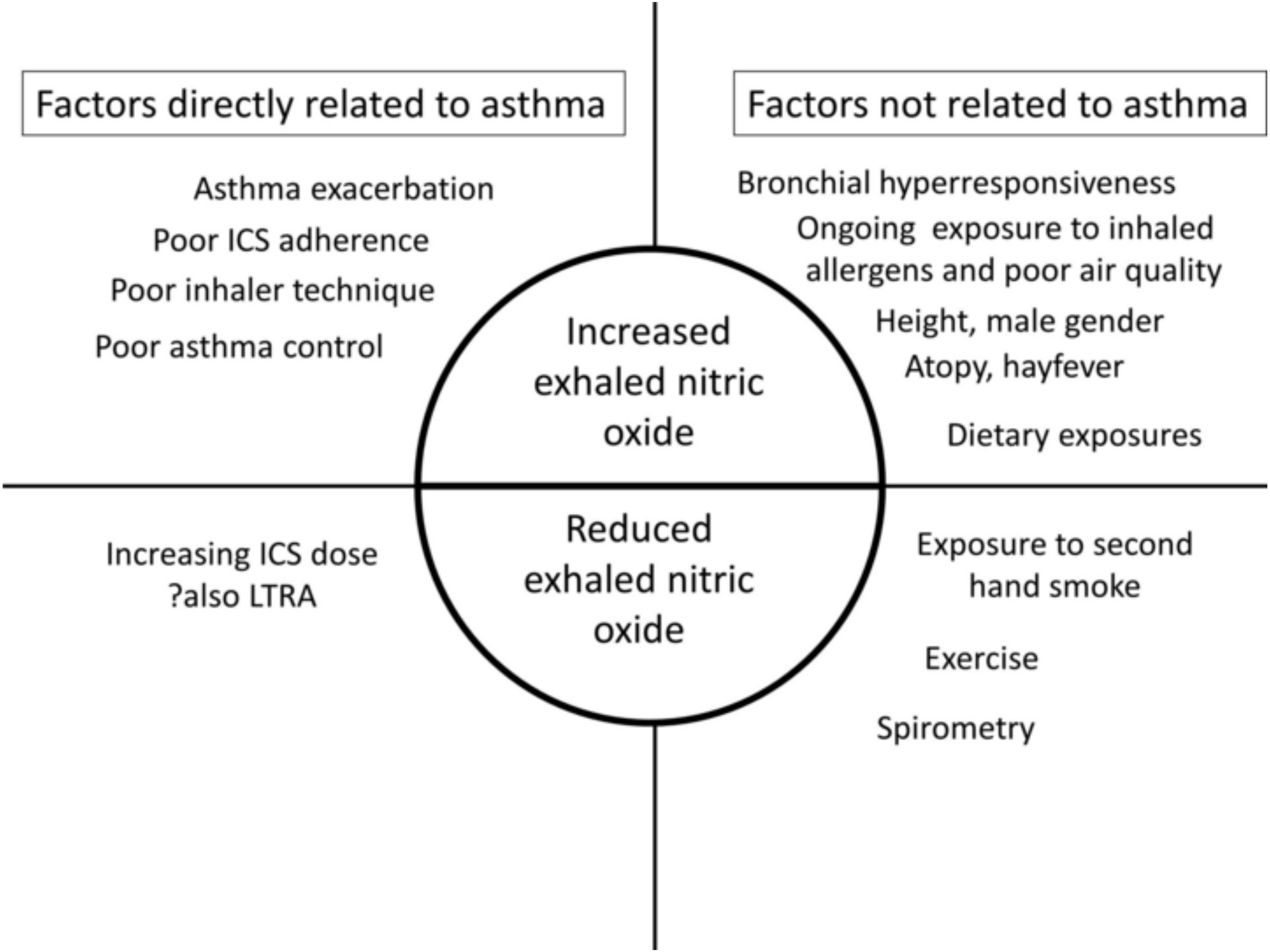

Table 1

Table 1. Clinically important questions in asthma management where FE_{NO} may give insight

Are these asthmatic symptoms in this child with asthma?
Should treatment be stepped up with inhaled corticosteroids or alternative medications?
When is it appropriate to step down inhaled corticosteroid treatment?
When is it safe to stop treatment with inhaled corticosteroids?

Table 2

Table 2. Summary of the literature suggesting that exhaled nitric oxide (FE_{NO}) may or may not be a good biomarker for childhood asthma.

Studies suggesting FE_{NO} may be a good biomarker for childhood asthma	**Studies suggesting FE_{NO} may NOT be a good biomarker for childhood asthma**
FE_{NO} is elevated in children with asthma [13]	FE_{NO} is elevated in atopic non-asthmatic children [45] [79] and in adolescents whose asthma has remitted [80]
Exhaled nitric oxide is positively correlated with three hallmarks for asthma, sputum eosinophils [44,81,82] (r=0.5), FEV_1 [44] and bronchial hyperresponsiveness (BHR) [45] [46]	Exhaled NO is not related to FEV_1 [45] or BHR [48]
Exhaled nitric oxide is positively correlated with airway eosinophilia after two weeks treatment with oral corticosteroids (r=0.5) [10]	
Elevated FE_{NO} is associated with poor asthma control (r=0.2) [41-43]	FE_{NO} is not correlated with asthma control [47]
FE_{NO} rises after withdrawal of ICS and before symptoms relapse [18]	FE_{NO} does not predict relapse after ICS withdrawal [83]
Treatment with inhaled corticosteroids reduces FE_{NO} in children with asthma [68].	FE_{NO} remains elevated in some individuals despite treatment with ICS [84,85].

Table 3

Table 3. Details of the six randomised controlled trials comparing standard symptom-based asthma management against standard management plus exhaled nitric oxide (FE_{NO}) in children with asthma.

Study	Population details	FE_{NO} Cut off(s) used	Study design	Primary outcome	Secondary outcomes
de Jongste[32]	Aged 6-18 attending academic centres or hospitals. Atopic (by plasma IgE or skin prick test). Stable mild-moderate asthma. 151 randomised.	≥20 ppb for 6-10 year olds ≥25 ppb for >10 year olds	30 week study, intervention arm made daily FE_{NO} measurements. Treatment reviewed each 3 weeks by telephone, physiological testing 1, 3, 5 months and at end of study	Symptom free days during last 3 months of trial; this improved equally in both arms of the trial.	No difference between control and intervention arm for ICS dose, FEV_1, FE_{NO} or exacerbations.
Peirsman[33]	Age range not stated. Mild to severe asthma attending hospital clinics. Atopic (by plasma IgE or skin prick testing). 99 randomised	≥20 ppb	52 week study. FE_{NO} and symptoms reviewed every three months	Symptom free days; no difference between groups	Exacerbation; reduced in intervention arm (18/49) compared to the control arm (35/50).
Fritsch[27]	Aged 6-18 years. 52 randomised.Attending hospital clinic. Skin prick positive.	Greater than or ≤20ppb	6 month duration, assessed each 6 weeks	FEV_1 – no difference	Exacerbations, mid expiratory flows, control. Mid expiratory flow 11 % higher in FE_{NO} group. Increased ICS doses (200 microg/day) in FE_{NO} group.
Petsky [31]	Aged >4 years 81 children invited 63	≥ or less than 10 ppb for	12 month study, monthly visits for	Exacerbation – FE_{NO} associated	Quality of life and spirometry did not significantly differ between

	randomised. Attending hospital clinic.	non atopic children ≥ or less than 12 ppb with one positive skin test ≥ or less than 20 ppb with more than one positive skin test	four months and alternate months thereafter.	with reduced exacerbations (19% versus 47%)	groups
Pijnenberg[30]	Aged 5-18 years. 108 screened 89 randomised. Attending hospital clinic. Atopic asthma treated with ICS.	Less than or ≥30ppb	12 month study with assessments each 3 months	ICS dose. No difference between groups.	FE_{NO} group had improved PD_{20} (1.3 doubling doses), lower FE_{NO} (geometric mean difference at end of study 32% lower) and trend for fewer exacerbations (20% versus 39%)
Pike[28]	Aged 6-17 years. 96 screened, 90 randomised. Attending hospital clinic with moderate-severe asthma.	≤15ppb 15.1-24.9ppb ≥25 ppb	12 month study, assessed each 2 months	ICS dose and exacerbation. No difference between groups.	Spirometry, no difference between groups.
Szeffler[26]	Aged 12-20 years. 780 screened. 546 randomised. Inner city area where ≥20% households below poverty level.	0-20 20.1-30 30.1-40 >40	46 week duration assessments each 6-8 weeks	Number of days with symptoms. No difference between FE_{NO} and control groups	FE_{NO} group had: Mean increased fluticasone treatment 119 microg/day. 10% reduction in proportion requiring OCS Among obese children 0.6 fewer days with symptoms. For those with multiple positive skin tests (ie >9 out of 14 tested) 0.8 fewer days

					with symptoms.
Verini[29]	Aged 6-17 years. 64 children. Referred to hospital and admitted.	12	12 month study with assessments at baseline and after 6 and 12 months	Severity score (mean reduced significantly from 1.1 to 0.6 and 0.8 after 6 and 12 months only in the FE_{NO} group). Exacerbation (mean number reduced from 2.0 to 1.0 and 0.8 only in FE_{NO} group), treatment (unchanged in FE_{NO} group but some evidence of increased treatment in control arm).	Spirometry – no difference

