



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

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Key Words:	nitric oxide (children), Asthma; child, Asthma (clinical aspects), Leukotriene receptor antagonists, obesity
Abstract:	<p>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.</p> <p>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.</p> <p>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</p>

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	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.

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Manuscripts

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10 4 Frischer T⁷, Szeffler SJ⁸, Gergen P⁹, Vermeulen F¹⁰, Vael R¹¹, Turner S¹².
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3 **27 ABSTRACT**
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6 **28** Introduction. Fractional exhaled nitric oxide ($F_{E}NO$), a biomarker of eosinophilic airway
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8 **29** inflammation, may be useful to guide asthma treatment. $F_{E}NO$ guided treatment may be more
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10 **30** effective in certain subgroups for improving asthma outcomes compared to standard treatment.
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13 **31** Methods. An individual patient data analysis was performed using data from seven randomised
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15 **32** clinical trials (RCT) which used $F_{E}NO$ to guide asthma treatment. The incidence of an asthma
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17 **33** exacerbation and loss of control, and the time to first exacerbation and loss of control were
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19 **34** described between five subgroups of RCT participants.
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23 **35** Results. Data were available in 1112 RCT participants. Among those not treated with LTRA (but not
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25 **36** among those who were treated with LTRA), $F_{E}NO$ guided treatment was associated with reduced
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27 **37** exacerbation risk (odds ratio (OR) 0.68 [95% CI 0.49, 0.94]), longer time to first exacerbation (hazard
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29 **38** ratio (HR) 0.76 [0.57, 0.99]) and borderline reduced risk for loss of control (OR 0.70 [0.49, 1.00]).
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32 **39** Non-obese children, compared to obese children, were less likely to lose asthma control when
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34 **40** treatment was guided by $F_{E}NO$ (OR 0.69 [0.48, 0.99]) and time to loss of control was longer (HR 0.77
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36 **41** [0.61, 0.99]).
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39 **42** Conclusions. Asthma treatment guided by $F_{E}NO$ may be more effective in achieving better asthma
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41 **43** outcomes for patients who are not treated with LTRA and who are not obese compared to standard
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43 **44** practice.
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47 **45** Keywords: Asthma, Child, Monitoring, Nitric oxide
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50 INTRODUCTION

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

56 Fractional exhaled nitric oxide ($F_{E}NO$) in exhaled breath has many of the characteristics required of
57 an objective tool to measure asthma symptoms. For example, $F_{E}NO$ rises before symptoms occur
58 [8,9], falls when asthma treatment is administered [10,11], can be measured with minimal
59 discomfort to the patient and results are available within a few minutes using commercially available
60 apparatus [12]. A meta-analysis including eight clinical trials in children and young adults found that
61 addition of $F_{E}NO$ measurements to symptom-guided treatment did not reduce asthma symptoms
62 [13], but that $F_{E}NO$ guided treatment reduced asthma exacerbations [13].

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

70 Our group has pooled the data collected from seven of the eight published RCTs where the efficacy
71 of $F_{E}NO$ used to guide asthma treatment was examined, compared to standard management [16].
72 Here we use data from 1112 participants to test the hypothesis that there are particular subgroups
73 of patients where $F_{E}NO$ guided treatment is more effective in improving asthma outcomes
74 compared to standard treatment.

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56 76 **METHODS**7
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9 77 **Study design**

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12 78 Authors of all published RCTs where measurements of F_ENO were used to guide asthma treatment in
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14 79 children [17] were contacted and asked to provide data as previously described [16]. The children
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16 80 who took part in the studies were recruited from hospital clinics and were followed up for between
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18 81 six and 12 months. The primary outcome was the presence of any asthma exacerbation during
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20 82 follow up [13]. Secondary outcomes were loss of control among those who were initially controlled
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22 83 and time to first exacerbation and time to first loss of control. Institutional ethical approval was
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24 84 provided for each trial which contributed data.

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28 85 **Details of each population (also see table one)**

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30 86 Fritsch *et al* [18] undertook a study of 47 children with asthma attending a hospital asthma clinic in
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32 87 Vienna, Austria and collected data (including F_ENO, asthma symptom score and history of recent
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34 88 exacerbations) at six-week intervals over six months. Peirsman *et al* [19] recruited 99 participants
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36 89 with persistent asthma attending hospital asthma clinics across Belgium and collected data at three-
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38 90 month intervals over twelve months. Petsky *et al* [20] recruited 63 children from hospital clinics in
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40 91 Australia and Hong Kong, and data were collected on eight occasions over twelve months (one, two,
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42 92 three, four, six, eight, ten and twelve months). Pijnenburg *et al* [21] included 86 participants
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44 93 attending a single hospital clinic in the Netherlands and data were collected at baseline, three, six,
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46 94 nine and twelve months. Pike *et al* [22] recruited 90 participants clinics in four UK hospitals and
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48 95 collected data at two-month intervals over a year. Szeffler *et al* [14] recruited 546 participants from
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50 96 the community in the USA and collected post-randomisation information over 46 weeks including at
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52 97 three months, six months, eight months and ten months. Voorend-van Bergen *et al* [23] undertook
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54 98 a study of 181 participants attending hospital clinics the Netherlands and collected data at four-
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99 month intervals over a year. The treatment algorithms in F_ENO-guided and standard practice arms in
100 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	Treatment options (same for both groups in all studies)	What did the trial find? (F _E NO treatment compared to standard care)
Fritsch <i>et al</i> 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 _E NO>20ppb	Combination of symptom score and FEV ₁ <80%	Four treatments steps	Higher mid expiratory flow, higher dose of ICS
Peirsman <i>et al</i> 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 _E NO>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 <i>et al</i> 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 _E NO> 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 <i>et al</i> 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 _E NO>30ppb. Treatment stepped down if symptoms	Symptom score alone	Nine steps (none including LABA or LTRA)	Reduced F _E NO and bronchial hyperresponsiveness No increase in ICS dose

				controlled and F _E NO ₂ ≤30ppb			
Pike <i>et al</i> 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 _E NO ₂ ≤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
Szeffler <i>et al</i> 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 _E NO ₂ 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 <i>et al</i> 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 _E NO ₂ <20, 20-50 or >50ppb	Symptom score alone	Seven treatment steps	Increased asthma control but not the primary outcome

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

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

104 †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.

