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

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Abstract:	Introduction. Fractional exhaled nitric oxide (FENO), a biomarker of eosinophilic airway inflammation, may be useful to guide asthma treatment. FENO 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 FENO 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 LTRA (but not among those who were treated with LTRA), FENO 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 FENO (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 FENO 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.

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

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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 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.

101 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)	Methodology for 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.	Unvalidated symptom diary	Combination of symptom score, FEV_1 <80% and F_ENO>20ppb	Combination of symptom score and FEV_1 <80%	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	First four (of seven) questions on ACT*	Combination of symptom >score, exacerbation in previous two weeks, FEV_1 <80% and F_ENO>20ppb	Combination of symptom score, exacerbation in previous two weeks and FEV_1 <80%	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	Validated symptom diary†	Combination of symptom score plus F_ENO> 10 for non atopic, >12 with one positive skin test, >20 for >1 positive skin test	Symptom score alone	Seven steps (none including LTRA)	Reduced exacerbation, increased ICS dose
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	Validated symptom diary‡	Treatment stepped up if F_ENO>30ppb. Treatment stepped down if symptoms	Symptom score alone	Nine steps (none including LABA or LTRA)	Reduced F_ENO and bronchial hyperresponsiveness No increase in ICS dose

				controlled and F_ENO≤30ppb			
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¥	Combination of symptoms, recent reliever medication use, FEV_1 >90%, 80-90% or <80% and F_ENO≤15, 15-25 or ≥25ppb	Combination of symptoms, recent reliever medication use, and FEV_1 >90%, 80-90% or <80%	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. Persistent or uncontrolled asthma if on long term preventer. Non-smoker.	ACT*	Combination of symptoms, FEV_1 ≥80, 70-79% or >70% and F_ENO 0-20, 20.1-30, 30.1-40 or >40ppb	Combination of symptoms and FEV_1 ≥80, 70-79% or >70%	Seven treatment steps (including low dose theophylline)	Reduced exacerbations, increased ICS dose. No difference in primary outcome.
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*	Combination of symptom score and F_ENO <20, 20-50 or >50ppb	Symptom score alone	Seven treatment steps	Increased asthma control but not the primary outcome

ICS=inhaled corticosteroids. LTRA=leukotriene receptor antagonist. LABA=long acting beta agonist. ppb=parts per billion. ITU=intensive care unit

*ACT=Asthma Control Test, Schatz M, et al *J Allergy Clin Immunol* 2006;117:549–556.

†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.

¥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]. 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.

152 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)

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

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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).

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

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.

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.

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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

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

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

The RCTs included in our study applied different inclusion criteria, F_ENO-guided treatment algorithms and asthma control scores, and these methodological differences will weaken any relationship between the intervention and asthma outcomes. The seven RCTs did apply a standard definition of exacerbation and apparatus for measuring F_ENO. Despite the differences between RCTs, we still observed differences in outcomes between some of the subgroups studied, and it is likely that the magnitude of difference that we report in outcomes between the subgroups stratified by LTRA 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) . 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. These findings support calls for individualised treatment for asthma [7].

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.

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

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

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

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

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

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

29. Jensen ME, Gibson PG, Collins CE, Wood LG. Airway and systemic inflammation in obese children 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.

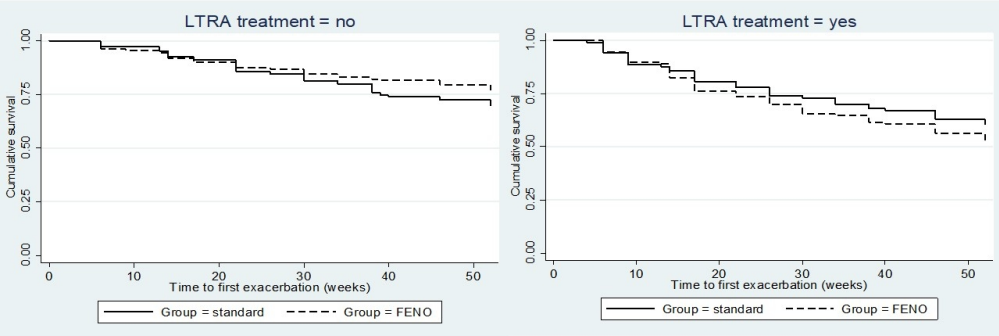
33. Calpin C, Macarthur C, Stephens D, Feldman W, Parkin PC. Effectiveness of prophylactic inhaled steroids in childhood asthma: a systemic review of the literature. *Journal of Allergy & Clinical Immunology* 1997; 100: 452-457.

