ORIGINAL ARTICLE

A SYSTEMATIC REVIEW AND META-ANALYSIS: TAILORING ASTHMA TREATMENT ON EOSINOPHILIC MARKERS (EXHALED NITRIC OXIDE OR SPUTUM EOSINOPHILS)

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Key words: asthma, exhaled airway markers, pulmonary eosinophilia, paediatric asthma.

ABSTRACT

Background: Asthma guidelines guide health practitioners to adjust treatments to the minimum level required for asthma control. As many people with asthma have an eosinophilic endotype, tailoring asthma medications based on airway eosinophilic levels (sputum eosinophils or exhaled nitric oxide, FeNO) may improve asthma outcomes.

Objective: To synthesise the evidence from our updated Cochrane systematic reviews, for tailoring asthma medication based on eosinophilic inflammatory markers (sputum analysis and FeNO) for improving asthma-related outcomes in children and adults.

Data sources: Cochrane reviews with standardised searches up to February 2017.

Study selection: The Cochrane reviews included randomised controlled comparisons of tailoring asthma medications based on sputum analysis or FeNO compared to controls (primarily clinical symptoms and/or spirometry/peak flow).

Results: The 16 included studies of FeNO-based management (7 in adults) and 6 of sputum-based management (5 in adults) were clinically heterogeneous. On follow-up, participants randomised to the sputum eosinophils strategy (compared to controls) were significantly less likely to have exacerbations (62 vs 82/100 participants with ≥1 exacerbation; OR=0.36, 95%CI 0.21 to 0.62). For the FeNO strategy, the respective numbers were; adults OR=0.60, 95%CI 0.43 to 0.84 and; children 0.58 (95%CI 0.45 to 0.75). However, there were no significant group differences for either strategy on daily inhaled corticosteroids dose (at end of study), asthma control or lung function.

Conclusion: Adjusting treatment based on airway eosinophilic markers reduced the likelihood of asthma exacerbations but had no significant impact on asthma control or lung function.

What is the key question?

What is the overall outcome of trials that utilise eosinophilic markers (sputum eosinophil counts or exhaled nitric oxide levels, FeNO) to tailor asthma treatment in children and adults?

What is the key point?

Treatment tailored using eosinophilic markers results in fewer asthma attacks when compared to traditional management but did not impact on day-to-day reported symptoms, lung function or final daily inhaled corticosteroid doses.

Why read on?

This systematic review combines 3 Cochrane reviews with 22 included studies, examining the updated evidence for objectively measuring inflammatory markers to personalise asthma management.

INTRODUCTION

The main aim of asthma guidelines is to provide an evidence-based approach to assist health professionals improve their patients' asthma management, which involve using the minimal amount of medications to optimize asthma outcomes (minimal symptoms and exacerbations and high quality of life).[1-3] Exacerbations are important as they cause anxiety to patients and are associated with increased healthcare cost.[4] Monitoring asthma control is important in asthma management, although there is no single outcome measure that can adequately assess asthma control.[5] Subjective measures usually involve a series of questions used for clinical assessment, and can include diary cards and quality of life (QoL) questionnaires. Traditional objective methods used to monitor asthma (but not control) include indices of spirometry/peak flow and airway hyperresponsiveness.[6] Newer methods include measurement of airway inflammation, such as airway cellularity in induced sputum or fractional exhaled nitric oxide (FeNO), as pheno/endotypes of asthma are increasingly appreciated.[7]

The inflammation in airways of people with asthma can be predominantly eosinophilic or non-eosinophilic (including neutrophilic).[8] Irrespective of the type of airway inflammation, inhaled corticosteroids (ICS) remain the major preventer therapy to control asthma symptoms, other than for children with mild intermittent asthma.[2] However ICS are more effective in reducing symptoms in patients with eosinophilic inflammation than those with neutrophilic inflammation.[9] Thus, treatment tailoring based on objective eosinophilic inflammation data may be helpful in improving asthma outcomes. Currently clinically available techniques are assessing airway cellularity and FeNO.[10]

The increased attention to personalised medicine, which for asthma includes basing treatment on objective airway inflammation[11] is reflected by interest in our previous systematic review .[12] We present an update to our previous review[12] by providing an overview of three recent related Cochrane reviews,[13-15] each of which addressed a different question as per the PICO framework. The objective of our systematic review is to evaluate the efficacy of tailoring asthma medications based on FeNO or sputum eosinophils (i.e. eosinophilic-based strategy) in comparison to controls (clinical symptoms with or without spirometry/peak flow) for asthma-related outcomes in children and adults.

METHODS

Inclusion criteria, outcomes and analyses were a-priori specified and documented in Cochrane review protocols and in the first versions of the three reviews on *The Cochrane Library*.[13-15]

Eligibility, Information Sources, Search Strategy and Study Selection

We used Cochrane methods and searched (up to February 2017) for eligible randomised controlled trials (RCTs) that compared adjustment of asthma medications based on sputum eosinophils or FeNO levels with adjustment according to clinical symptoms (with or without spirometry/peak flow). As outlined in the reviews,[13-15] searches used keywords in electronic sources (Cochrane Airways Group Specialised Register of Trials, the Cochrane Central Register of Controlled Trials (CENTRAL), Medline, EMBASE) and reference hand searching. Searches of bibliographies and texts were conducted to identify additional studies. Trials that included the use of other interventions were included if all participants had equal access to such interventions.

Participant inclusion criteria were children and adults with a diagnosis of asthma according to a guideline-defined criteria. Exclusion criteria were as follows: eosinophilic bronchitis, asthma related to an underlying lung disease such as bronchiectasis and chronic obstructive airway disease, or diagnostic categories such as 'cough variant asthma' and 'wheezy bronchitis' where controversies exist.

Data extraction

Titles and abstracts of all records returned by the literature search were reviewed independently in duplicate to identify potentially relevant trials. Searches of bibliographies

and texts were conducted to identify additional studies. Using the pre-specified criteria, two reviewers independently reviewed full texts to select trials for inclusion. There was no disagreement although it was planned that disagreement would have been resolved by third party adjudication. We extracted information from each trial on (a) study characteristics, (b) intervention type, and (c) outcomes, as described in our Cochrane reviews.[13-15]

Risk of bias

Risk of bias for each included study was assessed using the Cochrane Risk of Bias tool available in the RevMan5 software. Seven components were assessed in duplicate as low, unclear or high risk of bias: Sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias.

Summary (outcome) measures

Primary outcomes were indices reflective of asthma exacerbations (defined by study authors) during the follow-up period. Secondary outcomes were mean differences (MD) between groups in objective measurements of asthma (FEV_1, peak flow, airway hyper-responsiveness), FeNO level, symptoms of asthma (as reported in Asthma Control Test (ACT) or asthma-related QoL score) and ICS dose at final visit.

Methods of analyses

The results from studies that met the inclusion criteria and reported any of the outcomes of interest were included in the subsequent meta-analyses. We a-priori separated children from adult studies. All data were double entered (HP/AC or HP/KK) and triple checked (CC). We combined data for meta-analyses only where it was meaningful (i.e. based on clinical and

statistical criteria). We analysed dichotomous data as odds ratios (OR) and continuous data as MD, or as standardised mean difference (SMD) if different measurement scales were used across studies. For dichotomous data, we reported the proportion of participants contributing to each outcome in comparison with the total number randomised. Generic inverse variance was used for rate ratio (RR) analysis of common events, whereby one subject may have more than one event. The RRs were taken from the published papers and standard errors (SE) of the Log RR were calculated from confidence intervals (CI) or P-values published in the papers. Numbers needed to treat (NNT) were calculated from the pooled OR and its 95%CI applied to a specified baseline risk using an online calculator.[16] Fixed effects were used throughout unless stated otherwise.

Any heterogeneity between the study results was described and tested to see if it reached statistical significance using a chi-squared test. We included the 95%CI estimated using a random effects model whenever there were concerns about statistical heterogeneity. Heterogeneity was considered significant when the P value was <0.10.[17] We used the I^2 statistic to measure heterogeneity among the trials in each analysis. If we identified substantial heterogeneity (>50%), we reported it and explored possible causes. Subgroup analysis was planned for: 1. Basis for adjustment of ICS in the control group (guideline-driven monitoring versus non-guideline driven); 2. Use of spirometry or peak flow as an adjunctive monitoring tool for adjustment of medications (versus non-use of spirometry or peak flow); 3. Baseline ICS dose at commencement of intervention (<800 mcg/day versus >800 mcg/day budesonide equivalent); 4. Cut-offs for adjustment of medications.

We used the five GRADE considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) to assess the quality of a body of evidence as

it relates to the studies that contributed data to the meta-analyses for the pre-specified outcomes.

RESULTS

Study selection and study characteristics

The searches in 2017 identified 1208 publications for FeNO-based strategy and 1213 for sputum. After screening, 30 and seven papers respectively were retrieved but only 16 and six respectively fulfilled the inclusion criteria (Figure-1), including the nine studies from the previous review.[12] The 22 studies consisted of 16 FeNO-based trials (7 adults, 9 children) and six sputum-based trials (5 adults, 1 children), which included a total of 3500 participants, of whom 3208 completed the studies (91.7%).

Of the 22 studies included (Table 1, Supplement Table-1), nine were single centre studies,[18-26] two were dual-centred[27, 28] and 11 were multi-centred.[29-39] Ten studies were in children or adolescents,[18, 19, 20, 26, 28-30, 32-34] and twelve involved adult participants.[21-25, 27, 31, 35-39] We classified studies into children/adolescent studies based on the mean age reported as opposed to the entry criteria. Nine studies were double-blind, parallel group trials, [18, 24, 26-29, 31, 32, 35] seven were single-blind, parallel group trials,[19, 21, 22, 25, 33, 34, 38] and six studies had no blinding.[20, 23, 30, 36, 37, 39] Twenty-one papers were published in English and one was translated from Chinese.[22] Seven studies were supported by Aerocrine, the manufacturer of FeNO analyser (Supplement Table-1).

