Does treatment guided by fractional exhaled nitric oxide improve outcomes in subgroups of children with asthma?

Shona Fielding S[1], Marielle Pijnenburg[2], Johan de Jongste[2], Katherine Pike[3,4], Graham Roberts[3], Helen Petsky[5], Anne B Chang[6], Maria Fritsch[7], Thomas Frischer[7], Stanley J Szefler[8], Peter Gergen[9], Francoise Vermeulen[10], Robin Vael[11], Steve Turner S[12].

[1]Medical Statistics Team, Institute of Applied Health Sciences, University of Aberdeen, UK

[2]Department of Paediatric Respiratory Medicine and Allergology, Erasmus MC – Sophia Children's Hospital, Rotterdam, Netherlands

[3]Clinical and Experimental Science Academic Unit, University of Southampton, Southampton, UK

[4] Respiratory Critical Care and Anaesthesia group, Institute of Child Health, University College London, UK

[5]School of Nursing and Midwifery, Griffith University; Menzies Health Institute Queensland, Brisbane, Australia

[6]Department of Respiratory and Sleep Medicine, Queensland Children's Hospital, Queensland University of Technology, Brisbane; Child Health Division, Menzies School of Health Research, Charles Darwin University, Darwin, Australia

[7]University Children's Hospital, Vienna, Austria

[8]Breathing Institute, Children's Hospital Colorado, Department of Pediatrics, University of Colorado School of Medicine, Aurora, Colorado, USA

[9]National Institute of Allergy and Infectious Diseases, Bethesda, MD USA

[10]Pediatric Department, Hôpital Erasme, Université Libre de Bruxelles (U.L.B.), Brussels, Belgium

[11]Department of Paediatrics, Antwerp University Hospital, Antwerp, Belgium

[12]Child Health, University of Aberdeen, UK

25 Corresponding author: Professor Steve Turner, Child Health, Royal Aberdeen Children's Hospital,

26 Aberdeen, UK, AB25 2ZG. Tel +44 1224 438470. s.w.turner@abdn.ac.uk

27 Word count: 2611

ABSTRACT

Introduction. Fractional exhaled nitric oxide (F_ENO), a biomarker of eosinophilic airway inflammation, may be useful to guide asthma treatment. F_ENO guided treatment may be more effective in certain subgroups for improving asthma outcomes compared to standard treatment.

Methods. An individual patient data analysis was performed using data from seven randomised clinical trials (RCT) which used F_ENO to guide asthma treatment. The incidence of an asthma exacerbation and loss of control, and the time to first exacerbation and loss of control were described between five subgroups of RCT participants.

Results. Data were available in 1112 RCT participants. Among those not treated with Leukotriene Receptor Antagonist (LTRA), but not among those who were treated with LTRA, F_ENO guided treatment was associated with reduced exacerbation risk (odds ratio (OR) 0.68 [95% CI 0.49, 0.94]), longer time to first exacerbation (hazard ratio (HR) 0.76 [0.57, 0.99]) and borderline reduced risk for loss of control (OR 0.70 [0.49, 1.00]). Non-obese children, compared to obese children, were less likely to lose asthma control when treatment was guided by F_ENO (OR 0.69 [0.48, 0.99]) and time to loss of control was longer (HR 0.77 [0.61, 0.99]).

Conclusions. Asthma treatment guided by F_ENO may be more effective in achieving better asthma outcomes for patients who are not treated with LTRA and who are not obese compared to standard practice.

Keywords: Asthma, Child, Monitoring, Nitric oxide

INTRODUCTION

Asthma is a common chronic condition which affects one million children in the UK [1], six million in the US[2] and 235 million children and adults around the world [3]. There is effective treatment to control asthma symptoms and guidelines recommend that treatment should be titrated to asthma symptoms[4-6]. There remains a widely accepted recognition that an objective measurement to guide asthma treatment is required [7].

Fractional exhaled nitric oxide (F_ENO) in exhaled breath has many of the characteristics required of an objective tool to measure asthma symptoms. For example, F_ENO rises before symptoms occur [8,9], falls when asthma treatment is administered [10,11], can be measured with minimal discomfort to the patient and results are available within a few minutes using commercially available apparatus [12]. A meta-analysis including eight clinical trials in children and young adults found that addition of F_ENO measurements to symptom-guided treatment did not reduce asthma symptoms [13], but that F_ENO guided treatment reduced asthma exacerbations [13].

Asthma is a heterogeneous condition and what we do not know is whether there are patient sub-groups in whom using F_ENO to guide asthma treatment may be beneficial [7]. In one randomised controlled trial (RCT), the intervention was more effective in participants who had more positive skin tests and who were obese, but age, sex, asthma severity and initial F_ENO concentration were not associated with a different outcome from the intervention [14]. In a second RCT there was no evidence of improved outcomes between individuals who were concordant or discordant for FENO and symptoms [15].

Our group has pooled the data collected from seven of the eight published RCTs where the efficacy of F_ENO used to guide asthma treatment was examined, compared to standard management [16]. Here we use data from 1112 participants to test the hypothesis that there are particular subgroups of patients where F_ENO guided treatment is more effective in improving asthma outcomes compared to standard treatment.