Table 4

Table 4. Factors which are associated with changes in FE_{NO} in children independent of asthma

Factor	Approximate magnitude of effect
Height	Up to 1ppb rise per cm height gained [24]
Dietary exposures	Short lived rise of up to 5-10ppb [53,54]
Allergen exposure	Rise of up to 50% during birch pollen season [56]
Exposure to second hand smoke	Reduction of 100% (26ppb for exposed children versus 56ppb) [57] or absolute reduction of 10ppb [58]
Exposure to poor outdoor air quality	Rise of approximately 1ppb 4 hours after each increase of 10mg/m^3 fine particulate exposure ($PM_{2.5}$) [59]
Genetic variations	Variations in genes coding for NOS2 and NOS3 may lead to differences in FE_{NO} in adults of 10% [86] or 10ppb [87] but no association found for NOS1 variant and FE_{NO} in children [88]

Exhaled nitric oxide and the management of childhood asthma – yet another promising biomarker "has been" or a misunderstood gem

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Key words: Asthma, Child, Nitric oxide, Respiratory Symptoms

Conflicts of interest: Dr Turner has completed three studies where consumables were provided by Aerocrine.

ABSTRACT

Childhood asthma is a common chronic condition. Approximately five percent of all children in western countries are prescribed treatment with inhaled corticosteroids (ICS) to prevent asthma symptoms. Current guidelines advocate titrating ICS dose to symptoms but this approach is not without problem, e.g. how to discern asthmatic from non-asthmatic symptoms? And when to reduce ICS dose? This review describes the strengths and weaknesses of fractional exhaled nitric oxide (FE_{NO}) as an objective index for individualising asthma control in children. Epidemiological and mechanistic evidence suggest that FE_{NO} should be a promising biomarker for eosinophilic airway inflammation (a hall mark for asthma) but somewhat surprisingly, clinical trials in children have not consistently found benefit from adding FE_{NO} to a symptom-based approach to ICS treatment in children. There are a number of reasons why FE_{NO} has apparently failed to translate from promising biomarker to clinically useful tool, and one reason may be a lack of understanding of what merits a significant intrasubject change in FE_{NO}. This review describes the rise and apparent fall of FE_{NO} as biomarker for asthma and then focuses on more recent evidence which suggest that FE_{NO} may prove to have a role in the management of childhood asthma, and in particular preventing exacerbations.

Keywords: Asthma, Control, Child, Exhaled Nitric Oxide, Randomised Clinical Trial

EDUCATIONAL AIMS

- To summarise the literature from observational studies which support the role of fractional exhaled nitric oxide (FE_{NO}) as a biomarker for asthma control.
- To summarise the results from clinical trials which have used FE_{NO} to guide asthma treatment.
- To explore why there was an apparent failure to translate FE_{NO} from bench to bedside.
- To explore how FE_{NO} might be used in the future management of childhood asthma

1. A HISTORICAL BACKDROP TO ASTHMA AND NITRIC OXIDE

1.1 The search for an asthma control biomarker. Childhood asthma is a very common condition world wide[1] and approximately five percent of all children in western countries are prescribed inhaled corticosteroids (ICS) to prevent asthma symptoms[2]. Asthma remains a challenging condition to diagnose and manage in children (and adults) since there is no definition, diagnostic test or biomarker to objectively monitor disease control. Historically, several biomarkers have been evaluated as potential biomarkers for asthma control including peak flow, spirometry, bronchial hyperresponsiveness and eosinophil cationic protein but these tests all lack sufficient sensitivity and specificity. This review will focus on the potential for fractional exhaled nitric oxide (FE_{NO}) to be a biomarker for childhood asthma. This review will not explore the potential utility of FE_{NO} for diagnosing asthma which has been reviewed elsewhere[3,4]. A as a simple rule low FE_{NO} (<10ppb) can be considered a good screen to exclude allergic asthma in children aged ≥ five years and concentrations of ≥19ppb might have positive predictive value[4] but the interpretation of higher FE_{NO} remains challenging and this is predominantly due to confounding by atopy which leads to elevated FE_{NO} independent of asthma.

There is a pressing need for a biomarker for asthma management in children[5] due to a number of clinically important questions to which there are currently no answers (table 1). Currently the management of asthma is driven by symptoms and at times can be based on trial and error. One example of clinical uncertainty is the case of a child with asthma symptoms despite treatment with inhaled steroids – does the clinician increase

ICS dose or add in long acting beta agonist or leukotriene receptor antagonist? Children with asthma also get non-asthmatic respiratory symptoms[6] so how does the clinician deduce whether respiratory symptoms in a child with asthma are asthmatic or not? Third and fourth clinical scenarios are the decision-making behind stepping down or stopping ICS treatment in a child with no asthma symptoms on ICS treatment? Exhaled NO has the potential to give insight into these everyday clinical dilemmas.

1.2. Exhaled nitric oxide and asthma control, a brief summary of the evidence. Until the late 1980s, nitric oxide was thought to be just a pollutant generated from burning fossil fuels, but was subsequently found to be important to cellular function in many human organs and in 1992 was voted molecule of the year by Science magazine. Nitric oxide, a simple diatomic molecule, proved to be important in cellular communication and was the substance previously known as endothelial derived relaxing factor, a potent vasodilator. Nitric oxide is produced by two enzymes. Constitutive nitric oxide synthase (NOS) constantly produces NO at relatively low concentration and this activity is thought to be important to health and wellbeing; at low concentrations NO's properties in the respiratory system may include antimicrobial, immune regulation and possibly bronchodilation. The second enzymatic source of NO is inducible NOS which, on stimulation, can produce higher concentrations of NO compared to constitutive NOS which are associated with disease [7-9]. In the airways, higher concentrations of NO have no homeostatic role and are thought to be secondary to eosinophil inflammation[10]. The presence of gaseous nitric oxide in human exhaled breath was first reported in 1993[11] and shortly afterwards was found to be elevated in adults with asthma[12]; this observation was

replicated in children four years later [13]. A flurry of scientific activity relating exhaled nitric oxide to asthma was published during the early 2000s and this indicated both the potential [14,15] and the limitations [16] of using NO in exhaled breath as a biomarker for asthma (table 2).

With the epidemiology and cellular/molecular work pointing to FE_{NO} being a potential biomarker for asthma control in children and a standard methodology agreed, a number of studies explored where FE_{NO} might be used in asthma management. One study demonstrated how rising exhaled nitric oxide concentration (using a threshold concentration of >22ppb) and rising airway eosinophilia (using % eosinophil count as a continuous variable) were independently predictive of failure to step down inhaled corticosteroids in children with stable asthma [17]. A second study measured FE_{NO} four weeks after cessation of ICS treatment and found that concentrations in excess of 49ppb had the best sensitivity (71%) and specificity (93%) for subsequent asthma relapse [18]. By 2005 clinical trials were under way where FE_{NO} was applied to asthma management as an adjuvant to the standard symptom-based approach advocated by consensus guidelines.

1.3. A standard methodology for measuring NO in exhaled breath. This was agreed by the American Thoracic and European Respiratory Societies and published in 1999[19] and revised in 2005[20]. One of the challenges in measuring NO in exhaled breath is flow dependence, i.e. at higher expiratory flows, concentrations are reduced and *vice versa* . The flow dependence of exhaled NO does give insight into the origin of elevated NO in an individual (broadly from the proximal or distal airways) by deriving flow independent

parameters. Descriptions of derivation of flow independent parameters and their potential clinical relevance in children are available elsewhere [21,22]. The agreed standard was to measure the fractional exhaled nitric oxide at 50 ml/s. Using this methodology, a child without asthma would typically have FE_{NO} of 8-10 parts per billion (ppb) [23] but concentrations might be up to 25 ppb [24]. Not only was there evidence to support the paradigm that FE_{NO} was a biomarker for asthma control from epidemiological, observational and mechanistic studies, FE_{NO} measurements could be made quickly, with minimal discomfort, good reproducibility [25] and results were available within minutes.