Table 1: Characteristics of included studies

Study	Sample size	Description of intervention and control arms
Calhoun 2012[35]	FeNO group N=115. Control group N=114.	Control group: National Heart, Lung and Blood Institute guidelines. FeNO group: <22ppb treatment stepped down 22 to 35 maintain treatment >35 increase treatment
Cao 2007[22]	EOS strategy N=20. Control group N=21.	Control strategy: "Standard clinical guidelines" EOS strategy: decrease ICS <1% eosinophils, keep ICS the same 1-3% eosinophils, increase ICS if eosinophils >3%.
Chlumsky 2006[23]	EOS strategy N=30. Standard strategy N=25.	Standard strategy arm: GINA guidelines EOS strategy: decrease ICS if ≤3%, keep same if 4-8%, increase ICS if ≥8%.
deJongste 2009[30]	FeNO group N=75. Symptom group N=72.	All participants scored asthma symptoms in an electronic diary over 30 weeks. Aim to keep FeNO <20ppb Symptom group based on symptom score: Below range (< 10) = step down/discontinue, range 10 to 60 = no change and range > 60 = step up
Fleming 2012[26]	Inflammatory group N=27. Symptom group N=28.	Symptom group: Based on number of major exacerbations in the preceding 3 months and SABA use in preceding 2 weeks. Inflammatory group: Treatment aimed to keep sputum eosinophil counts <2.5%.
Fritsch 2006[19]	FeNO group N=22. Control group N=25.	FeNO group: therapy was based on symptoms, beta-agonists use, lung function and FeNO. Control group: therapy based on symptoms, beta-agonists and lung function only.
Green 2002[24]	Sputum management group N=37. BTS group N=37.	Sputum management group: anti-inflammatory treatment was based on maintenance of sputum eosinophil count below 3% with a minimum dose of anti-inflammatory treatment. BTS management group: BTS/SIGN guidelines.
Hashimoto 2011[36]	Internet strategy N=51. Conventional strategy N=38.	Internet strategy: Had steroid dose adjusted based on the 3 components: electronic diary, in-built algorithm (which includes FeNO levels), and monitoring support. Conventional strategy: GINA guidelines for the treatment of severe asthma.
Honkoop 2014[37]	FeNO group N=189. Controlled asthma group N=203.	Cluster randomization (at general practice level). FeNO strategy: Treatment targeted to keep FeNO <50ppb. Symptom strategy: ACT utilized including lung function
Jayaram 2006[31]	Sputum strategy group N=50.	Sputum strategy: Guided solely by induced sputum eosinophils to keep <2%.

	Clinical strategy group N=52.	Clinical strategy: Canadian Asthma Consensus Group Guidelines.
Malerba 2015[25]	Sputum strategy N=14. Clinical strategy N=14.	Sputum strategy: Treatment based on sputum eosinophil (%) and FeNO (ppb). Decrease ICS <2% & ≤10pbb Keep same 2-3% & 11-20ppb Increase ICS >3% & ≥20ppb Symptom strategy: Symptom scores, use of SABA and night time symptoms.
Peirsman 2014[34]	FeNO group N=49. Control group N=50.	FeNO group: Treatment aimed to keep FeNO below 20ppb. Control group: GINA guidelines
Petsky 2015[28]	FeNO group N=31. Symptom group N=32.	FeNO group: Treatment adjusted based on FeNO level and atopy status. Elevated FeNO defined as: ≥ 10ppb with no positive SPT ≥ 12ppb with 1 positive SPT ≥ 20ppb with ≥ 2 positive SPT Control group: Symptom diary cards
Pijnenburg 2005[18]	FeNO group N=39 Symptom group N=46	FeNO group: FeNO guided ICS dosing according to predetermined algorithm. Symptom group: Symptom scores influenced ICS dosing.
Pike 2013[32]	FeNO group N=44. Standard management group N=46.	FeNO group: FeNO measurements and symptom control. Standard management group: symptom control as per blinded clinician (reliever use, FEV_1).
Powell 2011[27]	FeNO group N=111. Control group N=109.	FeNO group: Sequential process, first FeNO concentrations used to adjust ICS dose, and second ACT score used to adjust the LABA dose. Clinical group: Juniper ACT cutoff points defined as: well-controlled asthma (ACT < 0.75), partially controlled asthma (0.75 to 1.50), and uncontrolled asthma (> 1.5)
Shaw 2007[38]	FeNO group N=58 Control group N=60.	FeNO group: FeNO >26ppb, ICS was increased. If FeNO <16ppb or <26ppb on 2 separate occasions, treatment was decreased. Control Group: Treatment was doubled if Juniper Asthma Control Score (JACS) >1.57 and treatment halved if JACS <1.57 for 2 consecutive months.
Smith 2005[21]	97 patients randomised from 110 patients	FeNO group: Based to keep FeNO <15ppb at 250mL/sec. Control group: dose adjustment based on asthma symptoms, night-time waking, bronchodilator use, variation in PEFR and FEV1.
Syk 2013[39]	FeNO group N=87. Control group N=78.	FeNO group: Keep FeNO level <24ppb for women, and <26ppb for men. Control group: Treatment adjusted based on patient reported symptoms, SABA use, physical examination and spirometry results.
Szefler 2008[29]	FeNO group N=276.	FeNO group: Standard treatment modified on the basis of measurements of FeNO

	Control group N=270	Control group: National Asthma Education and Prevention Program (NAEPP) guidelines
Verini 2010[20]	FeNO group N=32. GINA group N=32.	FeNO group at 6 month visit only: step treatment up if >12ppb. Control group: GINA guidelines.
Voorend-van Bergen 2015[33]	FeNO group N=92. Standard care group N=89.	FeNO group: Treatment adjusted according to FeNO levels and ACT results. <u>If ACT ≥ 20</u> and: FeNO < 25 = step down FeNO ≥ 25 to < 50 = no change FeNO ≥ 50 = step up <u>If ACT < 20</u> and: FeNO ≥ 25 = step up FeNO < 25 = no change Control group: Treatment adjusted based on ACT results < 20 = step up ≥ 20 = no change or step down

There was a degree of clinical heterogeneity among the studies (Table-1, Supplement Table-1), primarily with regard to the definition of an asthma exacerbation and the FeNO and sputum eosinophil cut-offs used for adjusting therapies. Although asthma exacerbations were an outcome measure in all papers, they differed in how they were defined ranging from unscheduled emergency visits[25] to defining an exacerbation using diary card data.[21, 28] Two studies defined an exacerbation as a decrease in morning lung function.[24, 36] Although there were variations in how exacerbations were defined, all included studies uniformly managed exacerbations with rescue oral steroids. Algorithms for adjustment of medications differed among studies and the cut-off values to step-up and down also varied across the FeNO studies (range 12[20, 28] to 50ppb[37]), and the sputum eosinophil percentages (range from 2[31] to 8[23]).

Outcomes and synthesis of results

Primary (Exacerbations)

In both adults and children, the number of participants with exacerbations (during the follow-up period 18-52 weeks) in the group whose treatment was adjusted according to FeNO were significantly lower than the control group; in adults OR was 0.60 (95%CI 0.43, 0.84, p=0.003; participants=1005; studies=5) and in children the OR was 0.58 (95%CI 0.45, 0.76, p<0.0001; participants=2284; studies=8) (Figure-2). Based on the number of participants who had at least one exacerbation over the study period (Table-2), the number to treat to benefit (NNTB) over 52 weeks was 12 (95%CI 8, 32) in adults; and 9 (95%CI 6, 15) in children.

Table 2: Number of participants who had ≥1 exacerbation over the study period

Adult studies	**FeNO group**		**Control group**	
	N with exacerbation	**N of group**	**N with exacerbation**	**N of group**
Honkoop 2014	23	189	30	203

Powell 2011	28	111	45	109
Shaw 2007	12	58	19	60
Smith 2005	14	46	11	48
Syk 2013	15	93	25	88
Paediatric studies				
de Jongste 2008	9	75	12	72
Peirsman 2014	11	49	22	50
Petsky 2015	6	31	15	32
Pijnenburg 2005	7	42	10	47
Pike 2013	37	44	38	46
Szefler 2008	91	276	115	270
Verini 2010	16	32	26	32
Voorend-van Bergen 2015	9	92	14	89

The exacerbation rate in the FeNO-strategy group was significantly lower than controls in the adult studies (RR=0.59, 95%CI 0.45, 0.76; participants=842; studies=5). There was no significant difference between groups in the paediatric data and as statistical heterogeneity among studies was present, we used random effects analysis to calculate the rate of exacerbations over 52 weeks (MD =-0.37, 95%CI -0.8, 0.06; participants=736; studies=4).

In the sputum-based meta-analysis (Figure-3), significantly fewer adults and children in the sputum-based strategy had asthma exacerbations compared to the control group (73 vs 100; p=0.0002), OR 0.36 (95%CI 0.21, 0.62); participants=173; studies=4. The NNT for one participant (adults) to avoid any exacerbations was 5 (95%CI 4, 11) over 16 months.

Secondary Outcomes

Inhaled Corticosteroid (ICS) Dose

For the FeNO-based studies, the meta-analysis found no significant group differences in the final ICS dose for adults or children (Figure-4). In adults, the direction favoured the FeNO strategy (MD between groups was -147.15ug budesonide equivalent; 95% CI -380.85, 86.56; p=0.22; participants=582; studies=4) but the direction in children favoured the control

strategy (MD 65.88ug budesonide equivalent, 95%CI -86.71, 218.47; p=0.40; participants=317; studies=3) (Figure-4).

All five studies that utilised sputum eosinophils to adjust treatment reported no differences in doses of ICS used between groups (Supplement Figure-1). The SDs for the groups were not available in Jayaram et al's paper[31] and were estimated based on the data from Green's paper.[24] The mean dose of ICS per person per day (ug budesonide equivalent) between groups was non-significant in adult studies, (MD 0.67, 95%CI -154.39, 155.73; p=0.99; participants=262; studies=4). Likewise, there was no difference in daily ICS doses in the sole paediatric study (MD 67.0, 95%CI -264.81, 398.81; p=0.69; participants=54).