105 ¥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 (FEV₁) was calculated according to the Global Lung Initiative standard [25] apart from participants in two trials [21,22] where only % FEV₁ 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 (\leq 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

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3 131 each RCT. For example, if a participant experienced an exacerbation after their three-month
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5 132 assessment but before the six month assessment, time was censored at six months. Logistic
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7 133 regression was used to relate any exacerbation or any loss of control to an interaction term between
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9 134 each baseline characteristic and treatment arm; a significant interaction term ($p < 0.05$) would
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11 135 indicate that outcomes were different between $F_E NO$ guided and standard treatment for a sub
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13 136 group. Cox proportional hazards models were used to investigate time to first exacerbation or time
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15 137 to first loss of control. Each subgroup was considered separately and all models included
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17 138 adjustment for covariates associated with the outcome including: age, a variable for each RCT and
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19 139 ICS dose at baseline (this was not included in the ICS dose subgroup model). Standard statistical
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21 140 software was used (STATA version 14) and significance was assumed at 5%. All analyses were
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23 141 exploratory, so no adjustment was made for multiple comparisons.
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143 **RESULTS**

144 **Study subjects**

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

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

	Fritsch[18]	Peirman[19]	Petsky[20]	Pijnenburg[21]	Pike[22]	Szeffler[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 _E NO (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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3 **155 F_ENO intervention and asthma exacerbation outcomes**
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5 156 *Any exacerbation.* Of the 1047 participants for whom exacerbation data were available, 296 (28%)
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7 157 had at least one exacerbation with the first occurring after a median (interquartile range IQR) 22 (14,
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9 158 38) weeks. Table 3 shows the effect of treatment group was different for the two LTRA subgroups
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11 159 (interaction p-value = 0.039). Those not treated with LTRA, had lower odds for ≥ 1 exacerbation in
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13 160 the F_ENO guided group compared to standard care (OR=0.68, 95%CI 0.49-0.94) but there was no
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15 161 difference observed between F_ENO guided and control groups for those on LTRA, table 3. The
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17 162 number needed to treat with F_ENO guided management to prevent one exacerbation among those
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19 163 not treated with LTRA was 15. Interactions between treatment arm and other baseline
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21 164 characteristics (ICS dose, obese, atopy and white ethnicity) were not significant when predicting
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23 165 exacerbation, table 3.
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30 167 *Time to first exacerbation.* Overall in the two treatment groups, the median time to first
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32 168 exacerbation was 22 (IQR 14, 38) weeks in the standard arm and 22 (IQR 13, 34) in the F_ENO guided
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34 169 arm. The interaction term between treatment arm and LTRA was of borderline significance for time
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36 170 for first exacerbation (p=0.049), and among those not treated with LTRA at baseline, the time to first
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38 171 asthma exacerbation was slightly longer for participants receiving F_ENO guided treatment compared
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40 172 to standard care (HR=0.76, 0.57-0.99, p=0.048), table 4 and figure 1. Time to first exacerbation was
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42 173 no different between treatment groups for those treated with LTRA. The interaction terms with
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44 174 treatment arm were not significant for ICS dose, atopy, obesity or ethnicity, table 4.
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175 Table 3. Proportion of individuals with any asthma exacerbation in F_ENO -guided and standard
 176 management arms of clinical trials with stratification for patient characteristics. ICS=inhaled
 177 corticosteroids, presented as ≤400 or >400 micrograms budesonide equivalent. Obesity was defined
 178 by International Obesity Task Force criteria.

Baseline characteristic		% with ≥1 exacerbation in each treatment arm		F _E NO vs standard		p value for interaction*
		F _E NO 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)	

179
 180 *adjusted for RCT population, age and (except the analysis for higher versus lower ICS dose) dose of
 181 inhaled corticosteroid (budesonide equivalent).

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

Sub group		Hazard Ratio for time to first exacerbation for participants where treatment was guided by F _E NO 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	

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

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3 **191 FeNO intervention and asthma control outcomes**
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5 **192** *Any loss of asthma control.* There were 787 participants who were controlled at baseline; 336 (43%)
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7 **193** remaining controlled until completion of the trial, 344 (44%) lost control and 107 (14%) were lost to
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10 **194** follow up for this outcome. The median (IQR) time to loss of control in these 344 patients was 22
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12 **195** (13, 30) weeks. There was no difference in mean age between those who did and did not lose
13
14 **196** control (12.8 (SD 3.0) and 12.6 (SD 2.9) years respectively) and no difference in baseline ICS dose
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16 **197** (median (IQR) 400 (400, 1000) for both those who did and did not lose control). The interaction
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18 **198** terms between treatment arm and the five baseline participant characteristics for loss of asthma
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20 **199** control were non-significant, supplemental table 3. However, there was an indication of reduced
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22 **200** odds of loss of control in the F_ENO arm versus standard arm in those subgroups of participants who
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24 **201** were not on LTRA at baseline, and in those who were not obese at baseline (supplemental table 3).
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27 **202** The number of controlled participants needed to treat with F_ENO guided management to prevent
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29 **203** one losing control among those not treated with LTRA was 11.
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33 **204**

34 **205** *Time to loss of control.* Within the subgroup who lost control (n=344) the median (IQR) time to loss
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36 **206** of control was 17 (13, 30) weeks with standard treatment and 22 (13, 34) weeks with F_ENO guided
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38 **207** treatment. The interaction terms with treatment arm were not significant for ICS dose ≤400 microg
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40 **208** versus >400 microg, atopy, LTRA treatment, white versus other race or obese (yes or no), table 5.
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43 **209** There was borderline evidence of a longer time to first loss of control for F_ENO guided compared to
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45 **210** standard treatment within subgroups who were not treated with LTRA (HR 0.77 [0.60, 0.99] figure
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47 **211** 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]
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49 **212** supplemental figure 1), table 5.
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215 Table 5. Results from cox regression models analysing time to first loss of control for subgroups of
 216 participants all of whom were controlled at baseline.

		Hazard Ratio for time to first exacerbation for participants where treatment was guided by F _E NO 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	

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

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DISCUSSION

We analysed data collected in seven RCTs to test the hypothesis that there are subgroups of patients where $F_{E}NO$ 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 $F_{E}NO$ -guided arm compared to standard treatment. The significant interaction term for LTRA treatment and treatment for exacerbation indicated that $F_{E}NO$ 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 $F_{E}NO$ 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), $F_{E}NO$ -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 $F_{E}NO$ -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 $F_{E}NO$ -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

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3 246 inclusion criterion for four cohorts [18,19,21,23] and the atopic subgroup were no more or less likely
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5 247 to have an exacerbation or to lose control within the trials. Since $F_{E}NO$ is considered to be a
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7 248 surrogate for allergic or eosinophilic airway inflammation [31] it is biologically plausible that $F_{E}NO$ -
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9 249 guided treatment algorithms are more likely to suppress airway inflammation and improve asthma
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11 250 control. Further evidence of biological plausibility comes from an RCT whose data are included in
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13 251 our analysis [14] which found fewer days with maximal symptoms among those with elevated IgE
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15 252 and multiple positive skin prick tests. Although non-atopic asthma is less common than atopic
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17 253 asthma, e.g. present in 18% of participants in the three trials which did not include only atopic
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19 254 participants [14,20,22], asthma is a very common condition and there are approximately 150-
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21 255 200,000 non-atopic asthmatic children in the UK [1]. There is a need to establish whether treatment
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23 256 and monitoring for atopic and nonatopic children should be the same.

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28 257 The magnitude of significantly reduced risk for exacerbations and loss of control in the intervention
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30 258 compared to standard treatment was typically 25-30% and this difference is clinically meaningful
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32 259 since it is consistent with the benefit seen from commonly-used asthma treatments such as LTRA
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34 260 and ICS. Knorr et al [32] report a 23% reduced incidence of exacerbations in young children treated
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36 261 with montelukast compared to placebo. The review by Calpin et al[33] reports a 32% reduced risk for
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38 262 oral steroid treatment for exacerbations among children treated with ICS compared to placebo.