2. EXHALED NO AS A BIOMARKER FOR ASTHMA MANAGEMENT IN CHILDREN

2.1. Results from clinical trials. At the time of writing there have been at least eight trials published which explored the clinical utility of FE_{NO} in the management of asthma in children [26-33]. These randomised clinical trials compared standard symptom-based management against standard management plus FE_{NO} (rather than symptom based versus FE_{NO} based management) and each study used absolute FE_{NO} values to guide changes in treatment (rather than relative or personalised FE_{NO} values). The clinical trials were undertaken by groups working independently and inevitably there is considerable heterogeneity between designs of the trials (table 3). The lower age limit for inclusion varied between 5 and 12 years, one recruited from the community[26] whilst the remainder recruited from hospital clinics [27-33]and some only included atopic children with asthma[27,30,32,33]. The absolute FE_{NO} values used as cut offs ranged between 10 and 40ppb,

some trials had only one cut off FE_{NO} value [27,29,30,32,33], whilst others had three or four FE_{NO} values to trigger escalation in asthma treatment [26,28] and one employed different single cut offs for an individual based on their atopic status[31]. One study also included FEV_1 in the decision making algorithm in addition to FE_{NO} [27]. The primary outcome for the studies, upon which the power calculations were based, were varied and included ICS dose[28-30] FEV_1[27], exacerbations[28,29,31], severity[29] and symptomatic [32,33]. None of the studies observed improved asthma control among the FE_{NO} arms, three found reduced exacerbations [26,29,31,33], two found improved physiological measurements (i.e. spirometry [27]and bronchial hyperresponsiveness [30]), two found increased doses of ICS among those randomised to FE_{NO} guided treatment [26,27] and one found reduced asthma severity over the course of the trial[29]. One very recent study, published only in abstract form at the time of writing [34] reported symptoms free days in 280 children aged 4-18 years randomised to (i) symptom driven treatment (ii) web-based monthly monitoring and (iii) symptom based treatment plus 4 monthly FE_{NO} measurement; here symptom free days increased marginally the FE_{NO} arm. Systematic reviews and meta-analyses using data from some of these studies have concluded that the evidence does not support the addition of FE_{NO} to standard symptom-based management of asthma for day-to-day control [35-37] but one finds evidence for FE_{NO} leading to reduced exacerbations[37]. In contrast, at least one expert group argues that FE_{NO} has an important role in the management of asthma[38]. Between evidence synthesis[35-37] and expert opinion[38], a recent report from the National Institute for Clinical Efficacy in the UK [39] has suggested that "it could be argued that the available evidence does point towards some benefit to the technology [FE_{NO} measurement]" and cites limitations in the current literature as

including “cut off values [which] are highly variable and largely based on derivation studies” and “unclear step-up/step-down protocols”.

2.2 Meta-analysis. Although this is not a systematic review, the eight papers identified in section 2.1 are likely to represent most papers published in this area and meta-analysis was undertaken using standard software was used (Review manager 5.2). The outcomes were (i) *risk for an individual requiring at least once course of oral corticosteroids*. Details of individuals requiring ≥1 course of OCS were provided by the author of one study [28] and was not available for a second [29]. Meta-analysis of seven studies demonstrated that risk for an individual having an exacerbation requiring OCS was reduced by treatment guided by FE_{NO} plus symptoms versus symptoms alone, odds ratio 0.67 [95% CI 0.51, 0.88] (figure 1). One study[26] contributed almost two thirds of data for this analysis and substantially influences the overall result from the meta analysis.

(ii) *risk for an individual having any exacerbation (however defined in the study design).* The risk for an individual having ≥1 exacerbation of any type could not be determined two studies (one reported total number of exacerbations[27] and a second did not report exacerbations[29]); treatment with FE_{NO} plus symptoms was associated with an identical reduction in risk compared to symptoms only as in (i) above (OR 0.67 [95% CI 0.51, 0.88].

(iii) *ICS dose at the end of the study*. Analysis for ICS dose at end of study was complicated by data being presented as median and interquartile range whereas the software (widely regarded as the gold standard) requires mean and standard deviation values. Data were transformed to mean and standard deviation [40] assuming that 25th and 75th centile values were low and high end of the range; these assumptions can be easily

challenged and should be considered when interpreting the results from this meta-analysis. Data were not available for three studies of which two[29, 30] reported (in the text) no increase in dose and one[26] which reported higher dose ICS (mean difference 119 microg budesonide equivalent [95% CI 49, 189]) associated with treatment guided by FE_{NO}. Among the remaining 5 studies there was an overall mean increase in ICS dose of 106 microg BUD equivalent [95% CI 75, 138], figure 2. The magnitude of this association is consistent with the one large study which dominated the meta analysis[26] and FE_{NO} guided treatment seems to be associated with an increased in ICS dose of approximately 100 microg BUD equivalent. In addition to the assumptions about mean and SD values (which resulted in an apparent dose reduction for the FENO arm of the study by de Jongste et al [32]where median values in the two arms were equal at 200 microg), there is an additional caveat to these results; the results are heterogeneous and when adjusted for (using random effects) the mean increase in ICS is 88 microg BUD equivalent [95% CI -10, 86].

3. **WHY MIGHT EXHALED NO NOT BE A USEFUL BIOMARKER?**

3.1 Exhaled NO is poorly specific for asthma. Elevated NO is a biomarker for eosinophilic inflammation rather than for asthma *per se* and this indirect relationship with asthma may explain why some studies find FE_{NO} is an index of asthma control scores[41-43], FEV_1[44] and bronchial hyper responsiveness (BHR) [45] [46], but FE_{NO} is not universally associated with control[47], FEV_1[45] or BHR[48]. There is the possibility that FE_{NO} is a more accurate index of asthma control for some individuals, eg those with atopy, or for individuals where there is discordance between symptoms and FEV_1. Eosinophilic inflammation may be asymptomatic and this most likely explains the relationship

between FE_{NO} and atopy and bronchial hyperreactivity in children without asthma [45,49,46,50]. It has been proposed that FE_{NO} is merely an index of atopy, i.e. a skin prick test, since concentrations are positively correlated with the number of skin tests [45] and age at onset of atopy [51] but this is probably over simplistic since FE_{NO} does change acutely after exposure to oral corticosteroid treatment[52], certain foods[53,54], exercise [55] and pollen[56]. What has been recognised is that factors other than asthma may acutely and chronically influence NO production in children (table 4, figure 3). Male gender and increasing height are consistently associated with modest increase in FE_{NO} concentrations and, although children are not likely to grow by more than a few cm between clinic visits, the association with anthropometric measurements challenges the logic behind having single FE_{NO} values to trigger changes in ICS throughout childhood; a teenager will grow by as much as 30cm during puberty and their FE_{NO} value will rise by approximately 5-10 ppb. As an aside, the association between height and increased FE_{NO} is an interesting observation since a measurement of concentration should adjust for size so this is not simply bigger people producing more NO. Dietary exposures have been associated with acute changes in FE_{NO} in children [53,54] but these changes are short-lived and of a small magnitude. Nitric oxide is derived from the amino acid L-arginine and ingestion of a dose of L-arginine equivalent to two chicken breasts is associated with a 5 ppb rise in FE_{NO} which lasts one hour[54]. Caffeine induces nitric oxide synthase and ingestion of a large drink of cola leads to a 9ppb increase in FE_{NO} after 30 minutes which resolves after one hour. [53] Inhaled exposures such as second hand tobacco smoke [57 58] and poor outdoor air quality[59] are associated with increased FE_{NO} but it is not known how long these changes last for. Respiratory infection with virus temporarily affects FE_{NO} values but the nature

of this association is not clear; FE_{NO} values are reduced in infants with respiratory syncitial virus[60] or rhinitis [61] but in adults with experimentally induced rhinovirus infection, FE_{NO} rises by approximately 5ppb [62]. There is little direct evidence of the effect of viral infection in children; indirect evidence comes from observations made during exacerbations, precipitated by rhinovirus, which are associated with elevated FE_{NO}[52,63]. The apparently inconsistent findings between virus infection and changing FE_{NO} might reflect differences in the host response to different virus which may be age related and also the retention of NO within secretions. Further evidence of almost continuous but small fluctuations in FE_{NO} is evidenced by the diurnal variability in concentrations[64]; concentrations are less than 1 ppb higher in the morning compared to the afternoon. In addition to variability over minutes and hours, FE_{NO} is elevated in children with asthma during periods when grass pollen exposure is present [41,56] and also is elevated during the autumn (when moulds cast spores) for those exposed to indoor moulds [43]. Children with hayfever have elevated FE_{NO}[65] and concentrations become particularly elevated during the spring when compared to those without hayfever [43]. In addition to the factors described in table 4 and figure 3, intrasubject variability in FE_{NO} measurements may also be introduced by the apparatus itself. As with all analytical processes, there is variability in repeated measurements using the same apparatus and this variability can be reduced by measuring two or three FE_{NO} values and reporting the mean value [20] but this requires time and also costs money. Further apparatus-dependent variability arises when different methods to derive NO are used; one study found an intrasubject difference of 4ppb between devices made by the same manufacturer[66]. Intrasubject variability becomes considerably greater when apparatus from different manufacturers are used[67] where a

typical difference might be 8ppb but range between -12 and +28ppb. At present it seems sensible to make repeated measurements for a given individual using the same apparatus.