Symptom scores and other outcomes

Symptom or ACT scores did not significantly differ between groups for FeNO-studies in either adults or children (Supplement Figure-2). In adults (4 studies), the direction of the difference in scores favoured the FeNO strategy, mean difference was -0.08 (95%CI -0.18, 0.01; p=0.09; participants=707) but the direction in children favoured the control group: mean difference was 0.14 (95%CI -0.18, 0.47; p=0.39; participants=724; studies=2). For the sputum-based studies, the two studies that reported on symptom scores also described no significant difference in symptoms scores between groups.[23, 24] Likewise for the outcome of asthma QoL scores, there were no significant group differences for the FeNO-based studies in adults and children (Supplement Figure-3). In adults, there were only two studies and the mean difference in children was 0.09 (95%CI -0.08, 0.26; p=0.29; studies=3).

There was insufficient data reported from the individual studies to undertake a meta-analyses for the other secondary outcomes (FEV_1, AHR, rescue B agonist use). While FEV_1 was

reported in all studies, data points were not provided; the studies described they found no difference between the participants who had treatment adjusted to inflammatory markers in comparison to the control group.

Subgroup analyses

As per Table 1, eight of the 16 FeNO-based studies[20, 21, 29, 32, 34-36, 38] utilised guideline-driven monitoring for the control group. In this subgroup analysis based on trials that utilised guideline driven monitoring, the significant difference was no longer present for the primary outcome of number of participants who had one or more exacerbations (OR 0.87, 95%CI 0.47, 1.61) in adults (4 studies) but that in children (4 studies) still significantly favoured the FeNO strategy (OR 0.67, 95%CI 0.51, 0.90). The subgroup analyses results for 'cut-off FeNO values' were similar to the main analyses; the FeNO group had significantly fewer exacerbations. As there was insufficient data, we could not undertake subgroup analyses for the other planned sub-groups.

Risk of bias in individual studies

The risk of bias diagram (Figure 5) shows that eight studies[18, 24-28, 31, 34, 38] were judged as having good methodological quality, but in all studies there was either insufficient details about allocation concealment and/or adequacy of blinding. Seven studies[20, 23, 30, 33, 36, 37, 39] were open label or single blinded (6 in FeNO studies, 1 in sputum driven studies). When data from the six open label FeNO driven studies[20, 30, 33, 36, 37, 39] were removed, the primary outcome results (exacerbations) did not change. In adults, the number of participants who had one or more exacerbations over the study period OR=0.63 (95%CI 0.41, 0.96; participants=432; studies=3) and exacerbation rates (RR=0.61, 95%CI 0.45, 0.82; participants=661, studies= 4). In children, the number of participants who had one or more

exacerbations over the study period OR=0.67 (95%CI 0.50, 0.89; participants=887, studies=5).

One sputum eosinophil driven study[23] did not use blinding, however removing the datum from this study did not alter the results of the primary outcome (exacerbations); occurrence of any exacerbation (RR 0.66, 95%CI 0.46, 0.93; participants=218; studies=3), or number of participants who had one or more exacerbations over the study period (OR 0.43, 95%CI 0.24, 0.79; participants=218; studies=3).

For the FeNO-based adult papers, the quality of evidence using the GRADE approach surmises that, of the three outcomes assessed, two were of moderate quality and one (ICS dose at final visit) was very low quality due to wide confidence intervals and the fact that one study[39] was open labelled, as well as heterogeneity between doses (Table 3). For the FeNO-based children studies, the quality was moderate for two outcomes and very low for one (exacerbation rates). This outcome was downgraded three levels for one open labelled study, [20] imprecision and heterogeneity (I^2=67%) (Table 4). For sputum-based studies, GRADE assessment shows that the quality of the three outcomes were moderate for two outcomes (exacerbations) and low (ICS dose) due to the lack of blinding in one study,[23] and the varied doses within and between studies (Table 5).

Table 3: Summary of findings for the main comparisons: FeNO based adult studies

Tailoring asthma treatment using FeNO versus clinical symptoms						
Patient or population: Adults with asthma **Setting:** outpatient **Intervention:** asthma treatment tailored on FeNO **Comparison:** asthma treatment tailored on clinical symptoms						
	Anticipated absolute effects* (95% CI)		**Relative effect (95% CI)**	**№ of participants (studies)**	**Quality of the evidence (GRADE)**	
Outcomes	**Risk with asthma**	**Risk with asthma**				**Comments**

	treatment tailored on clinical symptoms**	**treatment tailored on FeNO**				
Number of participants who had ≥ 1 exacerbations over study period Follow-up: range 18 weeks to 52 weeks	**25 per 100**	**17 per 100** (13 to 22)	**OR 0.60** (0.43 to 0.84)	1005 (5 RCTs)	⨁⨁⨁⨀ MODERATE[1]	-
Number of exacerbations per 52 weeks (exacerbation rates) Follow-up: mean 52 weeks	The control group ranged from 0.23 to 0.9 exacerbations per 52 weeks	**Rate ratio 0.59** (0.45 to 0.77)	-	842 (5 RCTs)	⨁⨁⨁⨀ MODERATE[1]	-
ICS dose at final visit Follow-up: range 18 weeks to 52 weeks	The mean ICS dose taken by the control group at final visit was **659 mcg**	The mean ICS dose taken in the FeNO groups was 17.01 lower (101.75 lower to 67.72 more) **577 mcg**	-	582 (4 RCTs)	⨁⨁⨀⨀ VERY LOW[2,3]	A random-effects sensitivity analysis gave a very imprecise result: MD -147.15 (95% CI -380.85 to 86.56)

***The risk in the intervention group** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
**The control group risks were calculated as a mean of the scores or events in the control groups of the studies contributing to each analysis. We could not calculate a control risk for the number of exacerbations per 52 weeks because we did not have information for each arm of the studies, just ratios between them.
CI: confidence interval; **FeNO:** fractional exhaled nitric oxide; **ICS:** inhaled corticosteroids; **MD:** mean difference; **OR:** odds ratio; **RCT:** randomised controlled trial

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect **Very low quality:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Table 4: Summary of findings for the main comparisons: FeNO based paediatric studies

Tailoring asthma treatment using fractional exhaled nitric oxide vs clinical symptoms						
Patient or population: Children with asthma **Setting:** outpatient **Intervention:** asthma treatment tailored on FeNO **Comparison:** asthma treatment tailored on clinical symptoms						
Outcomes	**Anticipated absolute effects* (95% CI)**		**Relative effect (95% CI)**	**No of participants (studies)**	**Quality of the evidence (GRADE)**	**Comments**
	Risk with clinical symptoms	**Risk with asthma treatment tailored on FeNO**				
Number of participants who had ≥ 1 exacerbations over study period (48.5 weeks)	40 per 100	28 per 100 (23 to 33)	OR 0.58 (0.45 to 0.75)	1279 (8 RCTs)	⊕⊕⊕⊖ Moderate[1]	-
Number of asthma exacerbations per 52 weeks (exacerbation rate)	The mean number of asthma exacerbations per 52 weeks (exacerbation rate) was 1.66	The mean number of asthma exacerbations per 52 weeks (exacerbation rate) in the intervention group was 0.37 lower (0.8 lower to 0.06 higher)	MD -0.37 (-0.8 to 0.06)	736 (4 RCTs)	⊕⊖⊖⊖ Very low[2]	-
ICS dose at final visit (budesonide equivalent)	The mean ICS dose at final visit (budesonide equivalent) was **483 µg/day**	The mean ICS dose at final visit (budesonide equivalent) in the intervention group was	-	317 (3 RCTs)	⊕⊕⊕⊖ Moderate[3]	-

		63.95 µg/day higher (51.89 lower to 179.79 higher)				

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **FeNO:** fractional exhaled nitric oxide; **ICS:** inhaled corticosteroid; **MD:** mean difference; **OR:** odds ratio; **RCT:** randomised controlled trial

Table 5: Summary of findings for the main comparisons: Sputum eosinophilia based studies

Tailored interventions based on sputum eosinophils compared to tailored interventions based on clinical symptoms for asthma in adults and children						
Patient or population: Adults and children with asthma **Settings:** hospital outpatients **Intervention:** based on sputum eosinophils count **Comparison:** based on clinical symptoms						
Outcomes	**Illustrative comparative risks* (95% CI)**		**Relative effect (95% CI)**	**No of Participants (studies)**	**Quality of the evidence (GRADE)**	**Comments**
	Assumed risk at one year	**Corresponding risk**				
	Tailored interventions based on clinical symptoms	**Tailored interventions based on sputum eosinophils**				
Number of participants who had one or more exacerbations over the study period Follow-up: 12 to 24 months	**82 per 100**	**62 per 100** (49 to 74)	**OR 0.36** (0.21 to 0.62)	228 (3 studies)	⊕⊕⊕⊖ **moderate**[1]	
Hospitalisations Follow-up: 12 to 24 months	**24 per 100**	**8 per 100** (3 to 21)	**OR 0.28** (0.09 to 0.84)	269 (4 studies)	⊕⊕⊕⊖ **moderate**[2]	
Mean dose of inhaled corticosteroids per person per day (BUD equivalent mcg/day) Follow-up: 12 to 24 months		The mean dose of inhaled corticosteroids per person per day in the intervention groups was **13 mcg/day higher** (128 lower to 153 higher)		316 (4 studies)	⊕⊕⊖⊖ **low**[3]	
*The basis for the **assumed risk** is the mean of the two studies with a duration of one year. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** Confidence interval; **OR:** Odds ratio;						

DISCUSSION

In this meta-analysis, we combined data from our 3 Cochrane reviews[13-15] that evaluated the efficacy of tailoring asthma medications (ICS predominantly) based on airway eosinophilic markers (FeNO or sputum eosinophils) in comparison to controls (clinical symptoms with or without spirometry/peak flow) for asthma-related outcomes in children and adults. Based on twenty-two studies involving 3500 adults and children (3208 completed), we found that children and adults randomised to either eosinophilic marker strategy (compared to controls) were significantly less likely to experience an exacerbation during the follow-up period (4.5-24 months). The exacerbation rate was also significantly lower in adults randomised to the FeNO or sputum strategy (compared to controls) but not in children. There was not a significant difference in the final dose of ICS in either children or adults. For both FeNO and sputum-based strategies, there was no difference between groups for all secondary outcomes (FEV_1, ACT, QoL, airway hyper-responsiveness or $beta_2$-agonist use).

