# Basic characteristics and clinical value of FeNO in smoking asthmatics -a systematic review

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Take home message

FeNO may be associated with eosinophilic inflammation and may be useful in diagnosing asthma also among smoking subjects, but there is lack of data on the clinical role of FeNO in smokers.

Registration in PROSPERO

The review has been registered in PROSPERO database with identifier CRD42018090112.

ABSTRACT

Exhaled nitric oxide (FeNO) reflects eosinophilic airway inflammation and it can be used to diagnose and phenotype asthma and predict treatment responses. However, smoking decreases FeNO and it is not clear if FeNO has clinical value in smoking subjects with asthma.

We conducted a systematic review focusing on four basic characteristics and five clinical questions on using FeNO in smokers with asthma. At least two authors independently screened search results, extracted data and assessed quality of the included studies. Data were synthesised mainly by qualitative methods.

Twenty-two studies were included. FeNO is lower in smoking than in non-smoking asthmatics, but importantly FeNO is higher in untreated smoking asthmatics than in healthy smokers. Information was incomplete but there is some indication that FeNO might be useful in detecting eosinophilic airway inflammation and in diagnosing asthma in smoking subjects. There was no data available to four of the five clinical questions.

In conclusion, at the moment there is insufficient data to give specific guidelines on using FeNO in smoking subjects, but although smoking decreases FeNO it does not seem to make FeNO measurement redundant. FeNO is associated with asthma also in smokers and current results encourage conducting clinical trials on FeNO in smokers with asthma.

INTRODUCTION

Asthma is a chronic airway disease usually characterised by mucosal inflammation, bronchial hyperresponsiveness and variable airway obstruction leading to symptoms such as cough, chest tightness and wheezing (1). The intensity and type of airway inflammation vary between individuals and many phenotypes of asthma have been identified (2). Eosinophilic airway inflammation is a common and best known inflammatory phenotype of asthma, but also neutrophilic and paucigranulocytic asthma have been described. Although inflammation is pivotal in the pathogenesis of asthma, the diagnosis and follow-up of asthma are currently mainly based on assessing symptoms and lung function.

As eosinophilic inflammation is particularly sensitive to treatment with inhaled corticosteroids (ICS) (3-6), non-invasive markers of eosinophilic phenotype of asthma have been developed in order to guide ICS-treatment in asthma. Nitric oxide (NO) is an important cellular signalling molecule that regulates pulmonary blood flow, mucus production, ciliary activity and inflammation. Under normal circumstances NO is produced at very low concentrations by constitutive NO synthases (endothelial NOS and neuronal NOS). In cases of airway inflammation pro-inflammatory cytokines upregulate expression of inducible NOS producing higher amounts of NO (7). Fractional exhaled nitric oxide (FeNO) is particularly associated with eosinophilic airway inflammation (8-12). Since allergic asthma is eosinophilic in nature, elevated FeNO is associated also with atopy among subjects with asthma (13). Treatment with ICS suppresses activity of eosinophilic inflammation, and it efficiently decreases FeNO level (14), probably by inhibiting NFκB (15), an important transcription factor regulating the expression of iNOS.

Active smoking is unfortunately almost as common among asthmatics as among healthy subjects (17). Smoking is known to induce a macrophage and neutrophil driven chronic airway inflammation, but many smoking asthmatics still have eosinophilic inflammation characteristic to asthma (18,19). As eosinophilic inflammation is found also in smoking asthmatics, FeNO might be used to find eosinophilic phenotype of asthma also in active smokers. However, smoking as such reduces FeNO (16,20) possibly by reducing the availability of a cofactor tetrahydrobiopterin needed in NO synthesis (21,22) or by increased superoxide synthesis by neutrophils that scavenges NO in chemical reactions preventing it from diffusing into exhaled air. Therefore, the cut-off value of FeNO to find active eosinophilic airway inflammation among smokers would probably have to be lower than among non-smokers. Due to the effect of smoking on FeNO, most of the clinical studies assessing the value of FeNO in asthma have been conducted in non-smoking asthmatics

only. In non-smokers with asthma, FeNO can be used to predict responsiveness to ICS and risk of exacerbations (23). Hence, in non-smoking asthmatics titrating ICS treatment based on FeNO seems to reduce number of exacerbations (24,25).