3.2 Trials were confounded by poor adherence with inhaled corticosteroid treatment. Adherence to ICS treatment is crucial to the interpretation of elevated FE_{NO}, as it currently is for standard symptom-based asthma management. Elevated FE_{NO} is associated with poor asthma control [41-43] and poor adherence with ICS treatment [26,68], whereas increasing ICS treatment leads to reduced FE_{NO} [68]. Adherence to treatment is always a challenge to measure in asthma, one paper found that typical FE_{NO} concentrations for adolescents with adherence was >50% was 24 ppb and was 31ppb for those with <50% compliance [26]. A second study of 17 children found that compliance with ICS of between 75 and 100% was associated with a relative reduction in FE_{NO} of 50-100% whereas compliance below 75% was associated with changes in FE_{NO} of less that 50% [68]. Observations of heterogeneity in FE_{NO} response to ICS [69,70] might reflect the presence of individuals with high FE_{NO} but little airway eosinophilia, a phenomenon seen in adults[71] but not described in children, or heterogeneity in adherence to ICS treatment. Although there is most likely to be incomplete adherence to ICS in the clinical trials, asthma outcomes improved in both FE_{NO} and standard arms of most trials suggesting that adherence was generally good.

3.3 Wrong study design. The clinical trials which have been completed in children to date all compared standard symptom-based treatment versus standard treatment plus FE_{NO} and perhaps trials should compare symptom-based treatment versus FE_{NO} only treatment. This bold study design has only been used in one trial of adult patients[72] and

found that FE_{NO} guided treatment was associated with reduced ICS doses and a non-significant trend for reduced symptoms compared to symptom based management. The poor correlation between asthma control and FE_{NO} reported in some studies[41-43] and the lack of correlation in at least one study[47] does question whether asthma treatment can be guided only by FE_{NO}. On the one hand, FE_{NO} and symptoms measure different outcomes and therefore an algorithm which captures both outcomes might be better than either alone. A more conservative approach might argue that there is a too much of a leap of faith involved in using FE_{NO} to guide treatment, and the symptom-based approach is patient-centred and therefore symptoms should predominate as the ultimate trigger for changing asthma treatment.

3.4 Insufficient power. Although studies justified their sample size by a power calculation, descriptions of the power calculations do not include a mean or median FE_{NO} value and associated variability. Pragmatically, only two published studies randomised more than 100 children[26 32] so it is possible that the remaining studies may have been underpowered.

3.5 Wrong cut offs used. Although increased FE_{NO} is associated with adverse asthma outcomes in children, the definition of what is "increased" remains unclear. . Evidence from population studies suggests that concentrations of >35ppb in children are "high" [38] but the question "what is a significant change in FE_{NO} for an individual?" remains poorly understood and has been explored in detail elsewhere [73]. One early study suggested that a change of 4 ppb might be clinically significant[74] but, as table 4 demonstrates, there are many factors other than asthma which can acutely change FE_{NO} by an order of at least 4ppb. Furthermore, a rise of 4ppb might be important in a child whose previous FE_{NO}

was 10ppb but not for a second individual whose FE_{NO} was 20ppb and relative change in FE_{NO} seems a more meaningful method for interpreting repeated measurements. Recent studies in adults have suggested that a relative change of <30% is unlikely to be clinically relevant [75] and a change from poor control to good control was associated with a FE_{NO} reduction of greater than 35% [76]. Having a "significant" magnitude of change in FE_{NO} of 30-35% would be consistent with a clinically meaningful change in bronchial hyperreactivity (a hallmark for asthma and correlated with FE_{NO}) of half a doubling dose [77]. In children, a FE_{NO} rise of 60% from baseline (with 95% confidence intervals of approximately 25, 140) was associated with an exacerbation [63] and by extrapolation, a rise in FE_{NO} of less than 60% might be indicative of increasing symptoms. A clinical practical guideline published by the American Thoracic Society in 2011 [38] acknowledged a weak evidence base and cautiously recommended that a rise in FE_{NO} of >20% or (in children) >20ppb may be significant and that a minimally important reduction in FE_{NO} was >20% for those with a FE_{NO} of ≥50ppb and <10ppb for those for those with lower values. In the adult literature there has been interest in expressing FE_{NO} as a percentage of predicted but this option is losing favour, mostly due to lack of precision and to differences between reference populations raising the question of which reference is the best for a given population? A fourth method to express FE_{NO} is a as percentage of lowest value and is measured after a two week course of oral corticosteroids, but this has an associated morbidity, might yield a low FE_{NO} value which cannot be achieved with ICS treatment and should be reserved for use only in special cases under expert supervision. Of the four methods described, percentage difference seems best suited for

individualising treatment since this recognises the relatively wide range of values within a population of children.

3.6 Insight into intrasubject variability. One recent study has given insight into the question "what is a significant change in FE_{NO}?" [43]. 178 children were recruited, of whom 47 had asthma, in a community-based observational study where FE_{NO} was measured over six two-month intervals. The difference between paired FE_{NO} measurements was expressed as an absolute value and limits of agreement. As might be expected, the limits of agreement for paired FE_{NO} measurements were greater for those with higher initial concentrations. Average FE_{NO} values were stable over eight months but did become significantly higher over a ten month interval, presumably due to the children becoming taller. Asthma was associated with *elevated* FE_{NO} in this population (27ppb versus 10 ppb for non-asthmatic) but when both time and baseline FE_{NO} value were considered, asthma was not independently associated with *change* in FE_{NO} value. As a rough rule of thumb, the authors suggested that FE_{NO} values may rise by up to 200% of the previous measurements over two to four months, independently of asthma. For example, in the 40 children with initial FE_{NO} between 11 and 20 ppb (median value 14ppb) the upper limits of agreement for measurements taken at a two and four month interval were +22ppb and +14 ppb respectively. As might be expected over time (and regression to the mean), low initial FE_{NO} concentrations became higher whilst higher concentrations became lower; thus the lower limits of agreement over two and four months for children whose initial FE_{NO} was 21-30 ppb were -19 and -25ppb. In keeping with the suggestion that a more permissive approach to interpretation of FE_{NO} values, a more liberal algorithm which allowed FE_{NO} concentrations to rise by up to 100% (from

16 to 29ppb) was found to be effective in reducing exacerbations and improving quality of life among pregnant women [78].

In addition to describing variability in FE_{NO} over time, this study related FE_{NO} to asthma control (both present and future) and also to environmental exposures which might affect FE_{NO} values [43]. There was weak correlation between FE_{NO} and current and future asthma control measured over a four month interval (correlation coefficient approximately 0.2). Compared with maintained good asthma control over two months, children who were poorly controlled but became well controlled had elevated FE_{NO}; in contrast, neither those who had good asthma control which became poorly controlled nor those whose asthma control remained poor had elevated FE_{NO}. These observations suggested that elevated FE_{NO} is an index of poor current control but not poor control in two month's time. Additionally the findings suggested that the mechanism for persistently poorly controlled symptoms in children with asthma may not involve eosinophilic airway inflammation.

Future research directions - so where do we go beyond 2014 with FE_{NO}?

It is too early to consign FE_{NO} to the dust bin where failed biomarkers for asthma are placed. There is still sufficient evidence to indicate that FE_{NO} may have a role in helping to address the current situation where there are too many children treated with inappropriately high doses of inhaled corticosteroids and conversely, too many children with poorly controlled asthma whose quality of life can be improved with ICS treatment. The inconsistency between the epidemiology and mechanistic studies (supportive of a role for FE_{NO} in asthma management) and the clinical trials to date (which are generally

not supportive of adding FE_{NO} to standard symptom-based management) suggests either FE_{NO} lacks precision or we have not properly understood how to interpret FE_{NO} as a clinical tool. Time will show whether FE_{NO} does have role or not in the management of childhood asthma. If FE_{NO} does prove to have a role in the management of childhood asthma then clinicians will have to place trust in FE_{NO} since guidelines will have to use FE_{NO} to step treatment down as well as up. Now that insight is being gained into what merits a significant change in FE_{NO}, clinical trials are needed which test cut offs to treatment algorithms. Future clinical trials designed to use FE_{NO} to improve asthma outcomes might consider the following:

1. Comparing symptom based management and FE_{NO} only based management. This might follow in the success of trials comparing symptoms versus FE_{NO} plus symptoms; the apparent failure of previous studies will understandably make clinicians very cautious in using only FE_{NO} to guide treatment.
2. Careful attention to treatment adherence. This needs to be integral to clinical trials since poor adherence has great potential to mask any true clinical benefit but in the long term, FE_{NO} may prove to give the clinician insight into adherence.
3. What is the "best" outcome. At present, the evidence would suggest that FE_{NO} may have a greater influence in reducing exacerbations rather than improving day-to-day control of symptoms. It is possible that one algorithm may lead to better control and another to fewer exacerbations for a given individual. On a practical note, having symptom control as an outcome and part of the algorithm is a potential flaw in study design.