In this review updated from our previous combined meta-analyses,[12] the data on sputum remained unchanged, i.e. using sputum to guide asthma therapies in adults is beneficial for the outcome of reducing exacerbations. The new single paediatric study[26] found no significant difference between the groups for this outcome, although favoured the sputum-based strategy. However, the OR for the combined adult and paediatric studies remained unchanged at 0.36 but the 95%CI was marginally smaller from 0.20 to0.64 to 0.21to 0.62.

In contrast to the data for sputum, the additional 10 studies included in the FeNO strategy analyses altered the previous 'no benefit' found in our previous review[12] to 'some benefit' as using a FeNO-based strategy reduced the number of participants with asthma exacerbations during the follow-up period in both children and adults. However, the benefit was

inconsistent as there was no longer any significant difference between groups in the sensitivity analyses for adults, whilst in children there was no group differences for exacerbation rate. While this new data is somewhat supportive of authors who previously advocated using FeNO levels to tailor medications,[40] we do not believe there is currently sufficient evidence to universally use FeNO to monitor airway inflammation recommended by others.[41]

In contrast to the favourable data in the outcome of exacerbations for both sputum and FeNO-based strategies, the data for other asthma outcomes (FEV_1, symptom scores, QoL and $beta_2$ agonist use) remained unchanged i.e. neither sputum and FeNO- based strategies were shown to confer any advantage over the control arms. There may be several reasons for this including the known discordance between asthma control and exacerbations.[1] While exacerbations are an important outcome, arguably subjective measures of asthma control are also important. Thus, although our findings demonstrate that using airway eosinophilic markers to guide medications future exacerbations, it is debatable whether either strategy should be universally advocated. Sputum analysis is restricted to laboratories with specific expertise, is relatively time consuming and is not always successful, particularly in young children. Use of FeNO universally will add a substantial cost to the millions of people who have asthma. Also, currently there is no evidence-based algorithm on how to adjust treatment based on FeNO levels (or indeed to sputum eosinophils levels) and the various guidelines (such as GINA[1], BTS[2], NAC[3]) differ on when and how to step up and down asthma therapies. Nevertheless, using airway eosinophilic markers to guide asthma therapy is most likely to be beneficial to the subset of people with frequent asthma exacerbations.

The data on the FeNO-based studies also need to be considered in light of several issues. Firstly, only one[28] of the 16 included studies utilising FeNO considered presence or severity of atopy in their algorithm of management although some but not all subjects were atopic. FeNO is higher when eosinophilic inflammation is present, however it is also higher in other conditions (eg. atopy, allergic rhinitis, eczema).[1] Secondly, the cut offs of FeNO utilised for stepping up or down therapy differed between studies (range 15-50 ppb). Pijnenburg et al[18] (paediatric study) subjects had the highest mean daily dose of ICS and subjects in this study also had quite high FeNO at the final visit (approximately 25.5 pbb in FeNO group, 36.7 in controls). Disconcertingly, use of FeNO strategy did not result in a lower FeNO level at the end of trial. Moreover some of the algorithms utilised a safety-net to avoid excessively high doses of ICS in some participants whose FeNO remained high. Thirdly, as reported in risk of bias table (Table-2) obtaining accurate FeNO measurements at each visit could not be obtained, either due to a faulty analyser[30] or technical issues.[19] Also, many aspects need to be considered when analysing FeNO; this includes the timing of spirometry (transiently reduces FeNO), food and beverage, circadian rhythm, smoking history, ambient NO and exercise.[43] Lastly, FeNO values may not always reflect levels of airway eosinophilia as shown in a RCT using mepoluzimab .[42]

Limitations of review

This systematic review is limited to 22 studies with 3208 subjects completing the trials. While the studies share some common issues, there are also substantial differences, notably, the definition of asthma exacerbation, the participants, how the decision to prescribe oral steroids was made, the cut-off levels for FeNO and sputum eosinophils were different, the control strategies (that often used uses multiple measures) and how medications were adjusted. Also,

7 of the 16 FeNO-based studies were supported by the FeNO manufacturers and although we are unaware of any publication bias, we cannot be certain of its existence.

CONCLUSION

Tailoring of asthma therapy based on FeNO or sputum eosinophils has been shown to be effective in decreasing asthma exacerbations in adults. Adjusting treatment based on FeNO levels for children tended to decrease asthma exacerbations at the expense of increased ICS doses. At present, despite their popularity, there is insufficient evidence to advocate their use in routine clinical practice.

Further, data starting with meta-analyses based on individual patient data (IPD) of all the studies may further inform the efficacy of strategies based on airway eosinophilic markers. If IPD meta-analysis does not shed more light e.g. the change in FeNO before medications are adjusted, further RCTs in both adults and children are then required. Ideally, these RCTs should include stratification e.g. high versus low doses of ICS, and eosinophilic versus non-eosinophilic asthma and cost effectiveness.

Figure Legend:

Figure 1: PRISMA flow chart

Figure 2: Number of subjects who had ≥1 exacerbation over study period (FeNO)

Figure 3: Number of subject who had ≥1 exacerbation over study period (SpEos)

Figure 4: Inhaled corticosteroid dose at final visit (FeNO)

Figure 5: Risk of bias summary

Supplement Figure 1: Mean dose of inhaled corticosteroid per person per day (SpEos)

Supplement Figure 2: Symptom score as per ACT (FeNO)

Supplement Figure 3: Quality of life score (FeNO)

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Competing interests: Two of the review authors (HP, AC) have conducted a randomised controlled trial in children on this subject.