*The aim and study questions*

The aim of the present systematic review was to assess the basic characteristics and evidence for clinical use of FeNO in smoking asthmatics. The detailed study questions were:

Basic characteristics of FeNO in relation to smoking and asthma:
1. Is FeNO associated with eosinophilic airway inflammation among smoking asthmatics?
2. Is FeNO associated with asthma control or asthma severity among smoking asthmatics?
3. Is FeNO lower in smoking than in non-smoking asthmatics?
4. Is FeNO among smoking asthmatics a) not on ICS-treatment or b) on ICS-treatment higher than among healthy smoking individuals?

Clinical value of FeNO in relation to smokers with asthma:

5. Can FeNO be used as aid in diagnosing asthma in smoking subjects?
6. Does FeNO predict response to ICS treatment in steroid-naïve smoking asthmatics?
7. During maintenance ICS treatment, does FeNO measurement identify those smoking asthmatic patients who are at risk of exacerbation?
8. During maintenance ICS treatment, does FeNO measurement identify those smoking asthmatic patients who would benefit from augmented glucocorticoid treatment?
9. In smoking asthmatics, what is the clinical value of FeNO measurement in tailoring ICS treatment compared to usual treatment strategy?

*METHODS*

As this review considers several kinds of study designs depending on the research question (on basic characteristics of FeNO, but also on the use of FeNO in diagnostics, prognostics and interventions), the review does not apply only one framework but is multi-methodological. For example, intended tools for risk of bias assessment of the included studies depend on the research question.

*1. Criteria for considering studies for this review*

Types of studies

We included studies in which FeNO measurement was conducted in smoking asthmatic subjects and the sample size was at least 10 subjects per group. We included systematic reviews, randomised controlled trials, controlled clinical trials, other comparative studies, observational studies, both prospective and retrospective designs, published in English, German, Scandinavian languages and Hungarian.

Types of participants

We included studies on smoking asthmatic subjects in whom the diagnosis of asthma was based on reversible or variable airway obstruction (1) in at least 80 % of participants with asthma. We placed no restrictions on comorbidities in included subjects. Subjects without any diagnosed respiratory disease were classified and included as healthy subjects. FeNO had to be measured using an online technique and measured at a healthcare unit excluding home measurements (the standard flow rate is 50 ml/s but we allowed also the use of other flow rates in the included studies). For reliable online FeNO measurement, the age of the subjects had to be at least 5 years (although this is obviously fulfilled in studies on active smokers).

In studies where FeNO was compared to other measures (e.g. questions 1 and 2), we placed no quality criteria for these comparative measures, such as induced sputum eosinophil counts or asthma control, but we accepted those as they were reported.

We decided to consider subjects as smokers if they were current smokers (without any restrictions e.g. on frequency and amount of smoking) and as non-smokers if they were never-smokers or ex-smokers who had not smoked for at least 6 months. Further, we decided that at least 80% of the non-smokers had to fulfil the above-mentioned criterion for the study to be included. The reason for these decisions is that we anticipated that the individual studies would have defined non-smokers very differently and useful information from the studies would have been lost if e.g. only studies considering never-smokers would have been included. In case the study reported characteristics and results separately both on never-smokers and ex-smokers, we used never-smokers as the control group. Unfortunately, there is no solid information how rapidly and to what extent quitting smoking affects FeNO, and therefore we decided in practical terms to set the time limit

for quitting of smoking as 6 months. A sensitivity analysis was conducted to see if inclusion of ex-smokers as non-smokers affects the results.

The criteria for anti-inflammatory medication and requirements for comparisons and outcome measures depended on the study question and are listed in detail in Online Supplement 1.

## 2. Search strategy

The electronic literature searches were based on the following keywords: nitric oxide, exhalation, FeNO, smoking, asthma. The searches were conducted in two stages. At first, we searched the following seven electronic databases up to May 25, 2017 to track down systematic reviews and individual trials: MEDLINE (OVID), Embase (OVID), Cochrane Central Register of Controlled Trials (CENTRAL), the Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects (DARE), Health Technology Assessment Database (HTA), and NHS Economic Evaluation Database (NHS EED).