METHODS

Study design

Authors of all published RCTs where measurements of F_ENO were used to guide asthma treatment in children [17] were contacted and asked to provide data as previously described [16]. The children who took part in the studies were recruited from hospital clinics and were followed up for between six and 12 months. The primary outcome was the presence of any asthma exacerbation during follow up [13]. Secondary outcomes were loss of control among those who were initially controlled and time to first exacerbation and time to first loss of control. Institutional ethical approval was provided for each trial which contributed data.

Details of each population (also see table one)

Fritsch *et al* [18] undertook a study of 47 children with asthma attending a hospital asthma clinic in Vienna, Austria and collected data (including F_ENO, asthma symptom score and history of recent exacerbations) at six-week intervals over six months. Peirsman *et al* [19] recruited 99 participants with persistent asthma attending hospital asthma clinics across Belgium and collected data at three-month intervals over twelve months. Petsky *et al* [20] recruited 63 children from hospital clinics in Australia and Hong Kong, and data were collected on eight occasions over twelve months (one, two, three, four, six, eight, ten and twelve months). Pijnenburg *et al* [21] included 86 participants attending a single hospital clinic in the Netherlands and data were collected at baseline, three, six, nine and twelve months. Pike *et al* [22] recruited 90 participants clinics in four UK hospitals and collected data at two-month intervals over a year. Szefler *et al* [14] recruited 546 participants from the community in the USA and collected post-randomisation information over 46 weeks including at three months, six months, eight months and ten months. Voorend-van Bergen *et al* [23] undertook a study of 181 participants attending hospital clinics the Netherlands and collected data at four-

month intervals over a year. The treatment algorithms in F_ENO-guided and standard practice arms in each RCT was different to other RCTs.

102 Table one. A summary of characteristics of the randomised controlled trials whose data were used for the present analysis.

	Mean age (SD), y	Inclusion criteria (in addition to child diagnosed with asthma)	Definition of asthma control	Treatment strategy for intervention group	Treatment strategy for control group group	Treatment options (same for both groups in all studies)	What did the trial find? (F_ENO treatment compared to standard care)
Fritsch *et al* 2006[1] Austria	11.5 (3.1)	Age 6-18 years. Sensitised to inhaled allergens. No systemic corticosteroids one month before recruitment.	FEV_1>80% predicted, <6 doses of SABA over 14 days and no or mild symptoms	Step up if control lost regardless of F_ENO. Step down if controlled and F_ENO ≤20ppb. If F_ENO>20ppb and controlled step up unless already on ICS (in which case no change)	Step up if either FEV_1 <80% or symptoms severe or ≥6 doses of SABA over previous 14 days. Step down if controlled	Four treatments steps	Higher mid expiratory flow, higher dose of ICS
Peirsman *et al* 2014[2] Belgium	10.7 (2.1)	Sensitised to inhaled allergens. No exacerbation or systemic corticosteroids three month before recruitment	A score of ≤3 from the first four (of seven) questions on ACT*, FEV_1>80% and ≤2 doses of SABA over a week	Step up if F_ENO>20ppb regardless of control. Consider stepping up if F_ENO ≤20ppb and partly/fully uncontrolled. Step down if controlled and F_ENO ≤20ppb.	Step up if uncontrolled, consider stepping up if partly controlled. Step down if controlled.	Step up and down options if on the following preventers: ICS alone; LTRA alone; ICS+LABA; ICS+LTRA	Reduced exacerbations, increased LTRA and ICS dose. No difference in primary outcome
Petsky *et al* 2015[3] Australia	10.0 (3.2)	Aged >4 years. Prescribed asthma preventer. Adherent to treatment	Symptom score† no more than 15% higher than previous assessment (only used for the control group)	Step up or down based only on F_ENO> or ≤10 ppb for non atopic, > or ≤12ppb with one positive skin test, > or ≤20 for	Step up if symptoms score >15% higher than previously. Step down if symptoms score <10.	Seven steps (none including LTRA)	Reduced exacerbation, increased ICS dose

				>1 positive skin test			
Pijnenburg *et al* 2005[4] Netherlands	12.3 (2.8)	Aged 6-18 years. Sensitised to inhaled allergens. ICS dose unchanged for ≥3 months at recruitment	Score of >14 on validated symptom diary‡	Step up if F_ENO>30ppb regardless of control. Treatment stepped down if symptoms controlled and F_ENO≤30ppb. No change if symptoms not controlled but F_ENO≤30ppb	Step up if symptoms uncontrolled, step down if controlled for second assessment. No change if controlled on first assessment	Nine steps (none including LABA or LTRA)	Reduced F_ENO and bronchial hyperresponsiveness No increase in ICS dose
Pike *et al* 2013[5] UK	11.9 (2.6)	Aged 6-17 years. Prescribed ≥400 microg ICS daily (budesonide equivalent). Adherent to treatment. No history of life-threatening asthma or requiring maintenance oral corticosteroids.	Modified validated symptom diary¥, FEV_1≥80% and < 1 SABA dose per week	Step up ICS if F_ENO≥25ppb (or >twice baseline value) regardless of control or FEV_1. Also step up with LABA if poorly controlled and F_ENO <25ppb. Step down if F_ENO ≤15 ppb and controlled on two consecutive assessments.	Step up if uncontrolled. Step down if controlled on two consecutive assessments.	Eight treatment steps	No differences in outcomes
Szefler *et al* 2008[6] USA	14.4 (2.1)	Aged 12-20 years. Living in community where ≥20% households were below poverty threshold.	Four levels of control depending on a symptom score (ACT*) and a	Step up by one, two or three treatment levels depending on	Step up by one, two or three treatment levels depending on	Seven treatment steps (including low dose theophylline)	Reduced exacerbations, increased ICS dose. No difference in primary outcome.