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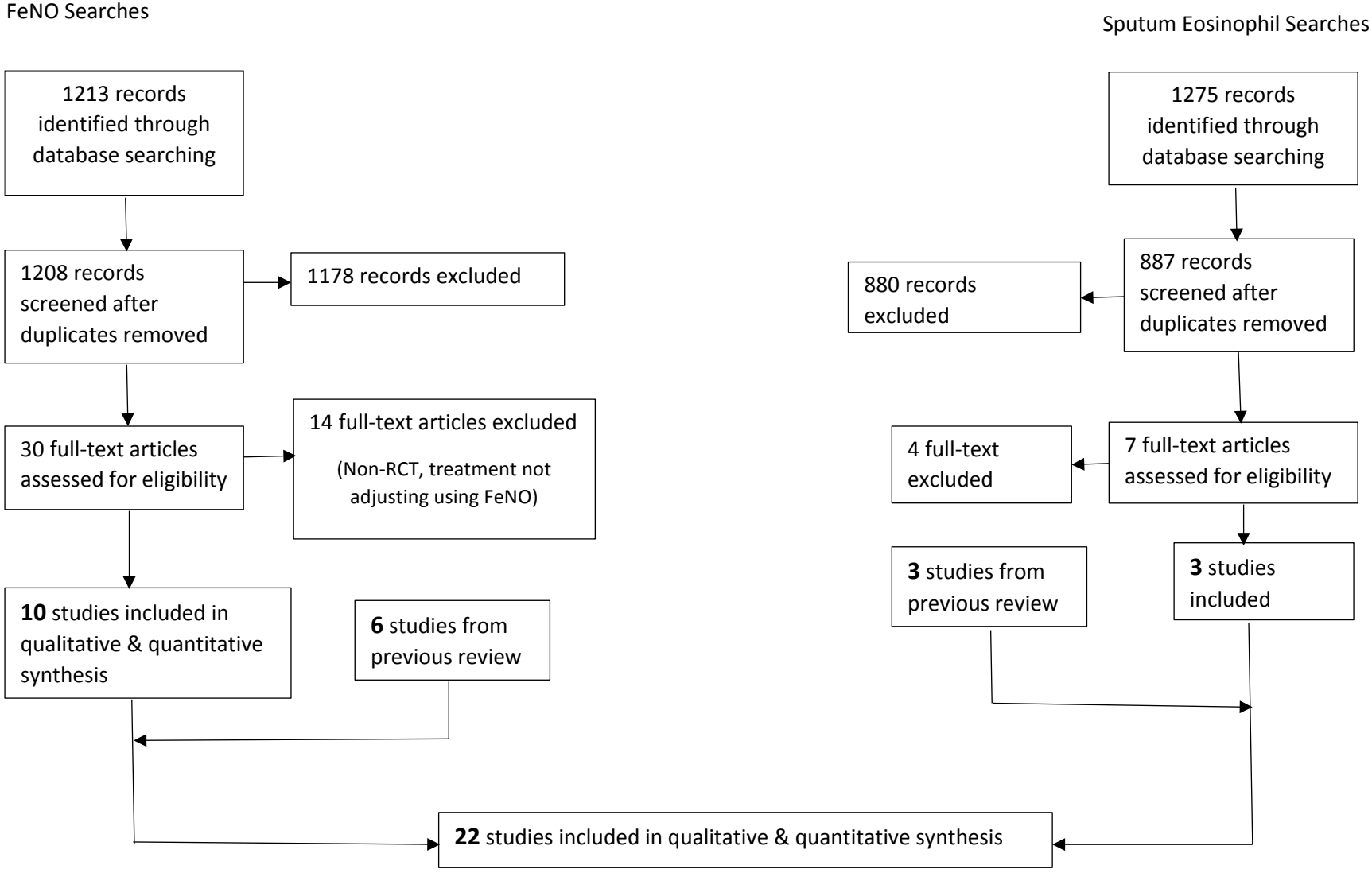
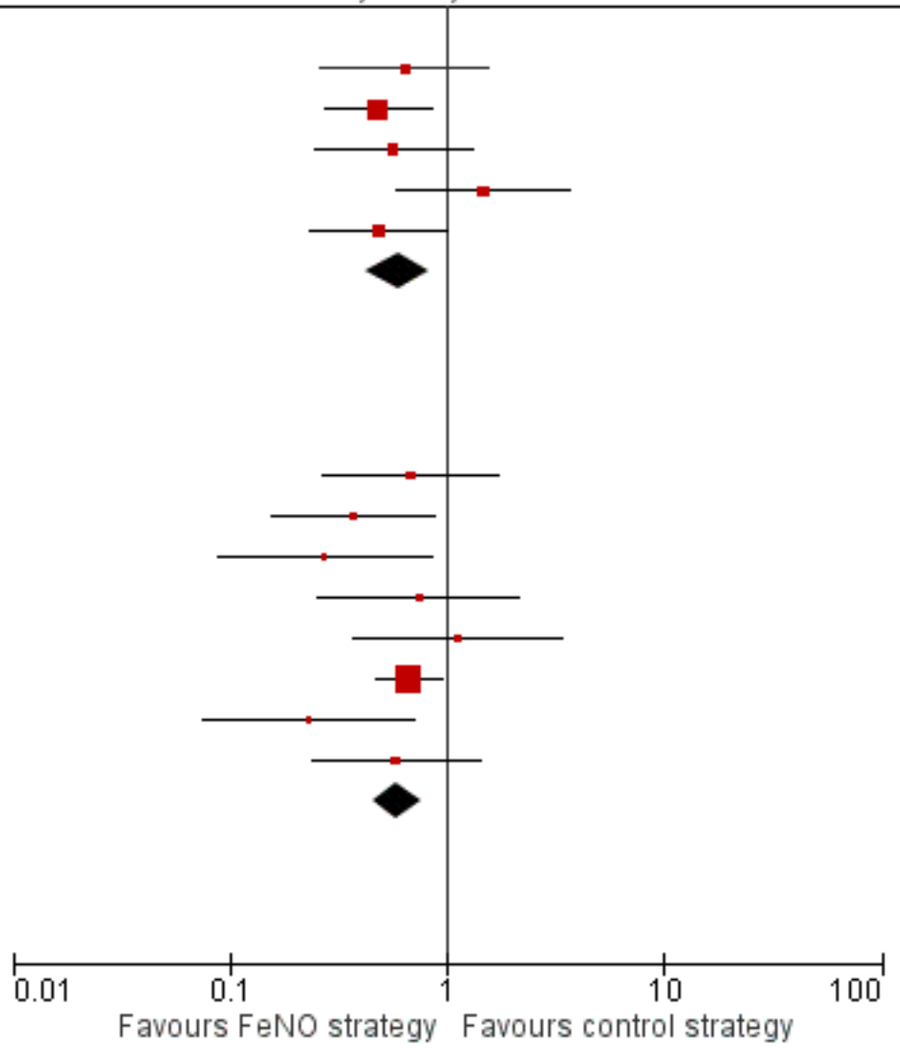
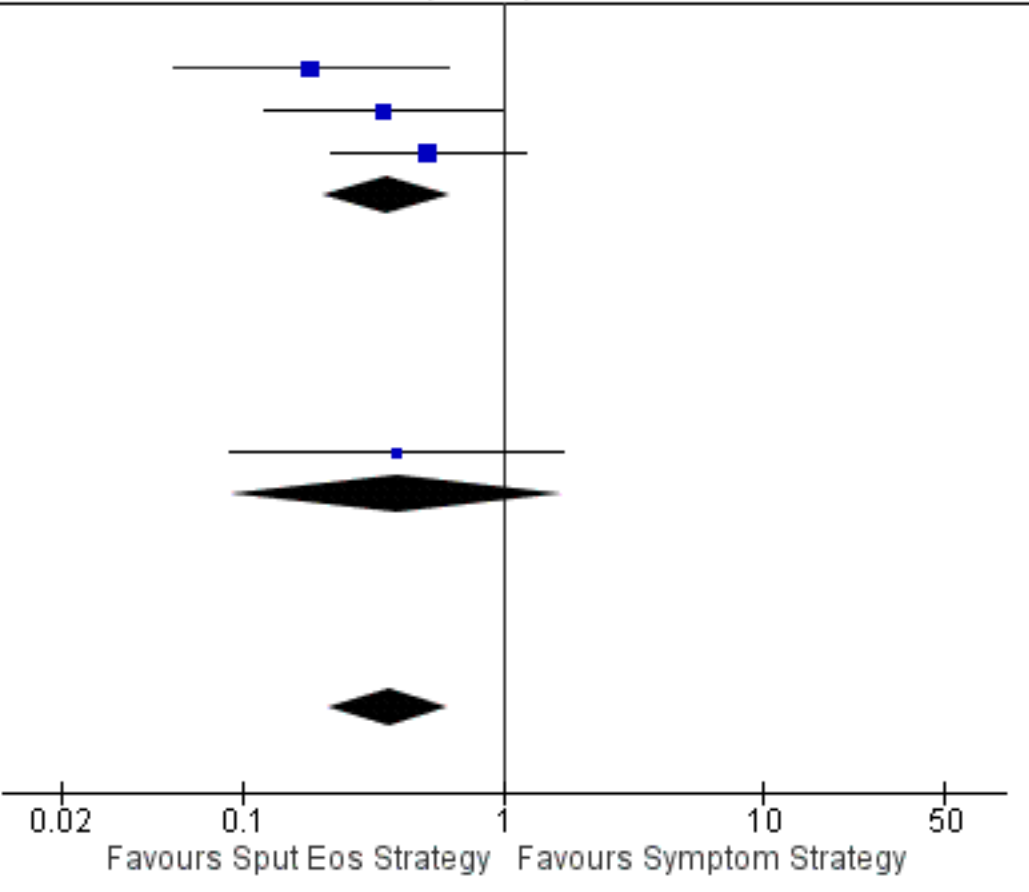

FeNO Searches
Sputum Eosinophil Searches
1213 records identified through database searching
1208 records screened after duplicates removed
1178 records excluded
30 full-text articles assessed for eligibility
14 full-text articles excluded
(Non-RCT, treatment not adjusting using FeNO)
10 studies included in qualitative & quantitative synthesis
6 studies from previous review
1275 records identified through database searching
887 records screened after duplicates removed
880 records excluded
7 full-text articles assessed for eligibility
4 full-text excluded
3 studies from previous review
3 studies included
22 studies included in qualitative & quantitative synthesis

Study or Subgroup	log[Odds Ratio]	SE	FeNO strategy Total	Control strategy Total	Weight	Odds Ratio IV, Fixed, 95% CI
1.1.1 Adults						
Honkoop 2014	-0.4463	0.4546	189	203	14.3%	0.64 [0.26, 1.56]
Powell 2011	-0.7344	0.2926	111	109	34.4%	0.48 [0.27, 0.85]
Shaw 2007	-0.5746	0.4267	58	60	16.2%	0.56 [0.24, 1.30]
Smith 2005	0.3863	0.4697	46	48	13.4%	1.47 [0.59, 3.69]
Syk 2013	-0.7244	0.3679	93	88	21.8%	0.48 [0.24, 1.00]
Subtotal (95% CI)			**497**	**508**	**100.0%**	**0.60 [0.43, 0.84]**
Heterogeneity: Chi² = 4.61, df = 4 (P = 0.33); I² = 13%						
Test for overall effect: Z = 3.00 (P = 0.003)						
1.1.2 Children						
de Jongste 2008	-0.383	0.4757	75	72	7.6%	0.68 [0.27, 1.73]
Peirsman 2014a	-0.9985	0.4454	49	50	8.6%	0.37 [0.15, 0.88]
Petsky 2015	-1.302	0.5763	31	32	5.1%	0.27 [0.09, 0.84]
Pijnenburg 2005a	-0.3011	0.5463	42	47	5.7%	0.74 [0.25, 2.16]
Pike 2013a	0.1069	0.5667	44	46	5.3%	1.11 [0.37, 3.38]
Szefler 2008a	-0.411	0.1776	276	270	54.2%	0.66 [0.47, 0.94]
Verini 2010a	-1.4663	0.5746	32	32	5.2%	0.23 [0.07, 0.71]
Voorend-van Bergen 2015	-0.5432	0.456	92	89	8.2%	0.58 [0.24, 1.42]
Subtotal (95% CI)			**641**	**638**	**100.0%**	**0.58 [0.45, 0.76]**
Heterogeneity: Chi² = 7.54, df = 7 (P = 0.38); I² = 7%						
Test for overall effect: Z = 4.11 (P < 0.0001)						
Test for subgroup differences: Chi² = 0.01, df = 1 (P = 0.92), I² = 0%						



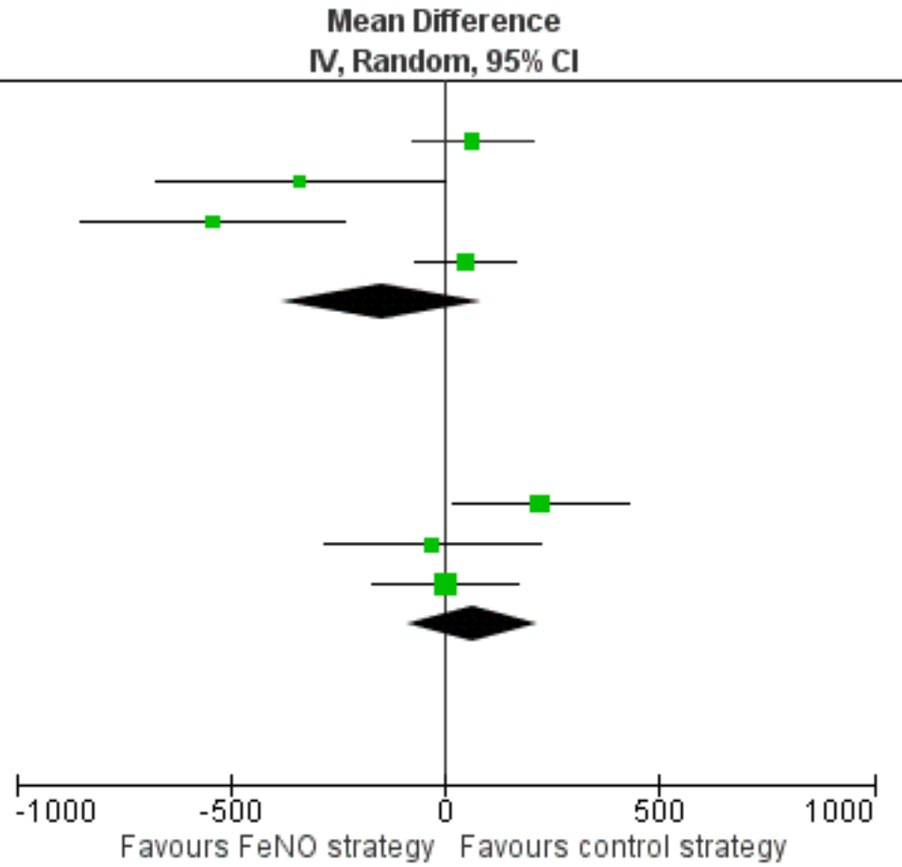
Study or Subgroup	Sputum Eos Strategy Events	Total	Symptom Strategy Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
1.2.1 Adult						
Chlumsky 2006	8	30	14	21	26.9%	0.18 [0.05, 0.61]
Green 2002 (1)	18	34	26	34	27.2%	0.35 [0.12, 0.98]
Jayaram 2006	26	45	37	51	32.6%	0.52 [0.22, 1.22]
Subtotal (95% CI)		**109**		**106**	**86.7%**	**0.36 [0.20, 0.64]**
Total events	52		77			
Heterogeneity: Chi² = 1.92, df = 2 (P = 0.38); I² = 0%						
Test for overall effect: Z = 3.49 (P = 0.0005)						
1.2.2 Children						
Fleming 2012	21	28	23	26	13.3%	0.39 [0.09, 1.71]
Subtotal (95% CI)		**28**		**26**	**13.3%**	**0.39 [0.09, 1.71]**
Total events	21		23			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.25 (P = 0.21)						
Total (95% CI)		**137**		**132**	**100.0%**	**0.36 [0.21, 0.62]**
Total events	73		100			
Heterogeneity: Chi² = 1.93, df = 3 (P = 0.59); I² = 0%						
Test for overall effect: Z = 3.70 (P = 0.0002)						
Test for subgroup differences: Chi² = 0.01, df = 1 (P = 0.92), I² = 0%						