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).



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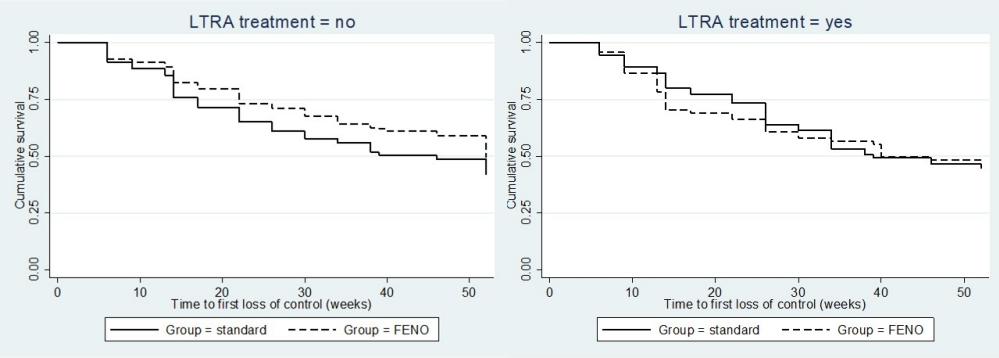

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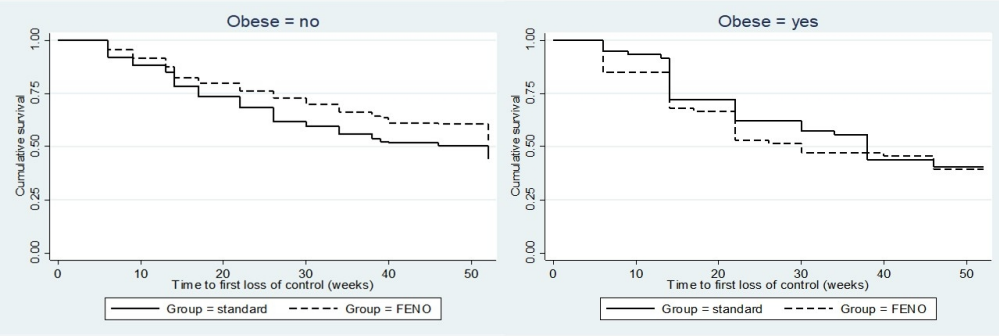

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SUPPLEMENT

Supplemental table 1. The interval in weeks between the baseline visit (when randomisation occurred) and subsequent follow up assessments in the seven randomised clinical trials whose data are included in the present analysis.

	Follow up visit 1	Follow up visit 2	Follow up visit 3	Follow up visit 4	Follow up visit 5	Follow up visit 6	Follow up visit 7	Follow up visit 8
Fritsch[1]	6	13	18	26				
Peirsman[2]	13	26	39	52				
Petsky[3]	4	9	13	17	26	32	40	52
Pijnenburg[4]	13	26	39	52				
Pike[5]	9	17	26	34	40	52		
Szefler[6]	6	14	22	30	38	46		
Voorend-van Bergen[7]	17	34	52					

Supplemental table 2. Characteristics of participants in the five subgroups where outcomes are compared between those in the standard treatment and F_ENO guided treatment arms.