4. Absolute versus relative FE_{NO} values. There is sufficient evidence to categorise individuals as having high FE_{NO} on study entry but more work is required in establishing whether cut offs for second and subsequent FE_{NO} values should be absolute or percent of previous values.
5. Algorithms could use FE_{NO} to guide treatment step up options for individuals with uncontrolled asthma despite compliance with ICS treatment, i.e. to further increase ICS or use alternative "add ons", as has been applied in adults[78].
6. Algorithms could use FE_{NO} to step down ICS treatment, even when (non-asthmatic) symptoms are present.
7. Clinical setting. Childhood asthma is a condition which is mostly managed in the community and trial design should ideally reflect this and aspire to an ideal of easily delivered personalised treatment algorithms
8. Preschool children. Methodologies are required to allow FE_{NO} to be measured in younger children – currently FE_{NO} can be measured in children aged 5-6 years

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FIGURE LEGENDS

Figure 1. Summary of the asthma-dependent and independent factors associated with increased or reduced concentrations of exhaled nitric oxide (FE_{NO}).

Figure 2. A forest plot comparing the effect on exacerbations requiring oral corticosteroid treatment where maintenance treatment is driven by exhaled nitric oxide (ENO) and symptoms versus symptoms alone.

Figure 3. A forest plot comparing the effect on inhaled corticosteroid dose at the time of study exit where maintenance treatment is driven by exhaled nitric oxide (ENO) and symptoms versus symptoms alone.

Exhaled nitric oxide and the management of childhood asthma – yet another promising biomarker "has been" or a misunderstood gem

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Key words: Asthma, Child, Nitric oxide, Respiratory Symptoms

Conflicts of interest: Dr Turner has completed three studies where consumables were provided by Aerocrine.

ABSTRACT

Childhood asthma is a common chronic condition. Approximately five percent of all children in western countries are prescribed treatment with inhaled corticosteroids (ICS) to prevent asthma symptoms. Current guidelines advocate titrating ICS dose to symptoms but this approach is not without problem, e.g. how to discern asthmatic from non-asthmatic symptoms? And when to reduce ICS dose? This review describes the strengths and weaknesses of fractional exhaled nitric oxide (FE_{NO}) as an objective index for individualising asthma control in children. Epidemiological and mechanistic evidence suggest that FE_{NO} should be a promising biomarker for eosinophilic airway inflammation (a hall mark for asthma) but somewhat surprisingly, clinical trials in children have not consistently found benefit from adding FE_{NO} to a symptom-based approach to ICS treatment in children. There are a number of reasons why FE_{NO} has apparently failed to translate from promising biomarker to clinically useful tool, and one reason may be a lack of understanding of what merits a significant intrasubject change in FE_{NO}. This review describes the rise and apparent fall of FE_{NO} as biomarker for asthma and then focuses on more recent evidence which suggest that FE_{NO} may prove to have a role in the management of childhood asthma and in particular preventing exacerbations.

Keywords: Asthma, Control, Child, Exhaled Nitric Oxide, Randomised Clinical Trial

EDUCATIONAL AIMS

- To summarise the literature from observational studies which support the role of fractional exhaled nitric oxide (FE_{NO}) as a biomarker for asthma control.
- To summarise the results from clinical trials which have used FE_{NO} to guide asthma treatment.
- To explore why there was an apparent failure to translate FE_{NO} from bench to bedside.
- To explore how FE_{NO} might be used in the future management of childhood asthma

1. A HISTORICAL BACKDROP TO ASTHMA AND NITRIC OXIDE

1.1 The search for an asthma control biomarker. Childhood asthma is a very common condition world wide[1] and approximately five percent of all children in western countries are prescribed inhaled corticosteroids (ICS) to prevent asthma symptoms[2]. Asthma remains a challenging condition to diagnose and manage in children (and adults) since there is no definition, diagnostic test or biomarker to objectively monitor disease control. Historically, several biomarkers have been evaluated as potential biomarkers for asthma control including peak flow, spirometry, bronchial hyperresponsiveness and eosinophil cationic protein but these tests all lack sufficient sensitivity and specificity. This review will focus on the potential for fractional exhaled nitric oxide (FE_{NO}) to be a biomarker for childhood asthma. This review will not explore the potential utility of FE_{NO} for diagnosing asthma which has been reviewed elsewhere[3,4]. A as a simple rule low FE_{NO} (<10ppb) can be considered a good screen to exclude allergic asthma in children aged ≥ five years and concentrations of ≥19ppb might have positive predictive value[4] but the interpretation of higher FE_{NO} remains challenging and this is predominantly due to confounding by atopy which leads to elevated FE_{NO} independent of asthma.

There is a pressing need for a biomarker for asthma management in children[5] due to a number of clinically important questions to which there are currently no answers (table 1). Currently the management of asthma is driven by symptoms and at times can be based on trial and error. One example of clinical uncertainty is the case of a child with asthma symptoms despite treatment with inhaled steroids – does the clinician increase

ICS dose or add in long acting beta agonist or leukotriene receptor antagonist? Children with asthma also get non-asthmatic respiratory symptoms[6] so how does the clinician deduce whether respiratory symptoms in a child with asthma are asthmatic or not? Third and fourth clinical scenarios are the decision-making behind stepping down or stopping ICS treatment in a child with no asthma symptoms on ICS treatment? Exhaled NO has the potential to give insight into these everyday clinical dilemmas.

1.2. Exhaled nitric oxide and asthma control, a brief summary of the evidence. Until the late 1980s, nitric oxide was thought to be just a pollutant generated from burning fossil fuels, but was subsequently found to be important to cellular function in many human organs and in 1992 was voted molecule of the year by Science magazine. Nitric oxide, a simple diatomic molecule, proved to be important in cellular communication and was the substance previously known as endothelial derived relaxing factor, a potent vasodilator. Nitric oxide is produced by two enzymes. Constitutive nitric oxide synthase (NOS) constantly produces NO at relatively low concentration and this activity is thought to be important to health and well being; at low concentrations NO's properties in the respiratory system may include antimicrobial, immune regulation and possibly bronchodilation. The second enzymatic source of NO is inducible NOS which, on stimulation, can produce higher concentrations of NO compared to constitutive NOS which are associated with disease [7-9]. In the airways, higher concentrations of NO have no homeostatic role and are thought to be secondary to eosinophil inflammation[10]. The presence of gaseous nitric oxide in human exhaled breath was first reported in 1993[11] and shortly afterwards was found to be elevated in adults with asthma[12]; this observation was

replicated in children four years later [13]. A flurry of scientific activity relating exhaled nitric oxide to asthma was published during the early 2000s and this indicated both the potential [14,15] and the limitations [16] of using NO in exhaled breath as a biomarker for asthma (table 2).

With the epidemiology and cellular/molecular work pointing to FE_{NO} being a potential biomarker for asthma control in children and a standard methodology agreed, a number of studies explored where FE_{NO} might be used in asthma management. One study demonstrated how rising exhaled nitric oxide concentration (using a threshold concentration of >22ppb) and rising airway eosinophilia (using % eosinophil count as a continuous variable) were independently predictive of failure to step down inhaled corticosteroids in children with stable asthma [17]. A second study measured FE_{NO} four weeks after cessation of ICS treatment and found that concentrations in excess of 49ppb had the best sensitivity (71%) and specificity (93%) for subsequent asthma relapse [18]. By 2005 clinical trials were under way where FE_{NO} was applied to asthma management as an adjuvant to the standard symptom-based approach advocated by consensus guidelines.

1.3. A standard methodology for measuring NO in exhaled breath. This was agreed by the American Thoracic and European Respiratory Societies and published in 1999[19] and revised in 2005[20]. One of the challenges in measuring NO in exhaled breath is flow dependence, i.e. at higher expiratory flows, concentrations are reduced and *vice versa* ~~(figure 1).~~. The flow dependence of exhaled NO does give insight into the origin of elevated NO in an individual (broadly from the proximal or distal airways) by deriving

flow independent parameters. Descriptions of derivation of flow independent parameters and their potential clinical relevance in children are available elsewhere [21,22]. The agreed standard was to measure the fractional exhaled nitric oxide at 50 ml/s. Using this methodology, a child without asthma would typically have FE_{NO} of 8-10 parts per billion (ppb) [23] but concentrations might be up to 25 ppb [24]. Not only was there evidence to support the paradigm that FE_{NO} was a biomarker for asthma control from epidemiological, observational and mechanistic studies, FE_{NO} measurements could be made quickly, with minimal discomfort, good reproducibility [25] and results were available within minutes.