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42 263 The RCTs included in our study applied different inclusion criteria, $F_{E}NO$ -guided treatment algorithms
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44 264 and asthma control scores, and these methodological differences will weaken any relationship
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46 265 between the intervention and asthma outcomes. The seven RCTs did apply a standard definition of
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48 266 exacerbation and apparatus for measuring $F_{E}NO$. Despite the differences between RCTs, we still
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50 267 observed differences in outcomes between some of the subgroups studied, and it is likely that the
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52 268 magnitude of difference that we report in outcomes between the subgroups stratified by LTRA
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54 269 treatment, obesity and atopy may be an underestimate of the true value.
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3 270 Our study was not designed to determine why F_ENO guided treatment was associated with improved
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5 271 asthma outcomes among those not treated with LTRA compared to participants receiving LTRA
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7 272 treatment. Treatment with LTRA is known to reduce F_ENO by approximately 25% in children with
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9 273 atopic asthma [27] and may plausibly confound F_ENO-guided treatment, especially since the RCT
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11 274 treatment algorithms did not consider the effect of LTRA on F_ENO. There is an alternative
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13 275 explanation for the differences in exacerbation outcomes associated with LTRA treatment in
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15 276 different RCT arms; those treated with LTRA were younger and had more severe asthma (including
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17 277 higher ICS dose, needing LABA treatment and almost twice the exacerbation prevalence) and F_ENO-
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19 278 guided asthma treatment may be less effective in more severe asthma rather than in children
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21 279 receiving LTRA treatment *per se*. Given that LTRA are commonly used in asthma treatment, there is
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23 280 a need to study the impact of LTRA treatment on F_ENO-guided asthma treatment.

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28 281 We observed that when data from the RCTs were combined, F_ENO-guided asthma treatment was
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30 282 associated with reduced risk for loss of control and time to loss of control among non-obese
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32 283 children. This contrasts with the findings of an RCT whose data are included in the present analysis
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34 284 [14] which reported fewer symptoms among obese participants (i.e. with BMI>30kg/m²) receiving
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36 285 F_ENO -guided treatment. This apparent inconsistency may be due to several factors. First the
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38 286 outcome in the paper by Szeffler *et al* [14] was days of maximal symptoms, but this variable was not
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40 287 available in all the RCTs included in the present paper and therefore loss of control was the outcome
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42 288 analysed here. Second, participants were all of African American or Hispanic ethnic origin, on higher
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44 289 ICS dose and had a considerably higher obesity prevalence[14], and some or all of these difference
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46 290 characteristics could explain different outcomes compared to the remaining six RCT participants. In
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48 291 our study, the reduced odds for loss of control and time to loss of control for non-obese children
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50 292 receiving F_ENO -guided treatment compared to standard treatment is likely to be underestimated
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52 293 due to inclusion of F_ENO and asthma control data from the RCT of Szeffler *et al* [14].
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3 294 There are some limitations to our study. First, the time to loss of control or first exacerbation was
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5 295 restricted to the predetermined assessment periods and this lack of precision will weaken the
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7 296 reported differences in these outcomes between sub groups. Secondly, the RCTs had different study
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10 297 designs with different step-up/step-down criteria and management regimes. Third, ethnicity data
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12 298 was only available for four of the seven RCTs and was therefore not included as a covariate in the
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14 299 models, but ideally we would have included ethnicity in our model since ethnicity was associated
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16 300 with differences between the other subgroups analysed(supplemental table 2) . A final limitation is
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18 301 that self-reported ICS adherence was available in only three RCTs included in our study [14,22,23]
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20 302 we were not able to compare outcomes between treatment arms between adherent and non-
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22 303 adherent participants. Future research could test the hypothesis that asthma outcomes are
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24 304 improved by F_ENO-guided treatment in adherent compared to non-adherent patients.
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31 306 In summary, we have used data from more than 1000 asthmatic children and report that F_ENO-
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33 307 guided treatment lead to better asthma outcomes among those not treated with LTRA. These
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35 308 findings support calls for individualised treatment for asthma [7].
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3 408 **FIGURE LEGEND**
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5 409 Figure 1. Kaplan Meier curves showing time to first exacerbation for patients whose asthma
6 410 treatment was guided by either fractional exhaled nitric oxide ("FENO") or by symptoms only
7 411 ("standard") and stratified by leukotriene receptor antagonist (LTRA) treatment. The difference
8 412 between treatment arms was significant for those not treated with LTRA ($p=0.048$) but not for the
9 413 patients treated with LTRA ($p=0.292$).
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14 415 Figure 2. Kaplan Meier curves showing time to loss of control for patients who were initially
15 416 controlled and whose asthma treatment was guided by either fractional exhaled nitric oxide
16 417 ("FENO") or by symptoms only ("standard") and stratified by leukotriene receptor antagonist (LTRA)
17 418 treatment. The difference between treatment arms was significant for those not treated with LTRA
18 419 ($p=0.038$) but not for the patients treated with LTRA ($p=0.822$).
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22 421 Figure 3. Kaplan Meier curves showing time to loss of control for patients who were initially
23 422 controlled and whose asthma treatment was guided by either fractional exhaled nitric oxide
24 423 ("FENO") or by symptoms only ("standard") and stratified by obese status. The difference between
25 424 treatment arms was significant for those who were not obese ($p=0.042$) but not for the patients who
26 425 were obese ($p=0.538$).
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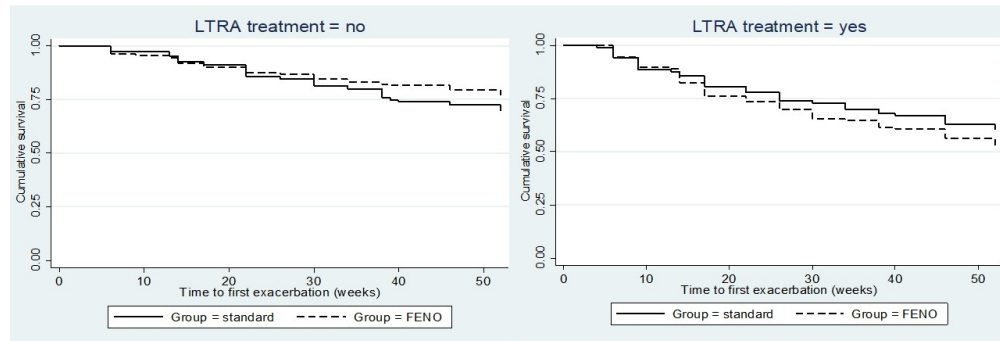


Figure 1. Kaplan Meier curves showing time to first exacerbation for patients whose asthma treatment was guided by either fractional exhaled nitric oxide ("FENO") or by symptoms only ("standard") and stratified by leukotriene receptor antagonist (LTRA) treatment. The difference between treatment arms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

338x190mm (96 x 96 DPI)

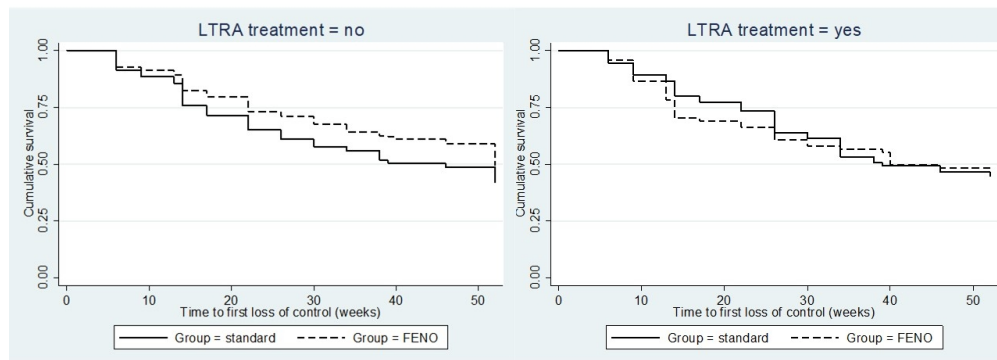


Figure 2. Kaplan Meier curves showing time to loss of control for patients who were initially controlled and whose asthma treatment was guided by either fractional exhaled nitric oxide ("FENO") or by symptoms only ("standard") and stratified by leukotriene receptor antagonist (LTRA) treatment. The difference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