	ICS dose		Atopy		LTRA treatment		Obese		White	
	≤400 microg	>400 microg	Yes	No	Yes	No	Yes	No	Yes	No
Male gender	359/596 (60%)	284/516 (55%)	588/991 (59%)	36/86 (42%)‡	127/227 (56%)	515/880 (58%)	97/187 (52%)	535/898 (60%)	209/312 (66%)	311/587 (53%)‡
Mean age (SD), y	12.1 (3.3)	13.2 (2.8) ‡	12.6 (3.1)	12.8 (3.3)	12.0 (3.1)	12.7 (3.1) ‡	13.9 (2.5)	12.2 (3.0) ‡	10.5 (2.8)	14.0 (2.4) ‡
Any exacerbation	106/568 (19%)	190/479 (40%)‡	251/939 (27%)	27/78 (35%)	89/213 (42%)	207/829 (25%)‡	66/169 (39%)	226/857 (26%)‡	65/312 (21%)	183/524 (31%)‡
Loss of control*	186/378 (49%)	158/302 (52%)	305/607 (50%)	23/50 (44%)	80/149 (54%)	264/531 (50%)	76/127 (60%)	261/538 (49%)‡	107/221 (48%)	185/375 (49%)
LTRA treatment	77/595 (13%)	150/512 (30%)‡	204/986 (21%)	11/86 (13%)	n/a	n/a	40/187 (21%)	185/894 (21%)	106/312 (34%)	96/583 (16%)‡
LABA treatment	185/595 (31%)	449/512 (88%)‡	553/986 (56%)	53/86 (62%)	168/227 (74%)	466/880 (53%)‡	130/187 (70%)	491/894 (55%)‡	161/312 (52%)	374/583 (64%)‡
Baseline FENO median (IQR)	21 (11.4, 40.2)	23.6 (12, 47.8) ‡	23.6 (12.6, 46.1)	10 (7.8, 16.2)	22.5 (11.2, 42.3)	21.8 (11.6, 43.0)	16.8 (10, 31.6)	23 (12, 46.3) ‡	19.9 (10.2, 38.4)	20.7 (11.2, 41.3)
Median (IQR) ICS dose†	n/a	n/a	400 (400,1000)	400 (400,1000)	1000 (400,2000)	400 (400,1000) ‡	1000 (400, 2000)	400 (400, 1000) ‡	400 (400, 800)	800 (400, 2000) ‡
Proportion white	204/483 (42%)	108/416 (26%)‡	286/790 (36%)	9/75 (12%)‡	106/202 (53%)	206/693 (30%)‡	10/172 (6%)	301/707 (43%)‡	n/a	n/a
Proportion obese	81/583(14%)	106/502 (21%)‡	165/967 (17%)	18/83 (22%)	40/225(18%)	147/856 (17%)	n/a	n/a	10/311 (3%)	162/568 (29%)‡
Proportion atopic	527/879 (91%)	464/498 (93%)	n/a	n/a	204/215 (95%)	782/857 (91%)	165/183 (90%)	802/867 (92.5%)	286/295 (97%)	504/570 (88%)‡

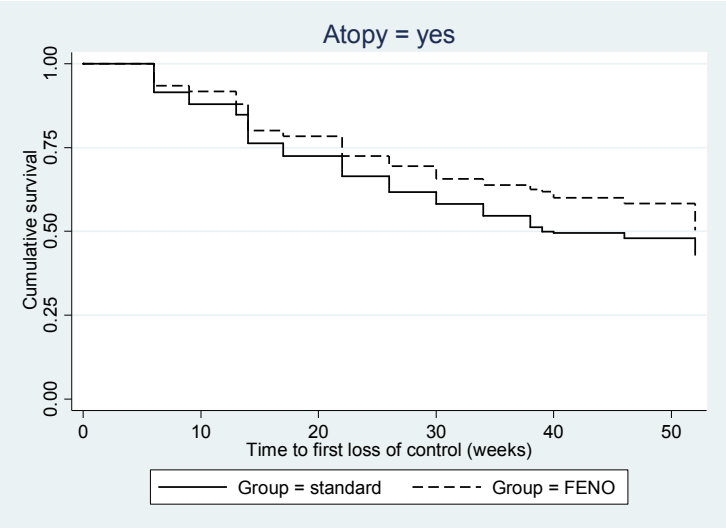
*After being controlled at baseline. †microg budesonide or equivalent. ‡ $p<0.05$.