We also searched the following two databases for ongoing studies: US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (clinicaltrials.gov; searched 25 May 2017), and World Health Organization International Clinical Trials Registry Platform (apps.who.int/trialsearch; searched 25 May 2017).

The searches were updated on 4 September 2018. Two new terms were added to Medical Subject Headings in 2018 (Tobacco smoking, Pipe smoking) and these terms were added to the strategies where appropriate. Search strategies are presented in Online Supplement 2.

In addition to searching the electronic databases, we screened the reference lists from the already identified studies and review articles for any additional relevant studies.

We placed no language restrictions in searches, although for inclusion we assessed only reports published in English, German, Scandinavian languages and Hungarian. If the information in the report was insufficient to make the final assessment of inclusion or exclusion, we contacted the authors of the studies to obtain additional information (authors of six studies were contacted).

We considered only studies with full-text reports for inclusion in this review because it has been shown that discrepancies occur between data reported in abstracts and published full reports (26,27).

## 3. Data extraction and quality assessment

At least two authors independently screened search results, extracted data and assessed risk of bias of the included studies. We extracted the following information: 1) on the study methods: study design, possible length of follow-up, inclusion and exclusion process of participants (question 5); 2) on participants' characteristics: location where the study was conducted, age, gender and numbers of participants in each group, severity of asthma, forced expiratory volume in 1 s ($FEV_1$), possible use of corticosteroids, atopy/allergy, smoking habits of smoking subjects; 3) on characteristics of FeNO measurement: device and exhalation flow rate; 4) details of reference standard in questions 1, 2 and 5; 5) analysis methods, results reported in each study, and funding source.

Assessment of risk of bias

Risk of bias was assessed by using different tools depending on the study questions. A quality assessment tool for diagnostic accuracy studies QUADAS-2 (28), Quality In Prognosis Studies tool QUIPS (29), and Cochrane risk of bias tool (30) were used where and when appropriate. Details of the assessment of risk of bias are given in Online Supplement 1.

## 4. Data synthesis

We synthesised data mainly by qualitative methods. The combined results in each research question and comparison were based on the similarity of the results of the individual studies and on descriptive statistics, if appropriate.

In the research question 3 and in one comparison of question 4 (with moderate numbers of included studies with codirectional results), we decided to calculate proportional difference in FeNO values between study groups in each study. Proportional difference in this review is defined as how many percentages lower the FeNO values (in the group with lower values) were compared to the FeNO values in the comparison group. We calculated 95% confidence intervals (CI) by Monte Carlo methods using R software version 3.4.2, ignoring possible factors influencing variation at study level. We chose proportional difference as the statistic to describe the results of different studies because there was much variability in flow rates and statistics used between studies. Studies reported their results e.g. as means with SDs or medians with interquartile ranges (IQR) or geometric means with 95% CIs. The distributions of FeNO values in most studies were skewed, and it was impossible to standardize different statistics of the studies in another way. We present only ranges of the percentages of differences in each comparison because the values varied much (and we do not present an average magnitude of the percentage difference in FeNO values based on all included data at each comparison).

In research question 3 with non-smokers as controls, we included never smokers and also ex-smokers who had quit smoking for at least 6 months earlier as non-smokers in the primary analysis. To assess the effect of including also ex-smokers and not only never smokers as non-smokers, we undertook a sensitivity analysis to assess the robustness of our results. In the sensitivity analysis we excluded studies with ex-smokers and studies not explicitly stating that only never-smokers had been included as controls.