		Persistent or uncontrolled asthma if on long term preventer. Non-smoker.	series of FEV_1, cut offs (FEV_1 ≥80, 70-79% or >70%)	symptoms score, FEV_1 and F_ENO 0-20, 20.1-30, 30.1-40 or >40ppb. Step down if controlled on two consecutive assessments and F_ENO≤20ppb.	symptoms score and FEV_1. Step down if controlled on two consecutive assessments		
Voorend-van Bergen *et al 2010*[8] Netherlands	10.2 (3.0)	Aged 4-18 years. Sensitised to inhaled allergens. >9% bronchodilator response. Prescribed ICS for ≥3 months. Non-smoker. No history of multiple ITU admissions for asthma.	ACT score ≥20*	Step up if controlled and F_ENO≥50ppb or uncontrolled and F_ENO≥25ppb. Step down if controlled and F_ENO<25ppb. Otherwise no change	Step up if uncontrolled. Step down/no change if controlled	Seven treatment steps	Increased asthma control but not the primary outcome

103 SABA=short acting beta agonist. ICS=inhaled corticosteroids. LTRA=leukotriene receptor antagonist. LABA=long acting beta agonist. ppb=parts per billion.
104 ITU=intensive care unit
105 *ACT=Asthma Control Test, Schatz M, et al *J Allergy Clin Immunol* 2006;117:549–556.
106 †Santanello NC, et al. *Eur Respir J* 1997;10:646–651. ‡Verberne AA, et al *Am J Respir Crit Care Med* 1997;156:688–695.
107 ¥Wasserfallen JB, et al. *J Allergy Clin Immunol* 1997;100: 16–22.

Data collected

Covariates collected at baseline in all trials included: age, gender, height, weight, treatment 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. Ethnicity was available in four cohorts[14,21-23]. Body Mass Index (BMI) was derived and International Obesity Task Force weight categories created [24]; obesity is defined as equivalent to adult BMI ≥30 kg/m^2. Percentage of predicted (%) Forced Expired Volume in one second (FEV_1) was calculated according to the Global Lung Initiative standard [25] apart from participants in two trials [21,22] where only % FEV_1 standardised to other references was available. F_ENO was measured in all studies in accordance with the 2005 guideline [26]. At each follow up visit an assessment of asthma control was made (see table 1) and history of any asthma attack since the previous assessment was recorded (defined as receipt of oral corticosteroids for an asthma exacerbation [16]). The trials used different symptom score methodology and loss of control was defined as per trial protocol by reaching a pre-agreed symptom score.

Analysis

Asthma outcomes were compared between participants in the F_ENO guided and standard treatment arms of RCTs for the following five subgroups defined at baseline and previously associated with differences in F_ENO. The five subgroups were stratified by: dose of ICS (≤400 microg budesonide equivalent or >400 microg)[10], use of LTRA [27], obesity [14], ethnicity (white versus other)[28] and atopic (i.e. positive skin prick test or positive type-specific IgE) [14]. Any exacerbation during follow up and time to first exacerbation and any loss of control and time to loss of control were calculated (the latter restricted to those who were controlled at baseline). Time to first exacerbation or to loss of control was determined using data collected at the scheduled study assessments, and table one in the supplement describes the time in weeks between baseline and each follow up assessment in

each RCT. For example, if a participant experienced an exacerbation after their three-month assessment but before the six month assessment, time was censored at six months. Logistic regression was used to relate any exacerbation or any loss of control to an interaction term between each baseline characteristic and treatment arm; a significant interaction term ($p<0.05$) would indicate that outcomes were different between F_ENO guided and standard treatment for a sub group. Cox proportional hazards models were used to investigate time to first exacerbation or time to first loss of control. Each subgroup was considered separately and all models included adjustment for covariates associated with the outcome including: age, a variable for each RCT and ICS dose at baseline (this was not included in the ICS dose subgroup model). Standard statistical software was used (STATA version 14) and significance was assumed at 5%. All analyses were exploratory, so no adjustment was made for multiple comparisons.

RESULTS

Study subjects

Data from seven RCTs were analysed [14,18-23], totalling 1112 participants. Characteristics of participants at baseline have previously been described [16] and are presented in table 2. The majority of participants (58%) were male and the mean age was 12.6 (standard deviation, SD 3.1) years. Characteristics of participants in the five subgroups are presented in supplemental table 2, i.e. LTRA treatment (yes/no), ICS dose ≤400 microg/>400 microg), obese (yes/no), atopic (yes/no) and white versus other ethnic group.