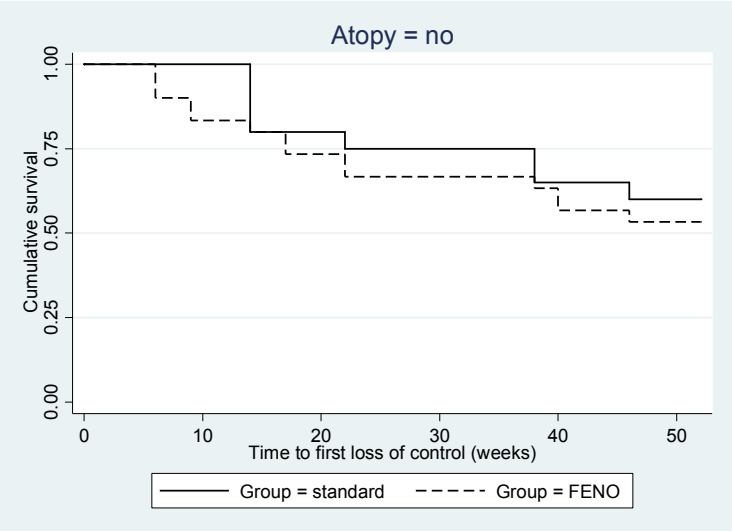
Supplemental table 3. Proportion of individuals who were initially controlled who lost control in F_ENO -guided and standard management arms of clinical trials with stratification for patient characteristics. ICS=inhaled corticosteroids, presented as ≤400 or >400 micrograms budesonide equivalent. Obesity was defined by International Obesity Task Force criteria.

Baseline characteristic		% with loss of control during follow-up# in each treatment arm		F_ENO vs standard		p value for interaction*
		F_ENO guided management	Standard management	OR	95% CI	
LTRA treatment	Yes	39/74 (53%)	41/75 (55%)	0.94	(0.48, 1.87)	0.453
	No	118/261/(45%)	146/270 (54%)	0.70	(0.49, 1.00)	
ICS dose	≤400 microg	88/191 (46%)	98/187 (52%)	0.80	(0.52, 1.22)	0.652
	>400 microg	69/144 (48%)	89/158 (56%)	0.69	(0.43, 1.99)	
Obese	Yes	40/66 (61%)	36/61 (59%)	1.08	(0.53, 2.22)	0.274
	No	116/264 (44%)	145/274 (53%)	0.69	(0.48, 0.99)	
Atopic	Yes	133/291 (46%)	172/316 (54%)	0.73	(0.52, 1.02)	0.457
	No	14/30 (47%)	8/20 (40%)	1.15	(0.36, 3.69)	
Ethnic group	White	48/106 (45%)	59/115 (51%)	0.76	(0.42, 1.37)	0.946
	Non-white	89/191 (47%)	96/184 (52%)	0.78	(0.52, 1.18)	

*adjusted for age, ICS at baseline (except ICS dose model) and RCT population; # from those controlled at baseline

Supplemental figure 1. 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 atopy. The difference between treatment arms was significant for those who were atopic (p=0.050) but not for the non-atopic patients (p=0.566).





REFERENCES

1. 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.

2. 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.

3. 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.

4. 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.

5. 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.

6. 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.

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

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

Fielding S[1], Pijnenburg M[2], de Jongste JC[2], Pike KC[3,4], Roberts G[3], Petsky H[5], Chang AB[6], Fritsch M[7], Frischer T[7], Szefler SJ[8], Gergen P[9], Vermeulen F[10], Vael R[11], Turner S[12].

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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 ~~plausible~~ subgroups of RCT participants.