## Results

### Search results

Twenty-two studies were included. The processing of search results is presented in Figure 1. Detailed reasons for exclusion of 16 studies are presented in Online Supplement 3 (main reasons for exclusions were: asthma diagnosis of subjects or smoking status did not fulfil the inclusion criteria assessed for this review).
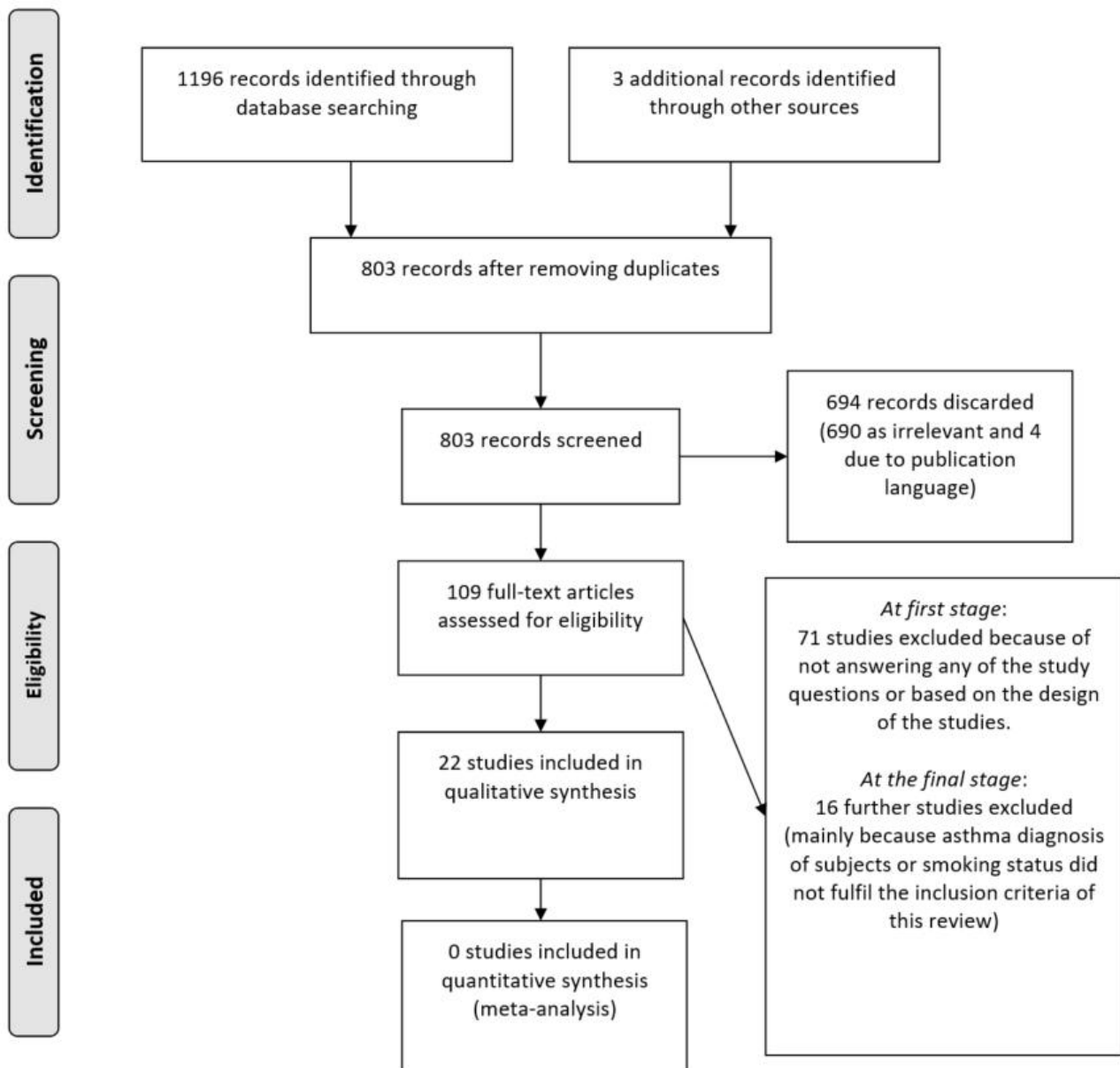


Figure 1. Flow chart of search results.