Footnotes

(1) p=0.058

Study or Subgroup	FeNO strategy Mean	SD	Total	Control strategy Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
1.9.1 Adults								
Powell 2011	423.42	561.65	111	359.63	516.24	109	29.3%	63.79 [-78.72, 206.30]
Shaw 2007	557	670.63	52	895	1,035.51	51	19.5%	-338.00 [-675.63, -0.37]
Smith 2005	740	720.63	46	1,282	792.09	48	21.0%	-542.00 [-847.91, -236.09]
Syk 2013	586	455.1236	87	540	319.3398	78	30.3%	46.00 [-73.03, 165.03]
Subtotal (95% CI)			**296**			**286**	**100.0%**	**-147.15 [-380.85, 86.56]**
Heterogeneity: Tau² = 43312.37; Chi² = 17.10, df = 3 (P = 0.0007); I² = 82%								
Test for overall effect: Z = 1.23 (P = 0.22)								
1.9.2 Children								
Petsky 2015	487	497	31	266	312	32	33.2%	221.00 [15.34, 426.66]
Pike 2013a	755	518	33	784	597	43	25.6%	-29.00 [-280.15, 222.15]
Voorend-van Bergen 2015	400	600	91	400	550	87	41.2%	0.00 [-168.98, 168.98]
Subtotal (95% CI)			**155**			**162**	**100.0%**	**65.88 [-86.71, 218.47]**
Heterogeneity: Tau² = 7263.39; Chi² = 3.32, df = 2 (P = 0.19); I² = 40%								
Test for overall effect: Z = 0.85 (P = 0.40)								
Test for subgroup differences: Chi² = 2.24, df = 1 (P = 0.13), I² = 55.3%								




Random sequence generation (selection bias)
Allocation concealment (selection bias)
Blinding of participants and personnel (performance bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective reporting (reporting bias)
Other bias
Calhoun 2012
Cao 2007
Chlumsky 2006
de Jongste 2008
Fleming 2012
Fritsch 2006
Green 2002
Hashimoto 2011
Honkoop 2014
Jayaram 2006
Malerba 2015
Peirsman 2014
Petsky 2015
Pijnenburg 2005
Pike 2013
Powell 2011
Shaw 2007
Smith 2005
Syk 2013
Szefler 2008
Verini 2010
Voorend-van Bergen 2015

Study	Sample size	Participant Age	Description of intervention and control arms	Primary Outcome and definition of exacerbation	Duration	Funding and support
Calhoun 2012[35]	342 randomised; FeNO group N=115. Control group N=114.	FeNO group: mean age 35(SD 11), 33 males. Control group: mean age 34 (SD12), 42 males.	Control group: Treatment decisions based on National Heart, Lung and Blood Institute guidelines. FeNO group: <22ppb treatment stepped down 22 to 35 maintain treatment >35 increase treatment	Primary outcomes: Time to first treatment failure, a clinically important worsening of asthma Exacerbation: Increased asthma symptoms resulting in use of oral corticosteroids, increased ICS, or additional asthma medications.	Participants were seen at week 2, 4, 6 and then every 6 weeks for 9 months. Follow-up duration: 9 months	National Institutes of Health and by National Institutes of Health Grants awarded by the National Heart, Lung, and Blood Institute Teva Pharmaceuticals provided the study drug and matching placebo.
Cao 2007[22]	41 randomised; EOS strategy N=20. Control group N=21.	EOS strategy: age 41 (SD2), 11 males. Control group: age 43 (SD4), 11 males.	Control strategy: “Standard clinical guidelines” EOS strategy: decrease ICS if <1% eosinophils, keep ICS the same if 1-3% eosinophils, increase ICS if eosinophils >3%.	Primary outcome: Total number of acute exacerbations. Exacerbation: Unknown	Participants had a 2 week run-in, then visits at months 2, 4 and 6. Follow-up duration: 6 months	Capital Medical Development Foundation (No. 2002-3004)

Chlumsky 2006[23]	55 randomised; EOS strategy N=30. Standard strategy N=25.	EOS strategy: mean age 42(SD 19) 13 males Standard strategy: mean age 48 (SD 16)	Standard strategy arm: GINA guidelines EOS strategy: decrease ICS if ≤3%, keep same if 4-8%, increase ICS if ≥8%.	Primary outcome: Rate of asthma exacerbations Exacerbation: a doubling of the frequency of symptoms or number of puffs of rescue salbutamol or a reduction in morning PEF by 30% or more on at least two consecutive days or two of the aforementioned or all three.	Participants were assessed every 3 months for 18 months	Internal Grant Agency of the Ministry of Health of the Czech Republic (Grant No. 5866/3)
deJongste 2009[30]	151 children randomised; FeNO group N=75. Symptom group N=72.	FeNO group: mean age 11.6 (SD 2.6), 46 males. Symptom group: mean age 11.8 (SD 4.3), 54 males.	All participants scored asthma symptoms in an electronic diary over 30 weeks. FeNO group received a portable nitric oxide analyser. Aim to keep FeNo <20ppb Symptom group based on symptom score: Below range (< 10) = step down/discontinue, range 10 to 60 = no change and range > 60 = step up	Primary outcome: Proportion of symptom free days over the last 12 study weeks. Exacerbation: emergency visit, hospitalization or prednisolone course	Children were seen at 3, 12, 21 and 30 weeks. Groups had their medications changed every 3 weeks based on electronic diary and/or FeNO levels. Follow-up duration = 30 weeks.	The study was supported by Aerocrine AB, Sweden.
Fleming 2012[26]	55 children randomised;	Inflammatory group: median age 13.4 yrs	Symptom group: Based on number of major exacerbations in the	Primary outcome: Rate of major exacerbations and asthma control as assessed by	Children were seen 3 monthly for 12 months.	British Lung Foundation

	Inflammatory group N=27. Symptom group N=28.	(range 11-15.8), 16 males. Symptom group: median age 12.6yrs (range 10.2-14.7), 13 males.	preceding 3 months and SABA use in preceding 2 weeks. Inflammatory group: Treatment aimed to keep sputum eosinophil counts <2.5%.	symptom-free days and SABA use. Minor exacerbation: Use of bronchodilators >5 times/wk (excl. routine or pre-exercise). Major exacerbation: Deterioration requiring high dose oral corticosteroids (≥20 mg/day) for at least 2 days.		
Fritsch 2006[19]	52 patients entered the study; FeNO group N=22. Control group N=25.	FeNO group: mean age 11.3 (SD 3.4), 14 males. Control group: mean age 12.1 (SD 2.8), 14 males.	FeNO group: therapy was based on symptoms, beta-agonists use, lung function and FeNO. Control group: therapy based on symptoms, beta-agonists and lung function only.	Primary outcome: FEV_1 Exacerbation defined by 4 parameters: oral steroid courses, and/or off-scheduled visit because of asthma symptoms over the past 4 weeks, and/or increase of asthma symptoms from a symptom score 0 or 1 to a symptom score 2 and/or decline of FEV_1 (L) more than 10% compared to the previous visit.	Visits were at 6, 12, 18 and 24 weeks after 4 week run-in. Follow-up duration = 24 months.	Aerocrine (analyser manufacturer) assisted with data analysis.
Green 2002[24]	74 randomised; Sputum management group N=37. BTS	Sputum management group: median age 50, range 19-73, 19 males.	Sputum management group: anti-inflammatory treatment was based on maintenance of sputum eosinophil count below 3% with a minimum	1.Number of severe asthma exacerbations 2.Control of eosinophilic airway inflammation measured by the induced sputum eosinophil count	Study duration was for 12 months with visits at month 1, 2, 3, 4, 6, 8, 10, 12.	Trent NHS Regional Research Scheme.

	management group N=37.	BTS management group: median age 47, range 20-75, 21 males.	dose of anti-inflammatory treatment. BTS management group: treatment decisions were based on traditional assessments of symptoms, peak expiratory flow and use of beta-2-agonists.	3.Exhaled nitric oxide concentrations 4.Symptom scores (0 to 3 for daytime and nighttime symptoms) 5.Total asthma quality of life scores 6.Peak flow amplitude as a proportion of the mean 7.FEV1 8.Changes from baseline of methacholine PC20 9.Drug use 10.Admissions for asthma Severe exacerbations defined as a decrease in morning peak expiratory flow to more than 30% below baseline value on = 2 consecutive days, or deterioration in symptoms needing rescue course of oral corticosteroid.	Follow-up duration = 12 months.	
Hashimoto 2011[36]	95 adults were randomised; Internet strategy N=51. Conventional strategy N=38.	Internet strategy: mean age 48.5 yrs (SD12.5), 23 males. Conventional strategy: mean age 52.4 yrs	Internet strategy: Had steroid dose adjusted based on the 3 components: electronic diary, in-built algorithm (which includes FeNO levels), and monitoring support, e.g. coaching by study nurse and	Primary outcomes: Cumulative sparing OCS (actual cumulative dose minus the expected dose), ACT, and AQLQ. Exacerbations: Decrease in morning FEV_1 >10% compared to mean FEV_1 from week before, increase in	Monthly visits with follow-up duration of 6 months. Participants daily registered their dose of OCS, lung	Netherlands Organisation for Health Research and Development (ZonMw). Equipment for the analysis of nitric