Results. Data were available in 1112 RCT participants. Among those not treated with 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]). ~~In atopic children, F_ENO guided treatment had no effect on the risk of loss of control per se, but increased the time to loss of control, compared to nonatopic children.~~

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 ~~or who are atopic~~ 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 ~~whilst~~ guidelines recommend that treatment should be titrated to asthma symptoms[4-6]. ~~,~~ ~~T~~there remains a~~is~~ 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 ~~since it~~ 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 ~~is~~ 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~~the~~ 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 ~~in~~ 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.

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)	Methodology for 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.	Unvalidated symptom diary	Combination of symptom score, FEV_1 <80% and F_ENO>20ppb	Combination of symptom score and FEV_1 <80%	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	First four (of seven) questions on ACT*	Combination of symptom >score, exacerbation in previous two weeks, FEV_1 <80% and F_ENO>20ppb	Combination of symptom score, exacerbation in previous two weeks and FEV_1 <80%	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	Validated symptom diary†	Combination of symptom score plus F_ENO> 10 for non atopic, >12 with one positive skin test, >20 for >1 positive skin test	Symptom score alone	Seven steps (none including LTRA)	Reduced exacerbation, increased ICS dose
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	Validated symptom diary‡	Treatment stepped up if F_ENO>30ppb. Treatment stepped down if symptoms	Symptom score alone	Nine steps (none including LABA or LTRA)	Reduced F_ENO and bronchial hyperresponsiveness No increase in ICS dose

				controlled and $F_ENO \leq 30ppb$			
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¥	Combination of symptoms, recent reliever medication use, FEV_1 >90%, 80-90% or <80% and $F_ENO \leq 15$, 15-25 or ≥25ppb	Combination of symptoms, recent reliever medication use, and FEV_1 >90%, 80-90% or <80%	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. Persistent or uncontrolled asthma if on long term preventer. Non-smoker.	ACT*	Combination of symptoms, FEV_1 ≥80, 70-79% or >70% and F_ENO 0-20, 20.1-30, 30.1-40 or >40ppb	Combination of symptoms and FEV_1 ≥80, 70-79% or >70%	Seven treatment steps (including low dose theophylline)	Reduced exacerbations, increased ICS dose. No difference in primary outcome.
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*	Combination of symptom score and F_ENO <20, 20-50 or >50ppb	Symptom score alone	Seven treatment steps	Increased asthma control but not the primary outcome

ICS=inhaled corticosteroids. LTRA=leukotriene receptor antagonist. LABA=long acting beta agonist. ppb=parts per billion. ITU=intensive care unit

*ACT=Asthma Control Test, Schatz M, et al *J Allergy Clin Immunol* 2006;117:549–556.

†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.

¥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]. 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~~, the following variables were collected:~~ 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 ~~inhaled corticosteroid (~~ICS ~~(,~~ ≤400 microg budesonide equivalent or >400 microg)[10], use of LTRA [27], obesity [14], ethnicity (white versus other)[28] and ~~skin prick positivity~~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 doese 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 21. 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), ~~skin prick positive~~atopic (yes/no) and white versus other ethnic group.

Table 21. 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
% ~~with positive skin prick test or positive aeroallergen sensitisation~~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~~889~~)
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)

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

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~~T~~) 22 (14, 38) weeks. Table 3~~2~~ shows the effect of treatment group was different for the two LTRA subgroups (interaction p-value = 0.0~~.~~39). 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~~2~~. 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, ~~skin prick positive~~atopy and white ethnicity) were not significant when predicting exacerbation, table 3~~2~~.

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~~shorter~~ for participants receiving F_ENO guided treatment compared to standard care (HR=0.76, 0.57-0.99, p=0.048), table 4~~3~~ 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, ~~skin prick test results~~atopy, obesity or ethnicity, table 4~~3~~.

Table 3~~2~~. 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)	
~~Skin prick positive~~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).

Table ~~43~~. Results from Cox regression models analysing time to first exacerbation for subgroups of 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	
~~Skin prick positive~~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	

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.