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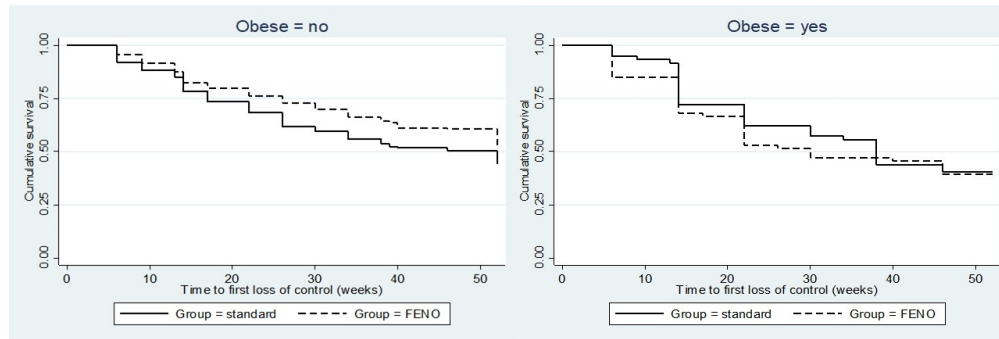


Figure 3. Kaplan Meier curves showing time to loss of control for patients who were initially controlled and whose asthma treatment was guided by either fractional exhaled nitric oxide ("FENO") or by symptoms only ("standard") and stratified by obese status. The difference between treatment arms was 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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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.

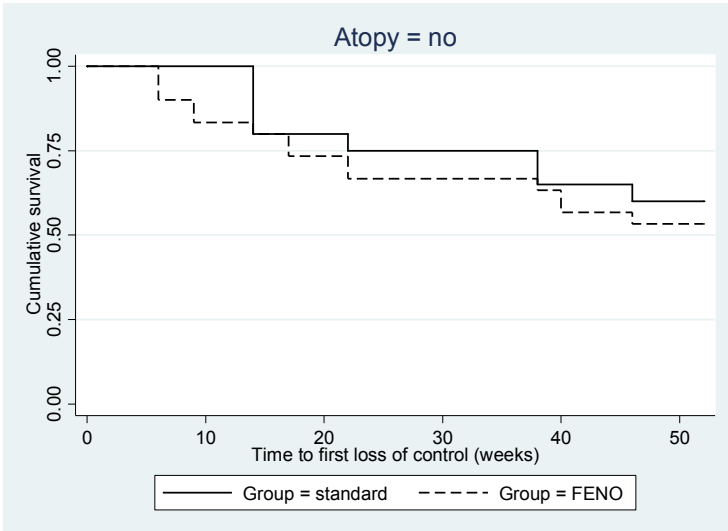
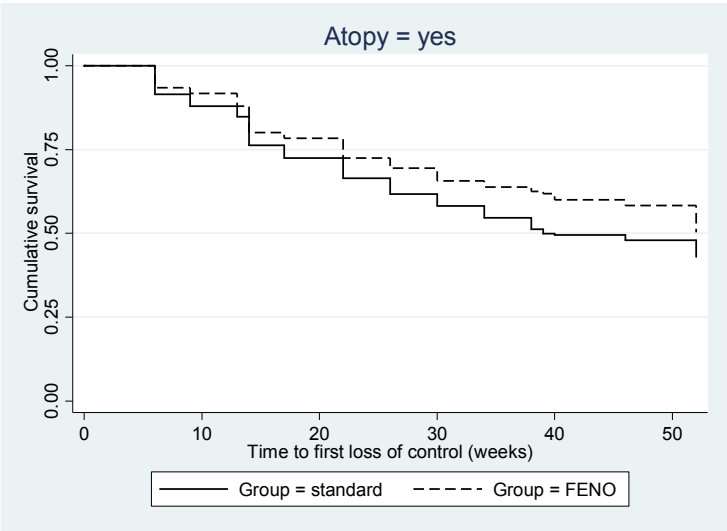
Supplemental table 3. Proportion of individuals who were initially controlled who lost control in F_ENO -guided and standard management arms of clinical trials with stratification for patient characteristics. ICS=inhaled corticosteroids, presented as ≤400 or >400 micrograms budesonide equivalent. Obesity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

Baseline characteristic		% with loss of control during follow-up [#] in each treatment arm		F _E NO vs standard		p value for interaction*
		F _E NO 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

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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 ($p=0.566$).



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3 1 Does treatment guided by fractional exhaled nitric oxide improve outcomes in subgroups of children
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26 Word count: 2611

27 **ABSTRACT**

28 Introduction. Fractional exhaled nitric oxide ($F_{E}NO$), a biomarker of eosinophilic airway
29 inflammation, may be useful to guide asthma treatment. $F_{E}NO$ guided treatment may be more
30 effective in certain subgroups for improving asthma outcomes compared to standard treatment.

31 Methods. An individual patient data analysis was performed using data from seven randomised
32 clinical trials (RCT) which used $F_{E}NO$ to guide asthma treatment. The incidence of an asthma
33 exacerbation and loss of control, and the time to first exacerbation and loss of control were
34 described between five ~~plausible~~ subgroups of RCT participants.

35 Results. Data were available in 1112 RCT participants. Among those not treated with LTRA (but not
36 among those who were treated with LTRA), $F_{E}NO$ guided treatment was associated with reduced
37 exacerbation risk (odds ratio (OR) 0.68 [95% CI 0.49, 0.94]), longer time to first exacerbation (hazard
38 ratio (HR) 0.76 [0.57, 0.99]) and borderline reduced risk for loss of control (OR 0.70 [0.49, 1.00]).

39 Non-obese children, compared to obese children, were less likely to lose asthma control when
40 treatment was guided by $F_{E}NO$ (OR 0.69 [0.48, 0.99]) and time to loss of control was longer (HR 0.77
41 [0.61, 0.99]). ~~In atopic children, $F_{E}NO$ guided treatment had no effect on the risk of loss of control
42 per se, but increased the time to loss of control, compared to nonatopic children.~~

43 Conclusions. Asthma treatment guided by $F_{E}NO$ may be more effective in achieving better asthma
44 outcomes for patients who are not treated with LTRA and who are not obese ~~or who are atopic~~
45 compared to standard practice.

46 Keywords: Asthma, Child, Monitoring, Nitric oxide

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56 51 **INTRODUCTION**

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9 52 Asthma is a common chronic condition which affects one million children in the UK [1], six million in
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11 53 the US[2] and 235 million children and adults around the world [3]. There is effective treatment to
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13 54 control asthma symptoms and ~~whilst~~ guidelines recommend that treatment should be titrated to
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15 55 asthma symptoms[4-6]. ~~There~~ remains a widely accepted recognition that an objective
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17 56 measurement to guide asthma treatment is required [7].