154 Table 2. Characteristic of study participants at the baseline visit in each study.

		Fritsch[18]	Peirsman[19]	Petsky[20]	Pijnenburg[21]	Pike[22]	Szefler[14]	Voorend-van Bergen[23]	All populations combined
Number of participants		47	99	63	86	90	546	181	1112
%(number) male		60% (28)	67% (66)	49% (31)	65% (56)	57% (51)	53% (288)	68% (123)	58% (643)
Mean age (SD)		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
% atopic		100%	100%	38% (24/63)	100%	76% (68/90)	88% (467/531)	100%	89% (972/1097)
% (number) obese		8% (4/47)	1% (1/99)	2% (1/58)	4% (4/85)	8% (7/89)	31% (165/526)	3% (5/181)	17% (187/1085)
% (number) 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)
% (number) 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)
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)
% (number) > 400ug BUD		30% (14/47)	15% (15/99)	49% (31/63)	66% (57/86)	59% (53/90)	53% (287/546)	33% (59/181)	46% (516/1112)
% White ethnic group		Not stated	82% (69/84)	Not stated	Not stated	92% (83/90)	0% (0/526)	89% (160/179)	35% (312/901)
Control status	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)
	Not Controlled	51% (24/47)	25% (16/65)	28% (16/57)	43% (33/77)	3% (3/90)	20% (107/528)	33% (59/181)	24% (258/1045)

155 SD=standard deviation, IQR=interquartile range, LTRA=leukotriene receptor antagonist, LABA=long acting beta agonist, BUD = budesonide equivalent ICS

156

F_ENO intervention and asthma exacerbation outcomes

Any exacerbation. Of the 1047 participants for whom exacerbation data were available, 296 (28%) had at least one exacerbation with the first occurring after a median (interquartile range IQR) 22 (14, 38) weeks. Table 3 shows the effect of treatment group was different for the two LTRA subgroups (interaction p-value = 0.039). Those not treated with LTRA, had lower odds for ≥1 exacerbation in the F_ENO guided group compared to standard care (OR=0.68, 95%CI 0.49-0.94) but there was no difference observed between F_ENO guided and control groups for those on LTRA, table 3. The number needed to treat with F_ENO guided management to prevent one exacerbation among those not treated with LTRA was 15. Interactions between treatment arm and other baseline characteristics (ICS dose, obese, atopy and white ethnicity) were not significant when predicting exacerbation, table 3.

Time to first exacerbation. Overall in the two treatment groups, the median time to first exacerbation was 22 (IQR 14, 38) weeks in the standard arm and 22 (IQR 13, 34) in the F_ENO guided arm. The interaction term between treatment arm and LTRA was of borderline significance for time for first exacerbation (p=0.049), and among those not treated with LTRA at baseline, the time to first asthma exacerbation was slightly longer for participants receiving F_ENO guided treatment compared to standard care (HR=0.76, 0.57-0.99, p=0.048), table 4 and figure 1. Time to first exacerbation was no different between treatment groups for those treated with LTRA. The interaction terms with treatment arm were not significant for ICS dose, atopy, obesity or ethnicity, table 4.

Table 3. Proportion of individuals with any asthma exacerbation in F_ENO -guided and standard management arms of clinical trials with stratification for patient characteristics. ICS=inhaled corticosteroids, presented as ≤400 or >400 micrograms budesonide equivalent. Obesity was defined by International Obesity Task Force criteria.

Baseline characteristic		% with ≥1 exacerbation in each treatment arm		F_ENO vs standard		p value for interaction*
		F_ENO guided management	Standard management	OR	95% CI	
LTRA treatment	Yes	49/109 (45%)	40/104 (38%)	1.46	(0.76, 2.79)	0.039
	No	88/410 (21%)	119/419 (28%)	0.68	(0.49, 0.94)	
ICS dose	≤400 microg	48/289 (17%)	58/279 (21%)	0.72	(0.46, 1.11)	0.493
	>400 microg	89/232(38%)	101/247 (41%)	0.88	(0.60, 1.28)	
Obese	Yes	30/88 (34%)	36/81 (44%)	0.63	(0.33, 1.21)	0.342
	No	107/425 (25%)	119/433 (27%)	0.90	(0.65, 1.24)	
Atopic	Yes	113/458 (25%)	138/481 (29%)	0.83	(0.61, 1.13)	0.391
	No	14/47 (30%)	13/31 (42%)	0.53	(0.20, 1.41)	
Ethnic group	White	34/148 (23%)	31/164 (17%)	1.28	(0.70, 2.33)	0.177
	Non-white	86/270 (32%)	97/254 (38%)	0.78	(0.54, 1.14)	

*adjusted for RCT population, age and (except the analysis for higher versus lower ICS dose) dose of inhaled corticosteroid (budesonide equivalent).

185 Table 4. Results from Cox regression models analysing time to first exacerbation for subgroups of

186 participants.