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 (I~~R~~QR) 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~~4~~. 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 t~~T~~able 3~~4~~). 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, ~~positive skin prick test~~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 ~~and supplemental figure 2, non-obese (HR 0.77 [95% CI 0.61, 0.99]) table 5 and supplemental figure 3~~.

Table 4. Proportion of individuals who were initially controlled who lost control 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 loss of control during follow-up# in each treatment arm		F_ENO vs standard		p value for interaction*
		F_ENO guided management	Standard management	OR	95% CI	
LTRA treatment	Yes	39/74 (53%)	41/75 (55%)	0.94	(0.48, 1.87)	0.453
	No	118/261/(45%)	146/270 (54%)	0.70	(0.49, 1.00)	
ICS dose	≤400 microg	88/191 (46%)	98/187 (52%)	0.80	(0.52, 1.22)	0.652
	>400 microg	69/144 (48%)	89/158 (56%)	0.69	(0.43, 1.99)	
Obese	Yes	40/66 (61%)	36/61 (59%)	1.08	(0.53, 2.22)	0.274
	No	116/264 (44%)	145/274 (53%)	0.69	(0.48, 0.99)	
Skin prick positive	Yes	133/291 (46%)	172/316 (54%)	0.73	(0.52, 1.02)	0.457
	No	14/30 (47%)	8/20 (40%)	1.15	(0.36, 3.69)	
Ethnic group	White	48/106 (45%)	59/115 (51%)	0.76	(0.42, 1.37)	0.946
	Non-white	89/191 (47%)	96/184 (52%)	0.78	(0.52, 1.18)	

*adjusted for age, ICS at baseline (except ICS dose model) and RCT population; # from those controlled at baseline

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	
~~Skin prick positive~~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

~~The role of F_ENO in guiding asthma treatment in children is unclear, and one potential explanation for this could be that F_ENO is more effective in improving asthma outcomes for some subgroups of the population.~~ 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 o~~O~~utcomes were no different between groups stratified by ~~LABA treatment,~~ 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 ~~some subgroups of~~ children with asthma not treated with LTRA.

A further ~~second~~ finding was that in non-obese participants (but not in obese participants), F_ENO-guided treatment was associated with a 31% reduction in ~~ed~~ 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 a~~A~~sthma associated with obesity may be a separate non-eosinophilic phenotype, especially in females~~,~~ [29]. ~~and this may explain why F_ENO guided treatment was more effective among non-obese children in our study.~~ 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. ~~A third finding was that among children who were atopic, the time to loss of control was longer for those receiving F_ENO-guided treatment compared to standard treatment~~. ~~Outcomes were no different between groups stratified by LABA treatment, 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 some subgroups of children with asthma.~~ The number of non-atopic participants included in our analysis was relatively small since atopy was an inclusion criterion for four cohorts [18,19,21,23] and the atopic subgroup were no more or less likely to have an exacerbation or to lose control within the trials. Since F_ENO is considered to be a surrogate for allergic or eosinophilic airway inflammation [31] it is biologically plausible that F_ENO-guided treatment algorithms are more likely to suppress airway inflammation and improve asthma control. Further evidence of biological plausibility comes from an RCT whose data are included in our analysis [14] which found fewer days with maximal symptoms among those with elevated IgE and multiple positive skin prick tests. Although non-atopic asthma is less common than atopic asthma, e.g. present in 18% of participants in the three trials which did not include only atopic participants [14,20,22], asthma is a very common condition and there are approximately 150-200,000 non-atopic asthmatic children in the UK [1]. There is a need to establish whether treatment and monitoring for atopic and nonatopic children should be the same.

The magnitude of significantly reduced risk for exacerbations and loss of control in the intervention compared to standard treatment was typically 25-30% and this difference is clinically meaningful since it is consistent with the benefit seen from commonly-used asthma treatments such as LTRA and ICS. Knorr et al [32] report a 23% reduced incidence of exacerbations in young children treated

with montelukast compared to placebo. The review by Calpin et al[33] reports a 32% reduced risk for oral steroid treatment for exacerbations among children treated with ICS compared to placebo.