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21 57 Fractional exhaled nitric oxide ($F_{E}NO$) in exhaled breath has many of the characteristics required of
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23 58 an objective tool to measure asthma symptoms. ~~For example, $F_{E}NO$ since it~~ rises before symptoms
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25 59 occur [8,9], falls when asthma treatment is administered [10,11], can be measured with minimal
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27 60 discomfort to the patient and results are available within a few minutes using commercially available
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29 61 apparatus [12]. A meta-analysis including eight clinical trials in children and young adults found that
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31 62 addition of $F_{E}NO$ measurements to symptom-guided treatment did not reduce asthma symptoms
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33 63 [13], but that $F_{E}NO$ guided treatment reduced asthma exacerbations [13].

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37 64 Asthma is a heterogeneous condition and what ~~is~~ we do not know is whether there are patient sub-
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39 65 groups in whom using $F_{E}NO$ to guide asthma treatment may be beneficial [7]. In one randomised
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41 66 controlled trial (RCT), the intervention was more effective in participants who had more positive skin
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43 67 tests and who were obese, but age, sex, asthma severity and initial $F_{E}NO$ concentration were not
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45 68 associated with a different outcome from the intervention [14]. In a second RCT there was no
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47 69 evidence of improved outcomes between individuals who were concordant or discordant for FENO
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49 70 and symptoms [15].

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53 71 Our group has pooled the data collected from seven of the eight published RCTs where the efficacy
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55 72 of $F_{E}NO$ used to guide asthma treatment was examined, compared to standard management [16].
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57 73 Here we use data from 1112 participants to test the hypothesis that there are particular subgroups
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3 74 of patients where F_ENO guided treatment is more effective in improving asthma outcomes
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11 77 **METHODS**

14 78 **Study design**

17 79 Authors of all published RCTs where measurements of F_ENO were used to guide asthma treatment in
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19 80 children [17] were contacted and asked to provide data as previously described [16]. The children
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21 81 who took part in the studies were recruited from hospital clinics and were followed up for between
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24 82 six and 12 months. The primary outcome was the presence of any asthma exacerbation during
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26 83 follow up [13]. Secondary outcomes were loss of control among those who were initially controlled
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28 84 and time to first exacerbation and time to first loss of control. Institutional ethical approval was
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30 85 provided for each trial which contributed data.
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33 86 **Details of each population (also see table one)**

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36 87 Fritsch *et al* [18] undertook a study of 47 children with asthma attending a hospital asthma clinic in
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38 88 Vienna, Austria and collected data (including F_ENO, asthma symptom score and history of recent
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40 89 exacerbations) at six-week intervals over six months. Peirsman *et al* [19] recruited 99 participants
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42 90 with persistent asthma attending hospital asthma clinics across Belgium and collected data at three-
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45 91 month intervals over twelve months. Petsky *et al* [20] recruited 63 children from hospital clinics in
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47 92 Australia and Hong Kong, and data were collected on eight occasions over twelve months (one, two,
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49 93 three, four, six, eight, ten and twelve months). Pijnenburg *et al* [21] included 86 participants
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51 94 attending a single hospital clinic in the Netherlands and data were collected at baseline, three, six,
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53 95 nine and twelve months. Pike *et al* [22] recruited 90 participants clinics in fourthe UK hospitals and
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55 96 collected data at two-month intervals over a year. Szeffler *et al* [14] recruited 546 participants from
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57 97 the community in the USA and collected post-randomisation information over 46 weeks including at
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3 98 three months, six months, eight months and ten months. Voorend-van Bergen *et al* [23] undertook
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5 99 a study of 181 participants [attending hospital clinics](#) in the Netherlands and collected data at four-
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8 100 month intervals over a year. The treatment algorithms in F_ENO-guided and standard practice arms in
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10 101 each RCT was different to other RCTs.
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102 Table one. A summary of characteristics of the randomised controlled trials whose data were used for the present analysis.

	<u>Mean age (SD), y</u>	<u>Inclusion criteria (in addition to child diagnosed with asthma)</u>	<u>Methodology for asthma control</u>	<u>Treatment strategy for intervention group</u>	<u>Treatment strategy for control group</u>	<u>Treatment options (same for both groups in all studies)</u>	<u>What did the trial find? (F_ENO treatment compared to standard care)</u>
<u>Fritsch <i>et al</i> 2006¹ Austria</u>	<u>11.5 (3.1)</u>	<u>Age 6-18 years. Sensitised to inhaled allergens. No systemic corticosteroids one month before recruitment.</u>	<u>Unvalidated symptom diary</u>	<u>Combination of symptom score, FEV₁ <80% and F_ENO>20ppb</u>	<u>Combination of symptom score and FEV₁ <80%</u>	<u>Four treatments steps</u>	<u>Higher mid expiratory flow, higher dose of ICS</u>
<u>Peirsman <i>et al</i> 2014² Belgium</u>	<u>10.7 (2.1)</u>	<u>Sensitised to inhaled allergens. No exacerbation or systemic corticosteroids three month before recruitment</u>	<u>First four (of seven) questions on ACT*</u>	<u>Combination of symptom >score, exacerbation in previous two weeks, FEV₁ <80% and F_ENO>20ppb</u>	<u>Combination of symptom score, exacerbation in previous two weeks and FEV₁ <80%</u>	<u>Step up and down options if on the following preventers: ICS alone; LTRA alone; ICS+LABA; ICS+LTRA</u>	<u>Reduced exacerbations, increased LTRA and ICS dose. No difference in primary outcome</u>
<u>Petsky <i>et al</i> 2015³ Australia</u>	<u>10.0 (3.2)</u>	<u>Aged >4 years. Prescribed asthma preventer. Adherent to treatment</u>	<u>Validated symptom diary†</u>	<u>Combination of symptom score plus F_ENO> 10 for non atopic, >12 with one positive skin test, >20 for >1 positive skin test</u>	<u>Symptom score alone</u>	<u>Seven steps (none including LTRA)</u>	<u>Reduced exacerbation, increased ICS dose</u>
<u>Pijnenburg <i>et al</i> 2005⁴ Netherlands</u>	<u>12.3 (2.8)</u>	<u>Aged 6-18 years. Sensitised to inhaled allergens. ICS dose unchanged for ≥3 months at recruitment</u>	<u>Validated symptom diary‡</u>	<u>Treatment stepped up if F_ENO>30ppb. Treatment stepped down if symptoms</u>	<u>Symptom score alone</u>	<u>Nine steps (none including LABA or LTRA)</u>	<u>Reduced F_ENO and bronchial hyperresponsiveness No increase in ICS dose</u>

				<u>controlled and F_ENO≤30ppb</u>			
<u>Pike et al 2013⁵</u> <u>UK</u>	<u>11.9 (2.6)</u>	<u>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.</u>	<u>Modified validated symptom diary</u> ‡	<u>Combination of symptoms, recent reliever medication use, FEV₁ >90%, 80-90% or <80% and F_ENO≤15, 15-25 or ≥25ppb</u>	<u>Combination of symptoms, recent reliever medication use, and FEV₁ >90%, 80-90% or <80%</u>	<u>Eight treatment steps</u>	<u>No differences in outcomes</u>
<u>Szeffler et al 2008⁶</u> <u>USA</u>	<u>14.4 (2.1)</u>	<u>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.</u>	<u>ACT*</u>	<u>Combination of symptoms, FEV₁ ≥80, 70-79% or >70% and F_ENO 0-20, 20.1-30, 30.1-40 or >40ppb</u>	<u>Combination of symptoms and FEV₁ ≥80, 70-79% or >70%</u>	<u>Seven treatment steps (including low dose theophylline)</u>	<u>Reduced exacerbations, increased ICS dose. No difference in primary outcome.</u>
<u>Voorend-van Bergen et al 2010⁸</u> <u>Netherlands</u>	<u>10.2 (3.0)</u>	<u>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.</u>	<u>ACT*</u>	<u>Combination of symptom score and F_ENO <20, 20-50 or >50ppb</u>	<u>Symptom score alone</u>	<u>Seven treatment steps</u>	<u>Increased asthma control but not the primary outcome</u>

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

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

105 †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.