Sub group		Hazard Ratio for time to first exacerbation for participants where treatment was guided by F_ENO compared to standard care (95% CI)	Interaction p-value
LTRA	No	0.76 (0.57, 0.99) p= 0.048	0.049
	Yes	1.26 (0.82, 1.90) p= 0.292	
ICS	<=400 microg	0.76 (0.52, 1.12) p=0.166	0.393
	>400 microg	0.94 (0.71, 1.25) p=0.667	
Atopic	No	0.61 (0.29, 1.31) p=0.207	0.347
	Yes	0.90 (0.70, 1.16) p=0.412	
Obese	No	0.96 (0.74, 1.25) p=0.787	0.456
	Yes	0.78 (0.48, 1.27) p=0.321	
Ethnic group	White	1.24 (0.76, 2.02) p=0.391	0.268
	Non-White	0.90 (0.67, 1.20) p=0.469	

187

188 # These models are fitted as time = Subgroup+Treatment group + Subgroup*treatment+ Age +
189 StudyID + baseline ICS. Baseline ICS was not included in the model where outcomes between ICS
190 subgroups were analysed.

191

FeNO intervention and asthma control outcomes

Any loss of asthma control. There were 787 participants who were controlled at baseline; 336 (43%) remaining controlled until completion of the trial, 344 (44%) lost control and 107 (14%) were lost to follow up for this outcome. The median (IQR) time to loss of control in these 344 patients was 22 (13, 30) weeks. There was no difference in mean age between those who did and did not lose control (12.8 (SD 3.0) and 12.6 (SD 2.9) years respectively) and no difference in baseline ICS dose (median (IQR) 400 (400, 1000) for both those who did and did not lose control). The interaction terms between treatment arm and the five baseline participant characteristics for loss of asthma control were non-significant, supplemental table 3. However, there was an indication of reduced odds of loss of control in the F_ENO arm versus standard arm in those subgroups of participants who were not on LTRA at baseline, and in those who were not obese at baseline (supplemental table 3). The number of controlled participants needed to treat with F_ENO guided management to prevent one losing control among those not treated with LTRA was 11.

Time to loss of control. Within the subgroup who lost control (n=344) the median (IQR) time to loss of control was 17 (13, 30) weeks with standard treatment and 22 (13, 34) weeks with F_ENO guided treatment. The interaction terms with treatment arm were not significant for ICS dose ≤400 microg versus >400 microg, atopy, LTRA treatment, white versus other race or obese (yes or no), table 5. There was borderline evidence of a longer time to first loss of control for F_ENO guided compared to standard treatment within subgroups who were not treated with LTRA (HR 0.77 [0.60, 0.99] figure 2), non-obese (HR 0.77 [95% CI 0.61, 0.99] figure 3) and atopic (HR 0.80 [95% CI 0.63, 1.00] supplemental figure 1), table 5.

Table 5. Results from cox regression models analysing time to first loss of control for subgroups of participants all of whom were controlled at baseline.

		Hazard Ratio for time to first exacerbation for participants where treatment was guided by F_ENO compared to standard care (95% CI)	Interaction p value
LTRA	No	0.77 (0.60, 0.99) p=0.038	0.230
	Yes	1.05 (0.68, 1.64) p=0.822	
ICS	<=400	0.82 (0.62, 1.10) p=0.182	0.899
	>400	0.84 (0.62, 1.16) p=0.293	
Obese	No	0.77 (0.61, 0.99) p=0.042	0.130
	Yes	1.15 (0.73, 1.81) p=0.538	
Atopy	No	1.29 (0.54, 3.08) p=0.566	0.293
	Yes	0.80 (0.63, 1.00) p=0.050	
Ethnic group	White	0.85 (0.58, 1.24) p=0.396	0.970
	Non-White	0.85 (0.64, 1.14) p=0.289	

These models are fitted as time = Subgroup+Treatment group + Subgroup*treatment+ Age + StudyID + baseline ICS . Baseline ICS was not included in the model where outcomes between ICS subgroups were analysed.

DISCUSSION

We analysed data collected in seven RCTs to test the hypothesis that there are subgroups of patients where F_ENO guided treatment is more effective in improving asthma outcomes compared to standard treatment. The main finding was that within these RCTs, the odds for exacerbation and loss of control for those not treated with LTRA were 32% and 30% lower in the F_ENO-guided arm compared to standard treatment. The significant interaction term for LTRA treatment and treatment for exacerbation indicated that FeNO driven management may have reduced exacerbations for those not treated with LTRA but not among those treated with LTRA. A second finding was that outcomes were no different between groups stratified by ICS dose, and ethnic group. Collectively these findings support the hypothesis that F_ENO is more useful for guiding treatment compared to standard practice in children with asthma not treated with LTRA.