The RCTs included in our study applied different inclusion criteria, F_ENO-guided treatment algorithms and asthma control scores, and these methodological differences will weaken any relationship between the intervention and asthma outcomes. The seven RCTs did apply a standard definition of exacerbation and apparatus for measuring F_ENO. Despite the differences between RCTs, we still observed differences in outcomes between some of the subgroups studied, and it is likely that the magnitude of difference that we report in outcomes between the subgroups stratified by LTRA 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. T~~, and t~~his 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].

~~Asthma associated with obesity may be a separate non-eosinophilic phenotype, especially in females, [29] and this may explain why F_ENO -guided treatment was more effective among non-obese children in our study. 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.~~

~~We found evidence of borderline significance that F_ENO-guided treatment was associated with longer time to loss of control compared to standard treatment among atopic children. This is consistent with an RCT whose data are included in our analysis [14] which found fewer days with maximal symptoms among those with elevated IgE and multiple positive skin prick tests. F_ENO is a biomarker of eosinophilic airway inflammation [31] and likely to be more effective in patients with atopy. The number of non-atopic participants included in our analysis was relatively small since atopy was an inclusion criterion for four cohorts [18,19,21,23]. Although non-atopic asthma is less common than atopic asthma, e.g. present in 18% of participants in the three trials which did not include only atopic participants [14,20,22], asthma is a very common condition and there are~~

~~approximately 150-200,000 non-atopic asthmatic children in the UK [1] There is a need to establish whether treatment and monitoring for atopic and nonatopic children should be the same.~~

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) . A final limitation is that ~~Thirdly, since~~ 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~~, who were not obese and who were atopic~~. These findings support calls for individualised treatment for asthma [7].

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.

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

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

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

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

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

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

29. Jensen ME, Gibson PG, Collins CE, Wood LG. Airway and systemic inflammation in obese children 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).

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(a)Indicate the study's design with a commonly used term in the title or the abstract. PAGE 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found. PAGE 3
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported. PAGE 4
Objectives	3	State specific objectives, including any prespecified hypotheses PAGE 4
Methods		
Study design	4	Present key elements of study design early in the paper PAGE 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection PAGES 5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. PAGES 5-6 *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed NOT APPLICABLE *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable PAGES 5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group PAGES 5-7
Bias	9	Describe any efforts to address potential sources of bias NOT APPLICABLE (NO COMPARABLE "WHOLE POPULATION")
Study size	10	Explain how the study size was arrived at PAGE 5 (THIS IS A SECONDARY ANALYSIS OF RCT DATA)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why. PAGES 5-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding PAGES 6-7
		(*b*) Describe any methods used to examine subgroups and interactions PAGES 6-7
		(*c*) Explain how missing data were addressed NOT APPLICABLE
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed PAGES 6-7 *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of

		sampling strategy
		(*e*) Describe any sensitivity analyses NOT APPLICABLE
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed PAGE 7
		(b) Give reasons for non-participation at each stage NOT APPLICABLE (SECONDARY ANALYSIS OF RCT DATA)
		(c) Consider use of a flow diagram A CONSORT DIAGRAM WOULD NOT ADD TO THE PAPER
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders PAGES 7, TABLE 1 AND SUPPLEMENTAL TABLE 2
		(b) Indicate number of participants with missing data for each variable of interest NOT APPLICABLE
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) NOT APPLICABLE
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure PAGE 6-7 AND TABLE 1
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included. PAGES 7-9 AND TABLES 2-5
		(*b*) Report category boundaries when continuous variables were categorized CONFIDENCE INTERVALS PRESENTED THROUGHOUT
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period NOT DONE
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses THIS IS AN ANALYSIS OF SUBGROUPS WITH INTERACTIONS
Discussion		
Key results	18	Summarise key results with reference to study objectives PAGES 9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias PAGES 10-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence PAGES 9-12
Generalisability	21	Discuss the generalisability (external validity) of the study results PAGE 12
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based NO FUNDING WAS OBTAINED

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at

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