106 ‡Wasserfallen JB, et al. *J Allergy Clin Immunol* 1997;100: 16–22.

107 **Data collected**

108 Covariates collected at baseline in all trials included: age, gender, height, weight, treatment arm,
109 dose of inhaled corticosteroid (ICS, as daily budesonide equivalent dose, BUD), prescribed long
110 acting beta agonist (LABA) or not, prescribed leukotriene receptor agonist (LTRA) or not, and an
111 asthma control score. Ethnicity was available in four cohorts[14,21-23]. Body Mass Index (BMI) was
112 derived and International Obesity Task Force weight categories created [24]. Percentage of
113 predicted (%) Forced Expired Volume in one second (FEV₁-) was calculated according to the Global
114 Lung Initiative standard [25] apart from participants in two trials [21,22] where only % FEV₁
115 standardised to other references was available. F_ENO was measured in all studies in accordance with
116 the 2005 guideline [26]. At each follow up visit, ~~the following variables were collected:~~ an
117 assessment of asthma control was made (see table 1) and history of any asthma attack since the
118 previous assessment was recorded (defined as receipt of oral corticosteroids for an asthma
119 exacerbation [16]). The trials used different symptom score methodology and loss of control was
120 defined as per trial protocol by reaching a pre-agreed symptom score.

122 **Analysis**

123 Asthma outcomes were compared between participants in the F_ENO guided and standard treatment
124 arms of RCTs for the following five subgroups defined at baseline and previously associated with
125 differences in F_ENO. The five subgroups were stratified by: dose of ~~inhaled corticosteroid (ICS (-~~
126 ~~≤400 microg budesonide equivalent or >400 microg)[10], use of LTRA [27], obesity [14], ethnicity~~
127 ~~(white versus other)[28] and skin-prick-positivityatopic (i.e. positive skin prick test or positive type-~~
128 ~~specific IgE) [14]. Any exacerbation during follow up and time to first exacerbation and any loss of~~
129 ~~control and time to loss of control were calculated (the latter restricted to those who were~~
130 ~~controlled at baseline). Time to first exacerbation or to loss of control was determined using data~~
131 ~~collected at the scheduled study assessments, and table one in the supplement describes the time in~~

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3 132 weeks between baseline and each follow up assessment in each RCT. For example, if a participant
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5 133 experienced an exacerbation after their three-month assessment but before the six month
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7 134 assessment, time was censored at six months. Logistic regression was used to relate any
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10 135 exacerbation or any loss of control to an interaction term between each baseline characteristic and
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12 136 treatment arm; a significant interaction term ($p < 0.05$) would indicate that outcomes were different
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14 137 between $F_{E}NO$ guided and standard treatment for a sub group. Cox proportional hazards models
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16 138 were used to investigate time to first exacerbation or time to first loss of control. Each subgroup
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18 139 was considered separately and all models included adjustment for covariates associated with the
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21 140 outcome including: age, a variable for each RCT and ICS dose at baseline (this was not included in the
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23 141 ICS dose sub group model). Standard statistical software was used (STATA version 14) and
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25 142 significance was assumed at 5%. All analyses were exploratory, so no adjustment was made for
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28 143 multiple comparisons.
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33 145 **RESULTS**

36 146 **Study subjects**

38 147 Data from seven RCTs were analysed [14,18-23], totalling 1112 participants. Characteristics of
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41 148 participants at baseline have previously been described [16] and are presented in table 21. The
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43 149 majority of participants (58%) were male and the mean age was 12.6 (standard deviation, SD 3.1)
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45 150 years. Characteristics of participants in the five subgroups are presented in supplemental table 2,
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48 151 i.e. LTRA treatment (yes/no), ICS dose ≤ 400 microg/ >400 microg), obese (yes/no), skin prick
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50 152 positive atopic (yes/no) and white versus other ethnic group.
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154 Table 21. Characteristic of study participants at the baseline visit in each study.

	Fritsch[18]	Peirman[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 _E NO (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% (312/901889)	
Control status	Controlled	49% (23/47)	75% (49/65)	72% (41/57)	57% (44/77)	97% (87/90)	80% (421/528)	67% (122/181)	75% (787/1045)
	Not Controlled	51% (24/47)	25% (16/65)	28% (16/57)	43% (33/77)	3% (3/90)	20% (107/528)	33% (59/181)	24% (258/1045)

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

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157 **F_ENO intervention and asthma exacerbation outcomes**

158 *Any exacerbation.* Of the 1047 participants for whom exacerbation data were available, 296 (28%)
159 had at least one exacerbation with the first occurring after a median ([interquartile range IQR](#)) 22
160 (14, 38) weeks. Table [32](#) shows the effect of treatment group was different for the two LTRA
161 subgroups (interaction p-value = 0.039). Those not treated with LTRA, had lower odds for ≥1
162 exacerbation in the F_ENO guided group compared to standard care (OR=0.68, 95%CI 0.49-0.94) but
163 there was no difference observed between F_ENO guided and control groups for those on LTRA, table
164 [32](#). [The number needed to treat with F_ENO guided management to prevent one exacerbation](#)
165 [among those not treated with LTRA was 15](#). Interactions between treatment arm and other baseline
166 characteristics (ICS dose, obese, [skin-prick-positive atopy](#) and white ethnicity) were not significant
167 when predicting exacerbation, table [32](#).

168
169 *Time to first exacerbation.* Overall in the two treatment groups, the median time to first
170 exacerbation was 22 (IQR 14, 38) weeks in the standard arm and 22 (IQR 13, 34) in the F_ENO guided
171 arm. The interaction term between treatment arm and LTRA was of borderline significance for time
172 for first exacerbation (p=0.049), and among those not treated with LTRA at baseline, the time to first
173 asthma exacerbation was slightly [longer shorter](#) for participants receiving F_ENO guided treatment
174 compared to standard care (HR=0.76, 0.57-0.99, p=0.048), table [43](#) and figure 1. Time to first
175 exacerbation was no different between treatment groups for those treated with LTRA. The
176 interaction terms with treatment arm were not significant for ICS dose, [skin-prick test results atopy](#),
177 obesity or ethnicity, table [43](#).

178 Table 32. Proportion of individuals with any asthma exacerbation in F_ENO -guided and standard
 179 management arms of clinical trials with stratification for patient characteristics. ICS=inhaled
 180 corticosteroids, presented as ≤400 or >400 micrograms budesonide equivalent. Obesity was defined
 181 by International Obesity Task Force criteria.