		(SD11.7), 18 males.	monitoring data, which was entered. Conventional strategy: GINA guidelines for the treatment of severe asthma.	symptoms requiring increased prednisolone >10mg/day, or course of antibiotics, regardless of hospitalisations.	function and FEV_1.	oxide was provided by Aerocrine AB.
Honkoop 2014[37]	GP practices cluster randomisation including 647 adults in 3 arms; FeNO group N=189. Controlled asthma group N=203.	FeNO group: mean age 39 yrs (SD 9), 62 males. Control group: mean age 40 yrs (SD 10), 69 males.	FeNO strategy: Treatment targeted to keep FeNO <50ppb. Symptom strategy: ACT utilized including lung function	Primary outcomes: Societal costs per QALY gained Severe exacerbation: Hospitalisation, emergency department visit because of asthma, or use of OCS for >3 days.	Follow-up duration of 12 months with 3 monthly visits.	Netherlands Organisation for Health Research and Development and by the Netherlands Asthma Foundation. Aerocrine (Solna, Sweden) provided 20 of a total of 40 fraction of exhaled nitric oxide meters for free.
Jayaram 2006[31]	117 randomised; Sputum strategy group N=50. Clinical strategy group N=52.	Sputum strategy: group mean age 46 (SD 13.8), 15 males Clinical strategy: group mean age 43.5 (SD 13.9), 15 males	Sputum strategy: dose of inhaled steroid was guided solely by induced sputum eosinophils to keep <2%. Spirometry was used to identify clinical control, exacerbations and other treatment. Clinical strategy: guided by symptoms as per Canadian Asthma Consensus Group Guidelines.	1.Relative risk reduction for the first exacerbation 2.The length of time without exacerbations 3.Type and severity of exacerbations 4. The usefulness of monitoring sputum cell counts in relation to the overall serverity of asthma. Defined by the minimum dose of inhaled steroid to maintain control	2 year study duration with monthly visits in Phase 1 until control maintained with minimum treatment (variable duration) or at exacerbations. Phase 2: 3 monthly visits or at exacerbations.	Canadian Institutes of Health Research Clinical Trials Grant.

				5. The cumulative dose of inhaled steroid needed in Phase 2 adjusted for its duration. Exacerbation: Loss of symptomatic control requiring increased use of short acting beta2-agonists by = 4 extra puffs per day for a minimum of 48 hours, or by nocturnal symptoms, or early morning wakening due to respiratory symptoms two or more times in one week. Severe exacerbations were defined as requiring rescue courses of oral prednisone as defined by the investigator.		
Malerba 2015[25]	28 adults randomised; Sputum strategy N=14. Clinical strategy N=14.	Sputum strategy: mean age 45.2 yrs (SD31.2), 5 males. Clinical strategy: mean age 46.7 yrs (SD30.1), 6 males.	Sputum strategy: Treatment based on sputum eosinophil (%) and FeNO (ppb). Decrease ICS <2% & ≤10pbb Keep same 2-3% & 11-20ppb Increase ICS >3% & ≥20ppb Symptom strategy: Symptom scores, use of SABA and night time symptoms.	Primary outcome: Asthma exacerbations combined with changes in symptom score at end of study. Moderate exacerbations: Requiring an unscheduled visit with a course of OCS. Severe exacerbation: Course of OCS as determined by study investigator.	Follow-up duration was 24 months, with 6 monthly visits.	University of Brescia

Peirsman 2014[34]	99 children randomised; FeNO group N=49. Control group N=50.	FeNO group: mean age 10.6 yrs (SD 2.2), 33 boys. Control group: mean age 10.7 yrs (SD 2.1), 33 boys.	FeNO group: Treatment aimed to keep FeNO below 20ppb. Control group: Treatment adjusted according to GINA guidelines (i.e. reporting of symptoms, use of SABA and FEV_1)	Primary outcome: Symptom free days using the first 4 questions from childhood ACT. Exacerbation: As per GINA guidelines	Follow-up duration was 12 months with 3 monthly appointments.	Study funded partially by Merck & Co and FeNO analysers supplied by Aerocrine.
Petsky 2015[28]	63 randomised; FeNO group N=31. Symptom group N=32.	FeNO group: median age 10.2 yrs (IQR 6.6 to 12.7), 18 boys. Symptom group: median age 10.1 yrs (IQR 6.3 to 12.4), 13 boys.	FeNO group: Treatment adjusted based on FeNO level and atopy status. Elevated FeNO defined as: ≥ 10ppb with no positive SPT ≥ 12ppb with 1 positive SPT ≥ 20ppb with ≥ 2 positive SPT	Primary outcome: Severe exacerbations requiring course of OCS with or without hospitalization. Exacerbation: Respiratory events requiring OCS.	Study duration 12 months with visits month 1, 2, 3, 4, 6, 8, 10 and 12.	Asthma Foundation of Queensland
Pijnenburg 2005[18]	89 children randomised; FeNO group N=39 Symptom group N=46	FeNO group: median age 11.9 (SD 2.9), 25 males. Symptom group: mean age 12.6 (SD 2.8), 30 males.	FeNO group: FeNO guided ICS dosing according to predetermined algorithm. Symptom group: Symptom scores influenced ICS dosing.	Primary outcome: cumulative steroid dose (sum of mean daily steroid doses of visits 1 to 5) Exacerbation: Deterioration in symptoms requiring oral prednisone course.	Study duration was 12 months with 3 monthly visits.	Kroger Foundation/Sophia Children's Hospital Foundation

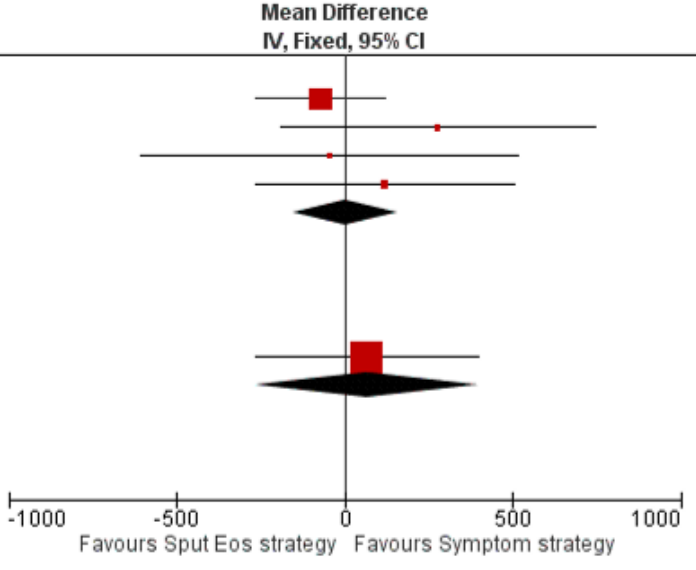
Pike 2013[32]	90 children randomised; FeNO group N=44. Standard management group N=46.	FeNO group: mean age 10.51 yrs (SD 2.62), 21 boys. Standard management group: mean age 11.42 yrs (SD 2.69), 30 boys.	FeNO group: FeNO measurements and symptom control. Standard management group: symptom control as per blinded clinician (reliever use, FEV_1).	Change in ICS dose, exacerbation frequency, FeNO measurements and lung function.	Study duration 12 months with study visits every 2 months.	Sparks
Powell 2011[27]	220 pregnant women randomised; FeNO group N=111. Control group N=109.	FeNO group: mean age 28 (range 27 to 29). Control group: mean age 29 (range 28 to 30).	FeNO group: Sequential process, first FeNO concentrations used to adjust ICS dose, and second ACT score used to adjust the LABA dose. Clinical group: Based on asthma control using Juniper ACT with cutoff points defined as: well-controlled asthma (ACT < 0.75), partially controlled asthma (0.75 to 1.50), and uncontrolled asthma (> 1.5)	Primary outcome: Total number of asthma exacerbations (i.e. moderate and severe). Secondary outcomes: QoL, asthma treatment, and fetal outcomes	Study duration was average of 4 months. Women were seen monthly until they delivered.	National Health and Medical Research Council of Australia
Shaw 2007[38]	118 adults were randomised;	FeNO group: median age 50	FeNO group: FeNO >26ppb, ICS was increased. If FeNO <16ppb or <26ppb	Primary outcome: Number of exacerbations	Study duration was 12 months with participants	Asthma UK

	FeNO group N=58 Control group N=60.	(range 20-75), 27 males. Control group: median age 52 (range 24-81), 27 males.	on 2 separate occasions, treatment was decreased. In Control Group treatment was doubled if Juniper Asthma Control Score (JACS) >1.57 and treatment halved if JACS <1.57 for 2 consecutive months.	Exacerbation: An increase in symptoms requiring oral steroids or antibiotics	being send at baseline, 2 weeks, months 1, 2, 3, 4, 6, 8, 10 and 12.	
Smith 2005[21]	97 patients randomised from 110 patients recruited.	N=46 in FeNO group achieved optimal dose in phase 1 and N=28 achieved optimal dose in control group. Mean age of randomised patients was 44.8 (range 12-73), 41 males.	Phase 1: Run-in period was for 6 weeks, after 2 weeks fluticasone 750ug/day was commenced. Visits were every 4 weeks until optimal dose was achieved. FeNO group: adjustment of dose of ICS was based soley to keep FeNO <15ppb at 250mL/sec. Control group: dose adjustment based on asthma symptoms, night-time waking, bronchodilator use, variation in PEFR and FEV1. Phase 2: visits every 2 months with upward adjustments made as per phase 1 but no downward adjustments would be made from optimal dose.	Primary outcome: Frequency of exacerbation Minor exacerbation was defined as a daily asthma score of 2 or more on 2 or more consecutive days, whereas a major exacerbation was a daily asthma score of 3 or more on 2 or more consecutive days.	2 phase study, with phase 1 varying in duration (3-12 months) depending when optimal dose was deemed to have been achieved. During phase 2 (12 months) optimal dose from phase 1 was continued and therapy stepped up if asthma control was lost.	Otago Medical Research Foundation, the Dean's Fund of the Dunedin School of Medicine, and a grant from the University of Otago. Supplies of fluticasone were provided by GlaxoSmithKline (New Zealand). Equipment for the analysis of nitric oxide in other studies was provided by Aerocrine.