Characteristics of included studies

The included 22 studies involved in total 5656 subjects (smoking and non-smoking asthmatics and smoking healthy subjects). The studies provided data for all the research questions on basic characteristics (three studies for question 1; two for question 2; 16 for question 3; and 9 for question 4) but for only one of the clinical questions (3 studies for question 5). The designs of the studies in questions 1 to 4 varied much but our research questions considered mainly cross-sectional data of the studies. There was marked variation between studies regarding inclusion criteria of subjects, severity of asthma and use of anti-inflammatory medication in asthmatics, proportion of atopics, smoking habits, and FeNO measurement (e.g. exhalation flow rate). In many studies, there was incomplete or missing information on these factors having influence on FeNO value. Detailed descriptions of the included 22 studies and results of each study per research question are provided in Online Supplements 4-9.

Results per research questions

1. Is FeNO associated with eosinophilic airway inflammation among smoking asthmatics?

*Summary: There is incomplete information, but based on three studies (two with positive and one with negative finding) there is some indication that FeNO may be useful in detecting eosinophilic airway inflammation in smoking asthmatics.*

Berry and colleagues (31) concluded that in smoking asthmatics, FeNO was not closely related to sputum eosinophil count and FeNO did not identify subjects with sputum eosinophil count > 3% (113 smoking asthmatics, AUC = 0.63, 95% CI 0.48 to 0.78, p = 0.10). On the contrary, Hillas and colleagues (32) found that the predictive performance of FeNO to detect purely eosinophilic phenotype (sputum eosinophilis ≥ 3% and neutrophils < 60%) was satisfactory (40 smoking asthmatics, AUC of ROC = 0.880, 95% CI 0.74 to 0.96, p < 0.0001). Schleich and colleagues (33) concluded that FeNO is able to identify the presence of sputum eosinophilia ≥ 3% in unselected patients with asthma with reasonable accuracy as long as FeNO thresholds are adjusted for high doses of ICS, atopy and smoking status. Based on multiple regression analyses, they found PPVs ranging from 52% to 62% and NPVs ranging from 76% to 89% depending on the covariates (such as smoking) included in the analyses. By simple regression analysis with smoking as the only covariate, the study reported PPV of 59% and NPV of 78%, for FeNO cut-off value of 28 ppb, (p-value 0.066). See Online Supplement 4 for details.

2. Is FeNO associated with asthma control or asthma severity among smoking asthmatics?

*Summary: Based on two studies FeNO is rather poorly associated with the level of asthma control in smoking asthmatics but based on one follow-up study change in FeNO between visits is related to simultaneous change in asthma control. There were no studies on FeNO and asthma severity.*

Kostikas and colleagues (34) evaluated the diagnostic performance of FeNO to identify patients with partly controlled or uncontrolled asthma defined according to GINA guidelines. FeNO identified asthma control better in ICS-untreated smokers than in ICS-treated smokers [AUC (95% CI) for the optimum cut-point of FeNO > 19 ppb was 0.680 (0.492 to 0.833, p=0.059) and for the optimum cut-point of FeNO > 23 ppb it was 0.597 (0.449 to 0.733, p=0.256)]. They further stated that high FeNO values (over 30 ppb) are indicative of poor asthma control even in ICS-treated smoking asthmatics (PPV of 83%).

Michils and colleagues (35) evaluated the diagnostic performance of FeNO for the identification of patients with controlled asthma defined as ACQ score < 1.5. The study reported PPV of 30%, NPV of 81%, accuracy of 53% and p-value of 0.39 for the optimal FeNO cut-off value of 25ppb.

Michils and colleagues studied also whether change in FeNO value between two visits is related to simultaneous change in asthma control and they found that sequential changes in FeNO are related to changes in asthma control in smokers. The study showed that in smoking asthmatics with uncontrolled asthma a FeNO reduction of < 20% between visits would indicate that asthma remained uncontrolled (analysed as change from uncontrolled [ACQ score ≥ 1.5] to controlled [ACQ score < 1.5] asthma as a positive event, then the cut-off value for decrease in FeNO, which had the highest NPV [82%] for establishing control was 20%; accuracy 67%, p-value of 0.016). Conversely, when asthma is controlled, an FeNO increase of < 50% would indicate that asthma remained controlled (analysed as change from controlled [ACQ score < 1.5] to uncontrolled [ACQ score ≥ 1.5] asthma as a positive event, then the cut-off value for an increase in FeNO, which had the highest NPV [89%] for a change to uncontrolled asthma was 50%; accuracy 83%, p-value 0.017).