Baseline characteristic		% with ≥1 exacerbation in each treatment arm		F _E NO vs standard		p value for interaction*
		F _E NO 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)	

182
 183 *adjusted for RCT population, age and (except the analysis for higher versus lower ICS dose) dose of
 184 inhaled corticosteroid (budesonide equivalent).

186 Table 43. Results from Cox regression models analysing time to first exacerbation for subgroups of
 187 participants.

Sub group		Hazard Ratio for time to first exacerbation for participants where treatment was guided by F _E NO 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	

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

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3 194 **FeNO intervention and asthma control outcomes**
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5 195 *Any loss of asthma control.* There were 787 participants who were controlled at baseline; 336 (43%)
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7 196 remaining controlled until completion of the trial, 344 (44%) lost control and 107 (14%) were lost to
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10 197 follow up for this outcome. The median (IQR) time to loss of control in these 344 patients was 22
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12 198 (13, 30) weeks. There was no difference in mean age between those who did and did not lose
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14 199 control (12.8 (SD 3.0) and 12.6 (SD 2.9) years respectively) and no difference in baseline ICS dose
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16 200 (median (IQR) 400 (400, 1000) for both those who did and did not lose control). The interaction
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18 201 terms between treatment arm and the five baseline participant characteristics for loss of asthma
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20 202 control were non-significant, [supplemental table 34](#). However, there was an indication of reduced
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22 203 odds of loss of control in the FeNO arm versus standard arm in those subgroups of participants who
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24 204 were not on LTRA at baseline, and in those who were not obese at baseline ([supplemental table](#)
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26 205 [34](#)). [The number of controlled participants needed to treat with FeNO guided management to](#)
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28 206 [prevent one losing control among those not treated with LTRA was 11.](#)
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35 208 *Time to loss of control.* Within the subgroup who lost control (n=344) the median (IQR) time to loss
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37 209 of control was 17 (13, 30) weeks with standard treatment and 22 (13, 34) weeks with FeNO guided
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39 210 treatment. The interaction terms with treatment arm were not significant for ICS dose ≤ 400 microg
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41 211 versus >400 microg, ~~positive skin prick test~~atopy, LTRA treatment, white versus other race or obese
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43 212 (yes or no), table 5. There was borderline evidence of a longer time to first loss of control for FeNO
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45 213 guided compared to standard treatment within subgroups who were [not treated with LTRA \(HR 0.77](#)
46
47 214 [\[0.60, 0.99\] figure 2](#)), [non-obese \(HR 0.77 \[95% CI 0.61, 0.99\] figure 3\)](#) and atopic (HR 0.80 [95% CI
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49 215 0.63, 1.00] [supplemental figure 1](#)), table 5 ~~and supplemental figure 2, non-obese (HR 0.77 [95% CI~~
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51 216 [0.61, 0.99\]](#)) table 5 ~~and supplemental figure 3.~~
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218 Table 4. Proportion of individuals who were initially controlled who lost control in F_ENO-guided and standard management arms of clinical trials with
 219 stratification for patient characteristics. ICS=inhaled corticosteroids, presented as ≤400 or >400 micrograms budesonide equivalent. Obesity was defined by
 220 International Obesity Task Force criteria.

Baseline characteristic		% with loss of control during follow-up [#] in each treatment arm		F _E NO vs standard		p-value for interaction*
		F _E NO-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)	

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

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

		Hazard Ratio for time to first exacerbation for participants where treatment was guided by F _E NO 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	

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

228

229 **DISCUSSION**

230 ~~The role of F_ENO in guiding asthma treatment in children is unclear, and one potential explanation~~
231 ~~for this could be that F_ENO is more effective in improving asthma outcomes for some subgroups of~~
232 ~~the population.~~ We analysed data collected in seven RCTs to test the hypothesis that there are
233 subgroups of patients where F_ENO guided treatment is more effective in improving asthma
234 outcomes compared to standard treatment. The main finding was that within these RCTs, the odds
235 for exacerbation and loss of control for those not treated with LTRA were 32% and 30% lower in the
236 F_ENO-guided arm compared to standard treatment. The significant interaction term for LTRA
237 treatment and treatment for exacerbation indicated that FeNO driven management may have
238 reduced exacerbations for those not treated with LTRA but not among those treated with LTRA. A
239 second finding was that oOutcomes were no different between groups stratified by LABA treatment,
240 ICS dose, and ethnic group. Collectively these findings support the hypothesis that F_ENO is more
241 useful for guiding treatment compared to standard practice in some subgroups of children with
242 asthma not treated with LTRA.

243 A ~~further second~~ finding was that in non-obese participants (but not in obese participants), F_ENO-
244 guided treatment was associated with a 31% ~~reduction in ed~~ odds for loss of control compared to
245 standard treatment and when control was lost, time to loss of control was longer. Although the
246 interaction term for obesity and treatment for loss of control was not significant, we believe that the
247 improved outcomes for non-obese children merits further consideration. There was consistency in
248 our results (i.e. an association with any loss of control and time to loss of control) and also there is
249 biological plausibility whereby aAsthma associated with obesity may be a separate non-eosinophilic
250 phenotype, especially in females; [29]. and this may explain why F_ENO guided treatment was more
251 effective among non-obese children in our study. A recent systematic review found no evidence of
252 increased or reduced asthma control among children who were obese [30] and asthma guidelines do
253 not recommend different treatment approaches for obese patients with asthma [4-6]. Further

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3 254 research is required to clarify whether F_ENO-guided treatment is equally effective in obese and non-
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5 255 obese children.

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8 256 Our observation that time to loss of control was longer among children who were atopic receiving
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10 257 F_ENO-guided treatment compared to standard treatment deserves careful consideration. A third
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12 258 finding was that among children who were atopic, the time to loss of control was longer for those
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14 259 receiving F_ENO-guided treatment compared to standard treatment. Outcomes were no different
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16 260 between groups stratified by LABA treatment, ICS dose, and ethnic group. Collectively these findings
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18 261 support the hypothesis that F_ENO is more useful for guiding treatment compared to standard
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20 262 practice in some subgroups of children with asthma. The number of non-atopic participants included
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22 263 in our analysis was relatively small since atopy was an inclusion criterion for four cohorts
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24 264 [18,19,21,23] and the atopic subgroup were no more or less likely to have an exacerbation or to lose
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26 265 control within the trials. Since F_ENO is considered to be a surrogate for allergic or eosinophilic
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28 266 airway inflammation [31] it is biologically plausible that F_ENO-guided treatment algorithms are more
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30 267 likely to suppress airway inflammation and improve asthma control. Further evidence of biological
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32 268 plausibility comes from an RCT whose data are included in our analysis [14] which found fewer days
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34 269 with maximal symptoms among those with elevated IgE and multiple positive skin prick tests.
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36 270 Although non-atopic asthma is less common than atopic asthma, e.g. present in 18% of participants
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38 271 in the three trials which did not include only atopic participants [14,20,22], asthma is a very common
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40 272 condition and there are approximately 150-200,000 non-atopic asthmatic children in the UK [1].
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42 273 There is a need to establish whether treatment and monitoring for atopic and nonatopic children
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44 274 should be the same.