Syk 2013[39]	187 adults randomised; FeNO group N=87. Control group N=78.	FeNO group: mean age 40.9 yrs (SD 11.8), 48 males. Control group: mean age 41.1 yrs (SD 12.9), 46 males.	FeNO group: Keep FeNO level <24ppb for women, and <26ppb for men. Control group: Treatment adjusted based on patient reported symptoms, SABA use, physical examination and spirometry results.	Primary outcome: change in mAQLQ score during the study. Exacerbation: Increasing asthma symptoms requiring course of OCS.	Study duration was 12 months with visits at months 1, 2, 4, 8 & 12.	Stockholm county council (PickUP), Centre for Allergy Research, Karolinska Institute, and the Research Foundation of the Swedish Asthma and Allergy Association. Aerocrine AB (NIOX MINO instruments), Phadia AB (ImmunoCAP Rapid), Meda AB (Buventol Easyhaler), and MSD Sweden (small grant).
Szefler 2008[29]	546 participants randomised from 780 patients screened. FeNO group N=276. Control group N=270	FeNO group: mean age 14.4, 146 males. Control group: mean age 14.4, 142 males.	FeNO group: Standard treatment modified on the basis of measurements of FeNO Control group: Standard treatment based on the guidelines of National Asthma Education and Prevention Program (NAEPP).	Primary outcome: Number of days with asthma symptoms. Exacerbation: Combination of admissions to hospital, unscheduled visits and oral prednisone.	The study duration was 46 weeks with visits every 6-8 weeks.	US National Institute of Allergy and Infectious Diseases, US National Institutes of Health.

Verini 2010[20]	64 children randomised; FeNO group N=32. GINA group N=32.	FeNO group: mean age 10.7 yrs (SD 2.4), 18 boys. GINA group: mean age 11.3 yrs (SD 2.1), 18 boys.	FeNO group at 6 month visit only: step treatment up if >12ppb. Control group: As per GINA guidelines.	Primary outcome: No clear definition given of outcomes, however asthma severity score, asthma exacerbation frequency and asthma therapy score were the main items reported in results section. Exacerbation: According to ATS/ERS criteria and requiring SABA.	Study duration was 12 months with 6 monthly visits.	No information provided on funding
Voorend-van Bergen 2015[33]	272 children randomised into 3 arms; FeNO group N=92. Standard care group N=89.	FeNO group: mean age 10.3 yrs (SD 2.9), 62 boys. Standard care group: mean age 10.2 yrs (SD 3.2), 61 boys.	FeNO group: Treatment adjusted according to FeNO levels and ACT results. If ACT ≥ 20 and: FeNO < 25 = step down FeNO ≥ 25 to < 50 = no change FeNO ≥ 50 = step up If ACT < 20 and: FeNO ≥ 25 = step up FeNO < 25 = no change Control group: Treatment adjusted based on ACT results < 20 = step up ≥ 20 = no change or step down	Primary outcome: Changes from baseline of proportion of symptom-free days Exacerbation: No definition provided but OCS courses and hospitalization data included in the exacerbation results.	Children were run-in for 4 weeks, then 4 monthly visits for a total of 12 months.	Lung Foundation Netherlands, the Netherlands Organisation for Health Research (ZonMW) and Fund Nuts Ohra.

Study or Subgroup	Mean Difference	SE	Sputum Eos Strategy Total	Symptom Strategy Total	Weight	Mean Difference IV, Fixed, 95% CI
1.9.1 Adults						
Cao 2007	-71	98.1587	20	21	65.0%	-71.00 [-263.39, 121.39]
Chlumsky 2006	277	237.9686	30	21	11.1%	277.00 [-189.41, 743.41]
Green 2002	-45	286.2615	34	34	7.6%	-45.00 [-606.06, 516.06]
Jayaram 2006 (1)	120	195.6908	50	52	16.3%	120.00 [-263.55, 503.55]
Subtotal (95% CI)			**134**	**128**	**100.0%**	**0.67 [-154.39, 155.73]**
Heterogeneity: Chi² = 2.28, df = 3 (P = 0.52); I² = 0%						
Test for overall effect: Z = 0.01 (P = 0.99)						
1.9.2 Children						
Fleming 2012 (2)	67	169.2927	26	28	100.0%	67.00 [-264.81, 398.81]
Subtotal (95% CI)			**26**	**28**	**100.0%**	**67.00 [-264.81, 398.81]**
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.40 (P = 0.69)						



Test for subgroup differences: Chi² = 0.13, df = 1 (P = 0.72), I² = 0%

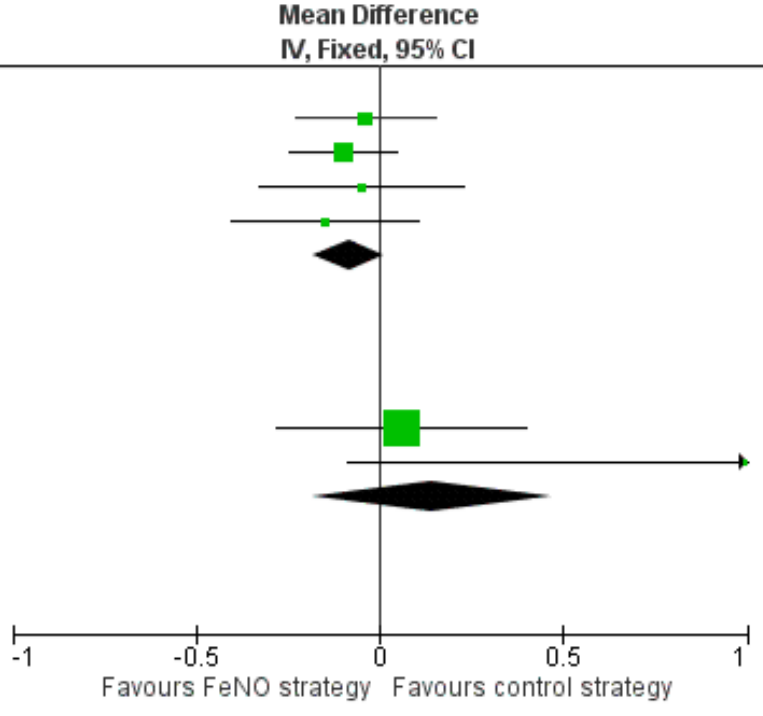
Footnotes

(1) Mean and SD reported as FP, therefore doubled to make Bud equiv

(2) Data obtained from author, Mean and SD given as FP, therefore doubled to make Bud equiv

Study or Subgroup	FeNO Mean	FeNO SD	FeNO Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Fixed, 95% CI
1.7.1 Adult								
Calhoun 2012	0.68	0.7335	115	0.72	0.7335	114	27.0%	-0.04 [-0.23, 0.15]
Powell 2011	0.5	0.5	111	0.6	0.6	109	45.6%	-0.10 [-0.25, 0.05]
Shaw 2007	1.1	0.72	52	1.15	0.71	51	12.8%	-0.05 [-0.33, 0.23]
Syk 2013	0.79	0.814	81	0.94	0.8201	74	14.7%	-0.15 [-0.41, 0.11]
Subtotal (95% CI)			**359**			**348**	**100.0%**	**-0.08 [-0.18, 0.01]**
Heterogeneity: Chi² = 0.56, df = 3 (P = 0.91); I² = 0%								
Test for overall effect: Z = 1.68 (P = 0.09)								
1.7.2 Children								
Szefler 2008a	21.89	2.0266	276	21.83	2.0266	270	91.1%	0.06 [-0.28, 0.40]
Voorend-van Bergen 2015	22.4	3.5	91	21.4	3.9	87	8.9%	1.00 [-0.09, 2.09]
Subtotal (95% CI)			**367**			**357**	**100.0%**	**0.14 [-0.18, 0.47]**
Heterogeneity: Chi² = 2.60, df = 1 (P = 0.11); I² = 62%								
Test for overall effect: Z = 0.87 (P = 0.39)								

Test for subgroup differences: Chi² = 1.74, df = 1 (P = 0.19), I² = 42.4%



Study or Subgroup	Mean Difference	SE	Weight	Mean Difference IV, Fixed, 95% CI
1.8.1 Adult				
Calhoun 2012	0	0.1122	15.2%	0.00 [-0.22, 0.22]
Honkoop 2014	0.001	0.0566	59.8%	0.00 [-0.11, 0.11]
Subtotal (95% CI)			**75.0%**	**0.00 [-0.10, 0.10]**
Heterogeneity: Chi² = 0.00, df = 1 (P = 0.99); I² = 0%				
Test for overall effect: Z = 0.02 (P = 0.99)				
1.8.2 Children				
de Jongste 2008	0	0.1225	12.8%	0.00 [-0.24, 0.24]
Petsky 2015	0.33	0.3674	1.4%	0.33 [-0.39, 1.05]
Voorend-van Bergen 2015	0.17	0.1327	10.9%	0.17 [-0.09, 0.43]
Subtotal (95% CI)			**25.0%**	**0.09 [-0.08, 0.26]**
Heterogeneity: Chi² = 1.33, df = 2 (P = 0.51); I² = 0%				
Test for overall effect: Z = 1.06 (P = 0.29)				
Total (95% CI)			**100.0%**	**0.02 [-0.06, 0.11]**
Heterogeneity: Chi² = 2.15, df = 4 (P = 0.71); I² = 0%				
Test for overall effect: Z = 0.54 (P = 0.59)				
Test for subgroup differences: Chi² = 0.82, df = 1 (P = 0.36), I² = 0%