A further finding was that in non-obese participants (but not in obese participants), F_ENO-guided treatment was associated with a 31% reduction in odds for loss of control compared to standard treatment and when control was lost, time to loss of control was longer. Although the interaction term for obesity and treatment for loss of control was not significant, we believe that the improved outcomes for non-obese children merits further consideration. There was consistency in our results (i.e. an association with any loss of control and time to loss of control) and also there is biological plausibility whereby asthma associated with obesity may be a separate non-eosinophilic phenotype, especially in females [29]. A recent systematic review found no evidence of increased or reduced asthma control among children who were obese [30] and asthma guidelines do not recommend different treatment approaches for obese patients with asthma [4-6]. Further research is required to clarify whether F_ENO-guided treatment is equally effective in obese and non-obese children.

Our observation that time to loss of control was longer among children who were atopic receiving F_ENO-guided treatment compared to standard treatment deserves careful consideration. . The number of non-atopic participants included in our analysis was relatively small since atopy was an

246 inclusion criterion for four cohorts [18,19,21,23] and the atopic subgroup were no more or less likely
247 to have an exacerbation or to lose control within the trials. Since F_ENO is considered to be a
248 surrogate for allergic or eosinophilic airway inflammation [31] it is biologically plausible that F_ENO-
249 guided treatment algorithms are more likely to suppress airway inflammation and improve asthma
250 control. Further evidence of biological plausibility comes from an RCT whose data are included in
251 our analysis [14] which found fewer days with maximal symptoms among those with elevated IgE
252 and multiple positive skin prick tests. Although non-atopic asthma is less common than atopic
253 asthma, e.g. present in 18% of participants in the three trials which did not include only atopic
254 participants [14,20,22], asthma is a very common condition and there are approximately 150-
255 200,000 non-atopic asthmatic children in the UK [1]. There is a need to establish whether treatment
256 and monitoring for atopic and nonatopic children should be the same.

257 The magnitude of significantly reduced risk for exacerbations and loss of control in the intervention
258 compared to standard treatment was typically 25-30% and this difference is clinically meaningful
259 since it is consistent with the benefit seen from commonly-used asthma treatments such as LTRA
260 and ICS. Knorr et al [32] report a 23% reduced incidence of exacerbations in young children treated
261 with montelukast compared to placebo. The review by Calpin et al[33] reports a 32% reduced risk for
262 oral steroid treatment for exacerbations among children treated with ICS compared to placebo.

263 The RCTs included in our study applied different inclusion criteria, F_ENO-guided treatment algorithms
264 and asthma control scores, and these methodological differences will weaken any relationship
265 between the intervention and asthma outcomes. The seven RCTs did apply a standard definition of
266 exacerbation and apparatus for measuring F_ENO. Despite the differences between RCTs, we still
267 observed differences in outcomes between some of the subgroups studied, and it is likely that the
268 magnitude of difference that we report in outcomes between the subgroups stratified by LTRA
269 treatment, obesity and atopy may be an underestimate of the true value.

Our study was not designed to determine why F_ENO guided treatment was associated with improved asthma outcomes among those not treated with LTRA compared to participants receiving LTRA treatment. Treatment with LTRA is known to reduce F_ENO by approximately 25% in children with atopic asthma [27] and may plausibly confound F_ENO-guided treatment, especially since the RCT treatment algorithms did not consider the effect of LTRA on F_ENO. There is an alternative explanation for the differences in exacerbation outcomes associated with LTRA treatment in different RCT arms; those treated with LTRA were younger and had more severe asthma (including higher ICS dose, needing LABA treatment and almost twice the exacerbation prevalence) and F_ENO-guided asthma treatment may be less effective in more severe asthma rather than in children receiving LTRA treatment *per se*. Given that LTRA are commonly used in asthma treatment, there is a need to study the impact of LTRA treatment on F_ENO-guided asthma treatment.

We observed that when data from the RCTs were combined, F_ENO-guided asthma treatment was associated with reduced risk for loss of control and time to loss of control among non-obese children. This contrasts with the findings of an RCT whose data are included in the present analysis [14] which reported fewer symptoms among obese participants (i.e. with BMI>30kg/m^2) receiving F_ENO -guided treatment. This apparent inconsistency may be due to several factors. First the outcome in the paper by Szefler *et al* [14] was days of maximal symptoms, but this variable was not available in all the RCTs included in the present paper and therefore loss of control was the outcome analysed here. Second, participants were all of African American or Hispanic ethnic origin, on higher ICS dose and had a considerably higher obesity prevalence[14], and some or all of these difference characteristics could explain different outcomes compared to the remaining six RCT participants. In our study, the reduced odds for loss of control and time to loss of control for non-obese children receiving F_ENO -guided treatment compared to standard treatment is likely to be underestimated due to inclusion of F_ENO and asthma control data from the RCT of Szefler *et al* [14].

There are some limitations to our study. First, the time to loss of control or first exacerbation was restricted to the predetermined assessment periods and this lack of precision will weaken the reported differences in these outcomes between sub groups. Secondly, the RCTs had different study designs with different step-up/step-down criteria and management regimes. Third, ethnicity data was only available for four of the seven RCTs and was therefore not included as a covariate in the models, but ideally we would have included ethnicity in our model since ethnicity was associated with differences between the other subgroups analysed(supplemental table 2) . Fourth, ideally we would have performed a sensitivity analysis by excluding participants in the RCT by Szefler *et al* [14] since their characteristics were different to the remaining RCTs for age, ethnicity and obesity, but this would have resulted in a 50% smaller sample size and the analysis would have been underpowered. A final limitation is that self-reported ICS adherence was available in only three RCTs included in our study [14,22,23] we were not able to compare outcomes between treatment arms between adherent and non-adherent participants. Future research could test the hypothesis that asthma outcomes are improved by F_ENO-guided treatment in adherent compared to non-adherent patients.