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46 275 The magnitude of significantly reduced risk for exacerbations and loss of control in the intervention
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48 276 compared to standard treatment was typically 25-30% and this difference is clinically meaningful
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50 277 since it is consistent with the benefit seen from commonly-used asthma treatments such as LTRA
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52 278 and ICS. Knorr et al [32] report a 23% reduced incidence of exacerbations in young children treated
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3 279 with montelukast compared to placebo. The review by Calpin et al[33] reports a 32% reduced risk for
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5 280 oral steroid treatment for exacerbations among children treated with ICS compared to placebo.
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11 282 The RCTs included in our study applied different inclusion criteria, F_ENO-guided treatment algorithms
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13 283 and asthma control scores, and these methodological differences will weaken any relationship
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15 284 between the intervention and asthma outcomes. The seven RCTs did apply a standard definition of
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17 285 exacerbation and apparatus for measuring F_ENO. Despite the differences between RCTs, we still
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19 286 observed differences in outcomes between some of the subgroups studied, and it is likely that the
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21 287 magnitude of difference that we report in outcomes between the subgroups stratified by LTRA
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23 288 treatment, obesity and atopy may be an underestimate of the true value.
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27 289 Our study was not designed to determine why F_ENO guided treatment was associated with improved
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29 290 asthma outcomes among those not treated with LTRA compared to participants receiving LTRA
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31 291 treatment. Treatment with LTRA is known to reduce F_ENO by approximately 25% in children with
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33 292 atopic asthma [27] and may plausibly confound F_ENO-guided treatment, especially since the RCT
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35 293 treatment algorithms did not consider the effect of LTRA on F_ENO. There is an alternative
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37 294 explanation for the differences in exacerbation outcomes associated with LTRA treatment in
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39 295 different RCT arms; those treated with LTRA were younger and had more severe asthma (including
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41 296 higher ICS dose, needing LABA treatment and almost twice the exacerbation prevalence) and F_ENO-
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43 297 guided asthma treatment may be less effective in more severe asthma rather than in children
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45 298 receiving LTRA treatment *per se*. Given that LTRA are commonly used in asthma treatment, there is
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47 299 a need to study the impact of LTRA treatment on F_ENO-guided asthma treatment.
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53 300 We observed that when data from the RCTs were combined, F_ENO-guided asthma treatment was
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55 301 associated with reduced risk for loss of control and time to loss of control among non-obese
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57 302 children. T, and t this contrasts with the findings of an RCT whose data are included in the present
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59 303 analysis [14] which reported fewer symptoms among obese participants (i.e. with BMI>30kg/m²)
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3 304 receiving F_ENO -guided treatment. This apparent inconsistency may be due to several factors. First
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5 305 the outcome in the paper by Szeffler *et al* [14] was days of maximal symptoms, but this variable was
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7 306 not available in all the RCTs included in the present paper and therefore loss of control was the
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10 307 outcome analysed here. Second, participants were all of African American or Hispanic ethnic origin,
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12 308 on higher ICS dose and had a considerably higher obesity prevalence[14], and some or all of these
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14 309 difference characteristics could explain different outcomes compared to the remaining six RCT
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16 310 participants. In our study, the reduced odds for loss of control and time to loss of control for non-
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18 311 obese children receiving F_ENO -guided treatment compared to standard treatment is likely to be
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20 312 underestimated due to inclusion of F_ENO and asthma control data from the RCT of Szeffler *et al* [14].
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24 313 ~~Asthma associated with obesity may be a separate non-eosinophilic phenotype, especially in~~
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26 314 ~~females, [29] and this may explain why F_ENO -guided treatment was more effective among non-~~
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28 315 ~~obese children in our study. A recent systematic review found no evidence of increased or reduced~~
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30 316 ~~asthma control among children who were obese [30] and asthma guidelines do not recommend~~
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32 317 ~~different treatment approaches for obese patients with asthma [4-6]. Further research is required~~
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34 318 ~~to clarify whether F_ENO-guided treatment is equally effective in obese and non-obese children.~~
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38 319 ~~We found evidence of borderline significance that F_ENO-guided treatment was associated with~~
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40 320 ~~longer time to loss of control compared to standard treatment among atopic children. This is~~
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42 321 ~~consistent with an RCT whose data are included in our analysis [14] which found fewer days with~~
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44 322 ~~maximal symptoms among those with elevated IgE and multiple positive skin prick tests. F_ENO is a~~
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46 323 ~~biomarker of eosinophilic airway inflammation [31] and likely to be more effective in patients with~~
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48 324 ~~atopy. The number of non-atopic participants included in our analysis was relatively small since~~
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50 325 ~~atopy was an inclusion criterion for four cohorts [18,19,21,23]. Although non-atopic asthma is less~~
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52 326 ~~common than atopic asthma, e.g. present in 18% of participants in the three trials which did not~~
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54 327 ~~include only atopic participants [14,20,22], asthma is a very common condition and there are~~
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3 328 ~~approximately 150-200,000 non-atopic asthmatic children in the UK [1] There is a need to establish~~
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5 329 ~~whether treatment and monitoring for atopic and nonatopic children should be the same.~~
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7
8 330 There are some limitations to our study. First, the time to loss of control or first exacerbation was
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10 331 restricted to the predetermined assessment periods and this lack of precision will weaken the
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12 332 reported differences in these outcomes between sub groups. Secondly, the RCTs had different study
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14 333 designs with different step-up/step-down criteria and management regimes. Third, ethnicity data
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16 334 was only available for four of the seven RCTs and was therefore not included as a covariate in the
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18 335 models, but ideally we would have included ethnicity in our model since ethnicity was associated
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20 336 with differences between the other subgroups analysed(supplemental table 2) . A final limitation is
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22 337 that Thirdly, since self-reported ICS adherence was available in only three RCTs included in our study
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24 338 [14,22,23] we were not able to compare outcomes between treatment arms between adherent and
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26 339 non-adherent participants. Future research could test the hypothesis that asthma outcomes are
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28 340 improved by F_ENO-guided treatment in adherent compared to non-adherent patients.
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36 342 In summary, we have used data from more than 1000 asthmatic children and report that F_ENO-
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38 343 guided treatment lead to better asthma outcomes among those not treated with LTRA, ~~who were~~
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40 344 ~~not obese and who were atopic~~. These findings support calls for individualised treatment for asthma
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445 **FIGURE LEGEND**

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

451
452 Figure 2. Kaplan Meier curves showing time to loss of control for patients who were initially
453 controlled and whose asthma treatment was guided by either fractional exhaled nitric oxide
454 ("FENO") or by symptoms only ("standard") and stratified by leukotriene receptor antagonist (LTRA)
455 treatment. The difference between treatment arms was significant for those not treated with LTRA
456 ($p=0.038$) but not for the patients treated with LTRA ($p=0.822$).

457
458 Figure 3. Kaplan Meier curves showing time to loss of control for patients who were initially
459 controlled and whose asthma treatment was guided by either fractional exhaled nitric oxide
460 ("FENO") or by symptoms only ("standard") and stratified by obese status. The difference between
461 treatment arms was significant for those who were not obese ($p=0.042$) but not for the patients who
462 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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. PAGES 5-6 <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed NOT APPLICABLE <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed PAGES 6-7 <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount) NOT APPLICABLE
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure PAGE 6-7 AND TABLE 1 <i>Cross-sectional study</i> —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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2 <http://www.annals.org/>, and *Epidemiology* at <http://www.epidem.com/>). Information on the STROBE Initiative is
3 available at www.strobe-statement.org.
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