2. EXHALED NO AS A BIOMARKER FOR ASTHMA MANAGEMENT IN CHILDREN

2.1. Results from clinical trials. At the time of writing there have been at least eight~~six~~ trials published which explored the clinical utility of FE_{NO} in the management of asthma in children [26-33]. These randomised clinical trials compared standard symptom-based management against standard management plus FE_{NO} (rather than symptom based versus FE_{NO} based management) and each study used absolute FE_{NO} values to guide changes in treatment (rather than relative or personalised FE_{NO} values). The clinical trials were undertaken by groups working independently and inevitably there is considerable heterogeneity between designs of the trials (table 3). The lower age limit for inclusion varied between 5 and 12 years, one recruited from the community[26] whilst the remainder recruited from hospital clinics [27-33]and some only included atopic children with

asthma[27,30,32,33]. The absolute FE_{NO} values used as cut offs ranged between 10 and 40ppb, some trials had only one cut off FE_{NO} value [27,29,30,32,33], whilst others had three or four FE_{NO} values to trigger escalation in asthma treatment [26,28] and one employed different single cut offs for an individual based on their atopic status[31]. One study also included FEV_1 in the decision making algorithm in addition to FE_{NO} [27]. The primary outcome for the studies, upon which the power calculations were based, were varied and included ICS dose[28-30] FEV_1[27], exacerbations[28,29,31], severity[29] and symptomatic [32,33]. None of the studies observed improved asthma control among the FE_{NO} arms, three found reduced exacerbations [26,29,31,33], two found improved physiological measurements (i.e. spirometry [27]and bronchial hyperresponsiveness [30]), ~~and~~ two found increased doses of ICS among those randomised to FE_{NO} guided treatment [26,27] and one found reduced asthma severity over the course of the trial[29]. One very recent study, published only in abstract form at the time of writing [34] reported symptoms free days in 280 children aged 4-18 years randomised to (i) symptom driven treatment (ii) web-based monthly monitoring and (iii) symptom based treatment plus 4 monthly FE_{NO} measurement; here symptom free days increased marginally the FE_{NO} arm. Systematic reviews and meta-analyses using data from some of these studies have concluded that the evidence does not support the addition of FE_{NO} to standard symptom-based management of asthma for day-to-day control [35-37] but one finds evidence for FE_{NO} leading to reduced exacerbations [37]. In contrast, at least one expert group argues that FE_{NO} has an important role in the management of asthma[38]. Between evidence synthesis[35-37] and expert opinion[38], a recent report from the National Institute for Clinical Efficacy in the UK [39] has suggested that "it could be argued that the available evidence does point towards some benefit to the

technology [FE_{NO} measurement]" and cites limitations in the current literature as including "cut off values [which] are highly variable and largely based on derivation studies" and "unclear step-up/step-down protocols".

2.2 Meta analysis. Although this is not a systematic review, the eight papers identified in section 2.1 are likely to represent most papers published in this area and meta analysis was undertaken. Standard software was used (Review manager 5.2). The outcomes were (i) risk for an individual requiring at least once course of oral corticosteroids. Details of individuals requiring ≥1 course of OCS were provided by the author of one study [28] and was not available for a second [29]. Meta-analysis of these seven studies demonstrated that risk for an individual having an exacerbation was reduced by treatment guided by FE_{NO} plus symptoms versus symptoms alone, odds ratio 0.67 [95% CI 0.51, 0.88] (figure 1). One study[26] contributed almost two thirds of data for this analysis and therefore substantially influences the overall result from the meta analysis. Overall, there is a reduction in exacerbations requiring OCS treatment where asthma treatment is informed by both FE_{NO} and symptoms

(ii) risk for an individual having any exacerbation (however defined in the study design). The risk for an individual having ≥1 exacerbation of any type could not be determined two studies (one reported total number of exacerbations[27] and a second did not report exacerbations [29]); treatment with FE_{NO} plus symptoms was associated with an identical reduction in risk compared to symptoms only as for need for OCS (OR 0.67 [95% CI 0.51, 0.88].

(iii) ICS dose at the end of the study. Analysis for ICS dose at end of study was complicated by data being presented as median and interquartile range whereas the

software (widely regarded as the gold standard) requires mean and standard deviation values. Data were transformed to mean and standard deviation [40] assuming that 25th and 75th centile values were low and high end of the range; these assumptions can be easily challenged and should be considered when interpreting the results from this meta analysis. Data were not available for three studies of which two[29, 30] reported (in the text) no increase in dose and one[26] reported higher dose ICS (mean difference 119 microg budesonide equivalent [95% CI 49, 189]) associated with treatment guided by FE_{NO}. Among the remaining 5 studies there was an overall mean increase in ICS dose of 106 microg BUD equivalent [95% CI 75, 138], figure 2. The magnitude of this association is consistent with the one large study which dominated the meta analysis[26] and FE_{NO} guided treatment seems to be associated with an increased in ICS dose of approximately 100 microg BUD equivalent. In addition to the assumptions about mean and SD values (which resulted in an apparent dose reduction for the FENO arm of the study by de Jongste et al [32]when median values in the two arms were equal at 200 microg), there is an additional caveat to these results; the results are heterogeneous and when adjusted for (i.e. random effects) the mean increase in ICS is 88 microg BUD equivalent [95% CI -10, 86].

3. **WHY MIGHT EXHALED NO NOT BE A USEFUL BIOMARKER?**

3.1 Exhaled NO is poorly specific for asthma. Elevated NO is a biomarker for eosinophilic inflammation rather than for asthma *per se* and this indirect relationship with asthma may explain why some studies find FE_{NO} is an index of asthma control scores[41-43], FEV_1[44] and bronchial hyper responsiveness (BHR) [45] [46], FE_{NO} is not universally associated with control[47], FEV_1[45] or BHR[48]. There is also the possibility that FE_{NO} is a more accurate index of asthma control for some individuals, eg those with atopy, or for individuals where there is discordance between symptoms and FEV_1. Eosinophilic inflammation may be asymptomatic and this most likely explains the relationship between FE_{NO} and atopy and bronchial hyperreactivity in children without asthma [45,49,46,50]. It has been proposed that FE_{NO} is merely an index of atopy, ie a skin prick test, since concentrations are positively correlated with the number of skin tests [45] and age at onset of atopy [51] but this is probably over simplistic since FE_{NO} does change acutely after exposure to oral corticosteroid treatment[52], certain foods[53,54], exercise [55] and pollen[56]. What has been recognised is that factors other than asthma may acutely and chronically influence NO production in children (table 4, figure 1~~2~~). Male gender and increasing height are consistently associated with modest increase in FE_{NO} concentrations and, although children are not likely to grow by more than a few cm between clinic visits, the association with anthropometric measurements challenges the logic behind having single FE_{NO} values to trigger changes in ICS throughout ~~for~~ childhood~~children~~; a teenager will grow by as much as 30cm during puberty and their FE_{NO} value ~~before puberty~~ will rise by approximately 5-10 ppb~~be of little relevance post puberty~~. As an aside, the association between height and increased FE_{NO} is an interesting observation since a

measurement of concentration should adjust for size so this is not simply bigger people producing more NO. Dietary exposures have been associated with acute changes in FE_{NO} in children [53,54] but these changes are short-lived and of a small magnitude. Nitric oxide is derived from the amino acid L-arginine and ingestion of a dose of L-arginine equivalent to two chicken breasts is associated with a 5 ppb rise in FE_{NO} which lasts one hour[54]. Caffeine induces nitric oxide synthase and ingestion of a large drink of cola leads to a 9ppb increase in FE_{NO} after 30 minutes which resolves after one hour. [53] Inhaled exposures such as second hand tobacco smoke [57] [58] and poor outdoor air quality[59] are associated with increased FE_{NO} but it is not known how long these changes last for. Respiratory infection with virus temporarily affects FE_{NO} values but the nature of this association is not clear; FE_{NO} values are reduced in infants with respiratory syncitial virus[60] or rhinitis [61] but in adults with experimentally induced rhinovirus infection, FE_{NO} rises by approximately 5ppb [62]. There is little direct evidence of the effect of viral infection in children; indirect evidence comes from observations made during exacerbations, precipitated by rhinovirus, which are associated with elevated FE_{NO}[52,63]. The apparently inconsistent findings between virus infection and changing FE_{NO} might reflect differences in the host response to different virus which may be age related and also the retention of NO within secretions. Further evidence of almost continuous but small fluctuations in FE_{NO} is evidenced by the diurnal variability in concentrations[6463]; concentrations are less than 1 ppb higher in the morning compared to the afternoon. In addition to variability over minutes and hours, FE_{NO} is elevated in children with asthma during periods when grass pollen exposure is present [41,56] and also is elevated during the autumn (when moulds cast spores) for those exposed to indoor moulds [43]. Children with

hayfever have elevated FE_{NO}[65~~64~~] and concentrations become particularly elevated during the spring when compared to those without hayfever [43]. In addition to the factors described in table 4 and figure 1~~2~~, intrasubject variability in FE_{NO} measurements may also be introduced by the apparatus itself. As with all analytical processes, there is variability in repeated measurements using the same apparatus and this variability can be reduced by measuring two or three FE_{NO} values and reporting the mean value [20] but this requires time and also costs money. Further apparatus-dependent variability arises when different methods to derive NO are used; one study found an intrasubject difference of 4ppb between devices made by the same manufacturer[66~~65~~]. Intrasubject variability becomes considerably greater when apparatus from different manufacturers are used[67~~66~~] where a typical difference might be 8ppb but range between -12 and +28ppb. At present it seems sensible to make repeated measurements for a given individual using the same apparatus.