In summary, we have used data from more than 1000 asthmatic children and report that F_ENO-guided treatment lead to better asthma outcomes among those not treated with LTRA.

REFERENCES

1. Asthma UK. Asthma facts and FAQs. http://www.asthma.org.uk/asthma-facts-and-statistics. 2017. Date last accessed: 08/31 2017.

2. Centers for Disease Control and Prevention. Asthma. Most recent data. https://www.cdc.gov/asthma/most_recent_data.htm. 2016. Date last accessed: 08/31 2017.

3. World Health Organisation. Asthma. https://www.who.int/respiratory/asthma/en/. 2011. Date last accessed: 12/07 2018.

4. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. https://ginasthma.org/gina-reports/. 2019. Date last accessed: 07/26 2019.

5. British Thoracic Society and Scottish Intercollegiate Guidelines Network. SIGN 158. The British Guideline on the Management of Asthma. https://www.brit-thoracic.org.uk/quality-improvement/guidelines/asthma/. 2019. Date last accessed: 07/27 2019.

6. National Institute for Health and Care Excellence. Asthma: diagnosis, monitoring and chronic asthma management. https://www.nice.org.uk/guidance/ng80. 2017. Date last accessed: 01/28 2018.

7. Pavord ID, Beasley R, Agusti A, Anderson GP, Bel E, Brusselle G, Cullinan P, Custovic A, Ducharme FM, Fahy JV, Frey U, Gibson P, Heaney LG, Holt PG, Humbert M, Lloyd CM, Marks G, Martinez FD, Sly PD, von Mutius E, Wenzel S, Zar HJ, Bush A. After asthma: redefining airways diseases. *Lancet* 2018; 391: 350-400.

8. Pijnenburg MW, Hofhuis W, Hop WC, De Jongste JC. Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission. *Thorax* 2005; 60: 215-218.

9. Zacharasiewicz A, Wilson N, Lex C, Erin EM, Li AM, Hansel T, Khan M, Bush A. Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children. *American Journal of Respiratory & Critical Care Medicine* 2005; 171: 1077-1082.

10. Beck-Ripp J, Griese M, Arenz S, Koring C, Pasqualoni B, Bufler P. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. *European Respiratory Journal* 2002; 19: 1015-1019.

11. Baraldi E, Azzolin NM, Zanconato S, Dario C, Zacchello F. Corticosteroids decrease exhaled nitric oxide in children with acute asthma. *J Pediatr* 1997; 131: 381-385.

12. Silkoff PE, Carlson M, Bourke T, Katial R, Ogren E, Szefler SJ. The Aerocrine exhaled nitric oxide monitoring system NIOX is cleared by the US Food and Drug Administration for monitoring therapy in asthma. *Journal of Allergy & Clinical Immunology* 2004; 114: 1241-1256.

13. Petsky HL, Kew KM, Chang AB. Exhaled nitric oxide levels to guide treatment for children with asthma. *Cochrane Database of Systematic Reviews* 2016: Art. No.: CD011439. DOI: 10.1002/14651858.CD011439.pub2.

14. Szefler SJ, Mitchell H, Sorkness CA, Gergen PJ, O'Connor GT, Morgan WJ, Kattan M, Pongracic JA, Teach SJ, Bloomberg GR, Eggleston PA, Gruchalla RS, Kercsmar CM, Liu AH, Wildfire JJ, Curry MD, Busse WW. Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomised controlled trial. *Lancet* 2008; 372: 1065-1072.

15. Voorend-van Bergen S, Vaessen-Verberne AA, Landstra AM, Brackel HJ, van den Berg NJ, Merkus PJ, de Jongste JC, Pijnenburg MW. Fractional Exhaled Nitric Oxide Monitoring Does Not Improve Asthma Management in Children with Concordant and Discordant Asthma Phenotypes. *American Journal of Respiratory & Critical Care Medicine* 2015; 192: 1016-1018.

16. Fielding S, Pijnenburg M, de Jongste JC, Pike K, Roberts G, Petsky H, Chang AB, Fritsch M, Frischer T, Szefler S, Gergen P, Vermeulen F, Vael R, Turner S. Change in FEV1 and FENO Measurements as Predictors of Future Asthma Outcomes in Children. *Chest* 2019; 155: 331-341.

17. Turner S. Exhaled nitric oxide and the management of childhood asthma – yet another promising biomarker "has been" or a misunderstood gem. *Paediatr Respir Rev* 2015; 16: 88-96.

18. Fritsch M, Uxa S, Horak FJ, Putschoegl B, Dehlink E, Szepfalusi Z, Frischer T. Exhaled nitric oxide in the management of childhood asthma: a prospective 6-months study. *Pediatr Pulmonol* 2006; 41: 855-862.