3.2 Trials were confounded by poor adherence with inhaled corticosteroid treatment. Adherence to ICS treatment is crucial to the interpretation of elevated FE_{NO}, as it currently is for standard symptom-based asthma management. Elevated FE_{NO} is associated with poor asthma control [41-43] and poor adherence with ICS treatment [26,68~~26,67~~], whereas increasing ICS treatment leads to reduced FE_{NO} [68~~67~~]. Adherence to treatment is always a challenge to measure in asthma, one paper found that typical FE_{NO} concentrations for adolescents with adherence was >50% was 24 ppb and was 31ppb for those with <50% compliance [26]. A second study of 17 children found that compliance with ICS of between 75 and 100% was associated with a relative reduction in FE_{NO} of

50-100% whereas compliance below 75% was associated with changes in FE_{NO} of less that 50% [66,67]. Observations of heterogeneity in FE_{NO} response to ICS [69,70,68,69] might reflect the presence of individuals with high FE_{NO} but little airway eosinophilia, a phenomenon seen in adults[71,70] but not described in children, or heterogeneity in adherence to ICS treatment. Although there is most likely to be incomplete adherence to ICS in the clinical trials, asthma outcomes improved in both FE_{NO} and standard arms of most trials suggesting that adherence was generally good.

3.3 Wrong study design. The clinical trials which have been completed in children to date all compared standard symptom-based treatment versus standard treatment plus FE_{NO} and perhaps trials should compare symptom-based treatment versus FE_{NO} only treatment. This bold study design has only been used in one trial of adult patients[72,71] and found that FE_{NO} guided treatment was associated with reduced ICS doses and a non significant trend for reduced symptoms compared to symptom based management. The poor correlation between asthma control and FE_{NO} reported in some studies[41-43] and the lack of correlation in at least one study[47] does question whether asthma treatment can be guided only by FE_{NO}. On the one hand, FE_{NO} and symptoms measure different outcomes and therefore an algorithm which captures both outcomes might be better than either alone. A more conservative approach might argue that there is a too much of a leap of faith involved in using FE_{NO} to guide treatment, and the symptom-based approach is patient-centred and therefore symptoms should predominate as the ultimate trigger for changing asthma treatment.

3.4 Insufficient power. Although studies justified their sample size by a power calculation, descriptions of the power calculations do not include a mean or median FE_{NO}

value and associated variability. Pragmatically, only two ~~one~~ published studies~~y~~ randomised more than 100 children[26] [32] so it is possible that the remaining studies may have been underpowered.

3.5 Wrong cut offs used. Although increased FE_{NO} is associated with adverse asthma outcomes in children, the definition of what is "increased" remains unclear. ~~although concentrations of >35ppb in children are, by consensus, thought to be high~~ [34]. Evidence from population ~~Whilst there is some guidance from population based~~ studies suggests that ~~to help address the question "what is a high FE_{NO}?"~~ concentrations of >35ppb in children are "high" [38] but the question "what is a significant change in FE_{NO} for an individual?" remains poorly understood and has been explored in detail elsewhere [73~~72~~]. One early study suggested that a change of 4 ppb might be clinically significant[74~~73~~] but, as table 4 demonstrates, there are many factors other than asthma which can acutely change FE_{NO} by an order of at least 4ppb. Furthermore, a rise of 4ppb might be important in a child whose previous FE_{NO} was 10ppb but not for a second individual whose FE_{NO} was 20ppb and relative change in FE_{NO} seems a more meaningful method for interpreting repeated measurements. ~~More R~~recent studies in adults have suggested that ~~rather than~~ a relative change of <30% is unlikely to be clinically relevant [75~~74~~] and a change from poor control to good control was associated with a FE_{NO} reduction of greater than 35% [76~~75~~]. Having a "significant" magnitude of change in FE_{NO} of 30-35% would be consistent with a clinically meaningful change in bronchial hyperreactivity (a hallmark for asthma and correlated with FE_{NO}) of half a doubling dose [77~~76~~]. ~~Variability in repeated measurements of FE_{NO} may be greater in children compared with adults. For example, I~~in ~~one study of~~ children, a FE_{NO} rise of 60% from baseline (with 95% confidence

intervals of approximately 25, 140) was associated with an exacerbation ~~where daily FE_{NO} measurements were made over 30 weeks observed that FE_{NO} rose by 60% (with 95% confidence intervals of approximately 25, 140) during an exacerbation~~ [63~~52~~] and by extrapolation, a rise in FE_{NO} of less than 60% might be indicative of increasing symptoms. A clinical practical guideline published by the American Thoracic Society in 2011 [38] acknowledged a weak evidence base and cautiously recommended that a rise in FE_{NO} of >20% or (in children) >20ppb may be significant and that a minimally important reduction in FE_{NO} was >20% for those with a FE_{NO} of ≥50ppb and <10ppb for those for those with lower values. ~~Although current guidelines consider changes in FE_{NO} expressed as an absolute figure or relative (percentage) change[34], I~~In the adult literature there has been interest in expressing FE_{NO} as a percentage of predicted but this option is losing favour, mostly due to lack of precision and to differences between reference populations raising the question of which reference is the best for a given population? A fourth method to express FE_{NO} is a as percentage of lowest value and is measured after a two week course of oral corticosteroids, but this has an associated morbidity, might yield a low FE_{NO} value which cannot be achieved with ICS treatment and should be reserved for use only in special cases under expert supervision. Of the four methods described, percentage difference seems best suited for individualising treatment since this recognises the relatively wide range of values within a population of children.

3.6 Insight into intrasubject variability. One recent study has given insight into the question "what is a significant change in FE_{NO}?" [43]. 178 children were recruited, of whom 47 had asthma, in a community-based observational study where FE_{NO} was measured over six two-month intervals. The difference between paired FE_{NO}

measurements was expressed as an absolute value and limits of agreement. As might be expected, the limits of agreement for paired FE_{NO} measurements were greater for those with higher initial concentrations. Average FE_{NO} values were stable over eight months but did become significantly higher over a ten month interval, presumably due to the children becoming taller. Asthma was associated with *elevated* FE_{NO} in this population (27ppb versus 10 ppb for non asthmatic) but when both time and baseline FE_{NO} value were considered, asthma was not independently associated with *change* in FE_{NO} value. As a rough rule of thumb, the authors suggested that FE_{NO} values may rise by up to 200% of the previous measurements over two to four months, independently of asthma. For example, in the 40 children with initial FE_{NO} between 11 and 20 ppb (median value 14ppb) the upper limits of agreement for measurements taken at a two and four month interval were +22ppb and +14 ppb respectively. As might be expected over time (and regression to the mean), low initial FE_{NO} concentrations became higher whilst higher concentrations became lower; thus the lower limits of agreement over two and four months for children whose initial FE_{NO} was 21-30 ppb were -19 and -25ppb. In keeping with the suggestion that a more permissive approach to interpretation of FE_{NO} values, a more liberal algorithm which allowed FE_{NO} concentrations to rise by up to 100% (from 16 to 29ppb) was found to be effective in reducing exacerbations and improving quality of life among pregnant women [78].

In addition to describing variability in FE_{NO} over time, this study related FE_{NO} to asthma control (both present and future) and also to environmental exposures which might affect FE_{NO} values [43]. There was weak correlation between FE_{NO} and current and future asthma control measured over a four month interval (correlation coefficient approximately 0.2).

Compared with maintained good asthma control over two months, children who were poorly controlled but became well controlled had elevated FE_{NO}; in contrast, neither those who had good asthma control which became poorly controlled nor those whose asthma control remained poor had elevated FE_{NO}. These observations suggested that elevated FE_{NO} is an index of poor current control but not poor control in two month's time. Additionally the findings suggested that the mechanism for persistently poorly controlled symptoms in children with asthma may not involve eosinophilic airway inflammation.

Future research directions - so where do we go beyond 2014 with FE_{NO}?

It is too early to consign FE_{NO} to the dust bin where failed biomarkers for asthma are placed. There is still sufficient evidence to indicate that FE_{NO} may have a role in helping to address the current situation where there are too many children treated with inappropriately high doses of inhaled corticosteroids and conversely, too many children with poorly controlled asthma whose quality of life can be improved with ICS treatment. The inconsistency between the epidemiology and mechanistic studies (supportive of a role for FE_{NO} in asthma management) and the clinical trials to date (which are generally not supportive of adding FE_{NO} to standard symptom-based management) suggests either FE_{NO} lacks precision or we have not properly understood how to interpret FE_{NO} as a clinical tool. Time will show whether FE_{NO} does have role or not in the management of childhood asthma. If FE_{NO} does prove to have a role in the management of childhood asthma then clinicians will have to place trust in FE_{NO} since guidelines will have to use FE_{NO} to step treatment down as well as up. Now that insight is being gained into what

merits a significant change in FE_{NO}, clinical trials are needed which test these percent of baseline cut offs to treatment algorithms. Future clinical trials designed to use FE_{NO} to improve asthma outcomes might consider the following:

1. Comparing symptom based management and FE_{NO} only based management. This might follow in the success of trials comparing symptoms versus FE_{NO} plus symptoms; the apparent failure of previous studies will understandably make clinicians very cautious in using only FE_{NO} to guide treatment.
2. Careful attention to treatment adherence. This needs to be integral to clinical trials since poor adherence has great potential to mask any true clinical benefit but in the long term, FE_{NO} may prove to give the clinician insight into adherence.
3. What is the "best" outcome. At present, the evidence would suggest that FE_{NO} may have a greater influence in reducing exacerbations rather than improving day-to-day control of symptoms. It is possible that one algorithm may lead to better control and another to fewer exacerbations for a given individual. On a practical note, having symptom control as an outcome and part of the algorithm is a potential flaw in study design.
4. Absolute versus relative FE_{NO} values. There is sufficient evidence to categorise individuals as having high FE_{NO} on study entry but more work is required in establishing whether cut offs for second and subsequent FE_{NO} values should be absolute or percent of previous values.
5. Algorithms could use FE_{NO} to guide treatment step up options for individuals with uncontrolled asthma despite compliance with ICS treatment, i.e. to further increase ICS or use alternative "add ons", as has been applied in adults[78].