19. Peirsman EJ, Carvelli TJ, Hage PY, Hanssens LS, Pattyn L, Raes MM, Sauer KA, Vermeulen F, Desager KN. Exhaled nitric oxide in childhood allergic asthma management: a randomised controlled trial. *Pediatr Pulmonol* 2014; 49: 624-631.

20. Petsky HL, Li AM, Au CT, Kynaston JA, Turner C, Chang AB. Management based on exhaled nitric oxide levels adjusted for atopy reduces asthma exacerbations in children: A dual centre randomized controlled trial. *Pediatr Pulmonol* 2015; 50: 535-543.

21. Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. *American Journal of Respiratory & Critical Care Medicine* 2005; 172: 831-836.

22. Pike K, Selby A, Price S, Warner J, Connett G, Legg J, Lucas JSA, Peters S, Buckley H, Magier K, Foote K, Drew K, Morris R, Lancaster N, Roberts G. Exhaled nitric oxide monitoring does not reduce exacerbation frequency or inhaled corticosteroid dose in paediatric asthma: a randomised controlled trial. *The clinical respiratory journal* 2013; 7: 204-213.

376 23. Voorend-van Bergen S, Vaessen-Verberne AA, Brackel HJ, Landstra AM, van den Berg NJ, Hop
377 WC, de Jongste JC, Merkus PJ, Pijnenburg MW. Monitoring strategies in children with asthma: a
378 randomised controlled trial. *Thorax* 2015; 70: 543-550.

379 24. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight
380 and obesity worldwide: international survey. *BMJ* 2000; 320: 1240-1243.

381 25. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MSM,
382 Zheng J, Stocks J, ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry
383 for the 3-95-yr age range: the global lung function 2012 equations. *European Respiratory Journal*
384 2012; 40: 1324-1343.

385 26. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for
386 standardized procedures for the online and offline measurement of exhaled lower respiratory nitric
387 oxide and nasal nitric oxide, 2005. *American Journal of Respiratory & Critical Care Medicine* 2005;
388 171: 912-930.

389 27. Montuschi P, Mondino C, Koch P, Barnes PJ, Ciabattoni G. Effects of a leukotriene receptor
390 antagonist on exhaled leukotriene E4 and prostanoids in children with asthma. *Journal of Allergy &*
391 *Clinical Immunology* 2006; 118: 347-353.

392 28. Koo S, Gupta A, Fainardi V, Bossley C, Bush A, Saglani S, Fleming L. Ethnic Variation in Response
393 to IM Triamcinolone in Children With Severe Therapy-Resistant Asthma. *Chest* 2016; 149: 98-105.

394 29. Jensen ME, Gibson PG, Collins CE, Wood LG. Airway and systemic inflammation in obese children
395 with asthma. *European Respiratory Journal* 2013; 42: 1012-1019.

30. Ahmadizar F, Vijverberg SJH, Arets HGM, de Boer A, Lang JE, Kattan M, Palmer CNA, Mukhopadhyay S, Turner S, Maitland-van der Zee AH. Childhood obesity in relation to poor asthma control and exacerbation: a meta-analysis. *European Respiratory Journal* 2016; 48: 1063-1073.

31. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *American Journal of Respiratory & Critical Care Medicine* 2001; 164: 1376-1381.

32. Knorr B, Franchi LM, Bisgaard H, Vermeulen JH, LeSouef P, Santanello N, Michele TM, Reiss TF, Nguyen HH, Bratton DL. Montelukast, a leukotriene receptor antagonist, for the treatment of persistent asthma in children aged 2 to 5 years. *Pediatrics* 2001; 108: E48.

33. Calpin C, Macarthur C, Stephens D, Feldman W, Parkin PC. Effectiveness of prophylactic inhaled steroids in childhood asthma: a systemic review of the literature. *Journal of Allergy & Clinical Immunology* 1997; 100: 452-457.

FIGURE LEGEND

Figure 1. Kaplan Meier curves showing time to first exacerbation for patients whose asthma treatment was guided by either fractional exhaled nitric oxide ("FENO") or by symptoms only ("standard") and stratified by leukotriene receptor antagonist (LTRA) treatment. The difference between treatment arms was significant for those not treated with LTRA (p=0.048) but not for the patients treated with LTRA (p=0.292).

Figure 2. Kaplan Meier curves showing time to loss of control for patients who were initially controlled and whose asthma treatment was guided by either fractional exhaled nitric oxide ("FENO") or by symptoms only ("standard") and stratified by leukotriene receptor antagonist (LTRA) treatment. The difference between treatment arms was significant for those not treated with LTRA (p=0.038) but not for the patients treated with LTRA (p=0.822).

Figure 3. Kaplan Meier curves showing time to loss of control for patients who were initially controlled and whose asthma treatment was guided by either fractional exhaled nitric oxide ("FENO") or by symptoms only ("standard") and stratified by obese status. The difference between treatment arms was significant for those who were not obese (p=0.042) but not for the patients who were obese (p=0.538).