~~5.~~6. Algorithms could use FE_{NO} to step down ICS treatment, even when (non-asthmatic) symptoms are present.

~~6.~~7. Clinical setting. Childhood asthma is a condition which is mostly managed in the community and trial design should ideally reflect this and aspire to an ideal of easily delivered personalised treatment algorithms

~~7.~~8. Preschool children. Methodologies are required to allow FE_{NO} to be measured in younger children – currently FE_{NO} can be measured in children aged 5-6 years

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Table 1. Clinically important questions in asthma management where FE_{NO} may give insight

Are these asthmatic symptoms in this child with asthma?
Should treatment be stepped up with inhaled corticosteroids or alternative medications?
When is it appropriate to step down inhaled corticosteroid treatment?
When is it safe to stop treatment with inhaled corticosteroids?

Table 2. Summary of the literature suggesting that exhaled nitric oxide (FE_{NO}) may or may not be a good biomarker for childhood asthma.

Studies suggesting FE_{NO} may be a good biomarker for childhood asthma	**Studies suggesting FE_{NO} may NOT be a good biomarker for childhood asthma**
FE_{NO} is elevated in children with asthma [13]	FE_{NO} is elevated in atopic non-asthmatic children [45] [79~~78~~] and in adolescents whose asthma has remitted [80~~79~~]
Exhaled nitric oxide is positively correlated with thre~~wo~~ hallmarks for asthma, sputum eosinophils [44,81,82~~44,80,81~~] (r=0.5), –FEV_1 [44] ~~and~~ and bronchial hyperresponsiveness (BHR) [45] [46]	Exhaled NO is not related to FEV_1 [45] or BHR [48]
Exhaled nitric oxide is positively correlated with airway eosinophilia after two weeks treatment with oral corticosteroids (r=0.5) [10]	
Elevated FE_{NO} is associated with poor asthma control (r=0.2) [41-43]	FE_{NO} is not correlated with asthma control [47]
FE_{NO} rises after withdrawal of ICS and before symptoms relapse [18]	FE_{NO} does not predict relapse after ICS withdrawal [83~~82~~]
Treatment with inhaled corticosteroids reduces FE_{NO} in children with asthma [68~~67~~].	FE_{NO} remains elevated in some individuals despite treatment with ICS [84,85~~83,84~~].

Table 3. Details of the six randomised controlled trials comparing standard symptom-based asthma management against standard management plus exhaled nitric oxide (FE_{NO}) in children with asthma. ~~*presented as abstract and additional data provided by Prof Chang (personal communication).~~

Study	Population details	FE_{NO} Cut off(s) used	Study design	Primary outcome	Secondary outcomes
de Jongste[32]	Aged 6-18 attending academic centres or hospitals. Atopic (by plasma IgE or skin prick test). Stable mild-moderate asthma. 151 randomised.	≥20 ppb for 6-10 year olds ≥25 ppb for >10 year olds	30 week study, intervention arm made daily FE_{NO} measurements. Treatment reviewed each 3 weeks by telephone, physiological testing 1, 3, 5 months and at end of study	Symptom free days during last 3 months of trial; this improved equally in both arms of the trial.	No difference between control and intervention arm for ICS dose, FEV_1, FE_{NO} or exacerbations.
Peirsman[33]	Age range not stated. Mild to severe asthma attending hospital clinics. Atopic (by plasma IgE or skin prick testing). 99 randomised	≥20 ppb	52 week study. FE_{NO} and symptoms reviewed every three months	Symptom free days; no difference between groups	Exacerbation; reduced in intervention arm (18/49) compared to the control arm (35/50).
Fritsch[27]	Aged 6-18 years. 52 randomised.Attending hospital clinic. Skin prick positive.	Greater than or ≤20ppb	6 month duration, assessed each 6 weeks	FEV_1 – no difference	Exacerbations, mid expiratory flows, control. Mid expiratory flow 11 % higher in FE_{NO} group. Increased ICS doses (200 microg/day) in FE_{NO} group.
Petsky* [31]	Aged >4 years 81 children invited 63 randomised. Attending hospital clinic.	≥ or less than 10 ppb for non atopic children ≥ or less than	12 month study, monthly visits for four months and alternate months thereafter.	Exacerbation – FE_{NO} associated with reduced exacerbations (19% versus 47%)	Quality of life and spirometry did not significantly differ between groups~~also improved marginally. Spirometry unchanged.~~

		12 ppb with one positive skin test ≥ or less than 20 ppb with more than one positive skin test			
Pijnenberg[30]	Aged 5-18 years. 108 screened 89 randomised. Attending hospital clinic. Atopic asthma treated with ICS.	Less than or ≥30ppb	12 month study with assessments each 3 months	ICS dose. No difference between groups.	FE_{NO} group had improved PD_{20} (1.3 doubling doses), lower FE_{NO} (geometric mean difference at end of study 32% lower) and trend for fewer exacerbations (20% versus 39%)
Pike[28]	Aged 6-17 years. 96 screened, 90 randomised. Attending hospital clinic with moderate-severe asthma.	≤15ppb 15.1-24.9ppb ≥25 ppb	12 month study, assessed each 2 months	ICS dose and exacerbation. No difference between groups.	Spirometry, no difference between groups.
Szeffler[26]	Aged 12-20 years. 780 screened. 546 randomised. Inner city area where ≥20% households below poverty level.	0-20 20.1-30 30.1-40 >40	46 week duration assessments each 6-8 weeks	Number of days with symptoms. No difference between FE_{NO} and control groups	FE_{NO} group had: Mean increased fluticasone treatment 119 microg/day. 10% reduction in proportion requiring OCS Among obese children 0.6 fewer days with symptoms. For those with multiple positive skin tests (ie >9 out of 14 tested) 0.8 fewer days with symptoms.

Verini[29]	Aged 6-17 years. 64 children. Referred to hospital and admitted.	12	12 month study with assessments at baseline and after 6 and 12 months	Severity score (mean reduced significantly from 1.1 to 0.6 and 0.8 after 6 and 12 months only in the FE_{NO} group). Exacerbation (mean number reduced from 2.0 to 1.0 and 0.8 only in FE_{NO} group), treatment (unchanged in FE_{NO} group but some evidence of increased treatment in control arm).	Spirometry – no difference

Table 4. Factors which are associated with changes in FE_{NO} in children independent of asthma

Factor	Approximate magnitude of effect
Height	Up to 1ppb rise per cm height gained [24]
Dietary exposures	Short lived rise of up to 5-10ppb [53,54]
Allergen exposure	Rise of up to 50% during birch pollen season [56]
Exposure to second hand smoke	Reduction of 100% (26ppb for exposed children versus 56ppb) [57] or absolute reduction of 10ppb [58]
~~Asthma exacerbation~~	~~Typical rise of approximately 60%~~ [41]
Exposure to poor outdoor air quality	Rise of approximately 1ppb 4 hours after each increase of $10mg/m^3$ fine particulate exposure ($PM_{2.5}$) [59]
Genetic variations	Variations in genes coding for NOS2 and NOS3 may lead to differences in FE_{NO} in adults of 10% [86~~85~~] or 10ppb [87~~86~~] but no association found for NOS1 variant and FE_{NO} in children [88~~87~~]

FIGURE LEGENDS

~~Figure 1. Diagram demonstrating the flow dependence of exhaled nitric oxide (FE_{NO}). At lower flows, concentrations are higher and *vice versa*. The figure also demonstrates how the absolute FE_{NO} value is derived from a plateau achieved over a ten second exhalation in older children and adults (six seconds in younger children).~~

Figure 1~~2~~. Summary of the asthma-dependent and independent factors associated with increased or reduced concentrations of exhaled nitric oxide (FE_{NO}).

Figure 2. A forest plot comparing the effect on exacerbations requiring oral corticosteroid treatment where maintenance treatment is driven by exhaled nitric oxide (ENO) and symptoms versus symptoms alone.

Figure 3. A forest plot comparing the effect on inhaled corticosteroid dose at the time of study exit where maintenance treatment is driven by exhaled nitric oxide (ENO) and symptoms versus symptoms alone.