Further, Michils and colleagues found that a decrease in FeNO of < 20% between two visits precluded asthma control improvement (defined as a decrease ACQ < 0.5, NPV of 70%) and that an increase in FeNO < 30% was unlikely to be associated with worsening in asthma control (ACQ improvement > 0.5, NPV of 86%). However, when subjects were treated with moderate to high ICS doses, change in FeNO lost its ability to reflect an improvement or worsening in asthma control. See Online Supplement 4 for details.

3. Is FeNO lower in smoking than in non-smoking asthmatics?

*Summary: Based on 15 studies FeNO levels are lower in smoking asthmatics than in non-smoking asthmatics with the median of proportional difference being about 46 %.*

In 15 out of the 16 studies providing data for this research question, FeNO levels were lower in smoking asthmatics than in non-smoking asthmatics (18,32,35-48). However, the magnitude of difference between smokers and non-smokers varied largely between studies (the proportional differences in FeNO values varied from 28.4% (95% CI 12.5% to 43.8%) to 71.8% (95% CI 57.5% to 85%); median of the proportional differences was 46.3 %; medians of the lower and upper limits of 95% CIs were 36.9 % and 57.1 %, respectively). A sensitivity analysis was undertaken to assess if inclusion of ex-smokers as non-smoking controls affected this result. Eight of the 15 studies clearly reported only never-smokers having been used as controls. When the analysis was restricted to these 8 studies only the results were similar (the proportional differences in FeNO values varied from 28.7% (95% CI 21.1% to 36.1%) to 71.8% (95% CI 57.5% to 85%); median of the proportional differences was 44.6 %; medians of the lower and upper limits of 95% CIs were 31.5 % and 57.7 %, respectively). There was incomplete information to deduce how known factors affecting FeNO value (such as use of steroids, proportion of atopics and severity of asthma) influenced the marked differences between studies (Online Supplement 6). In one study FeNO levels were similar among Japanese smokers and never-smokers with asthma (41). In that particular study, sputum eosinophil count was high both in smokers and in non-smokers (mean of 17.0 % (SD 18.4) and of 11.5 % (SD 20.0), respectively), and thus even higher in smokers than in non-smokers. Detailed results and description of studies are presented in Online Supplement 5.

Although in majority of studies FeNO levels in smoking groups were significantly lower than in non-smoking groups, it was not appropriate to synthetize quantitatively the average magnitude of the proportional difference in FeNO levels between smoking asthmatics and non-smoking asthmatics because of clinical diversity of participants e.g. regarding smoking habits and marked variation of differences in FeNO levels between the studies.

4. Is FeNO among smoking asthmatics a) not on ICS-treatment or b) on ICS-treatment higher than among healthy smoking individuals?

*Summary: As compared to smoking healthy subjects, FeNO is increased in smoking asthmatics not on ICS-treatment (six studies) but similar in ICS-treated smoking asthmatics (four studies).*

*4a Smoking asthmatics not on ICS-treatment vs. smoking healthy subjects*

In all six studies providing data for this comparison, FeNO levels were significantly higher in smokers with asthma not on ICS-treatment than in healthy smokers (37,38,42,44,46,49). Detailed results and description of studies are presented in Online Supplement 7. In five of these studies the proportional differences in FeNO varied from 41.7% (95% CI 24.1 to 58.6) to 81.8% (95% CI 72.3 to 90.9) (Online Supplement 8). The largest difference in FeNO values was in the Japanese study by Shimoda in 2016 (in smoking asthmatics not on ICS mean FeNO was 77 ppb (SD 55 ppb) and in smoking healthy subjects mean FeNO was 14 ppb (SD 4 ppb), flow rate of 50 mL/s) (44). In that study, sputum eosinophil count was high in smoking asthmatics (mean of 21% (SD 18)). In one of the six studies 25 % of all asthmatics used ICS but there was no information how many smoking asthmatics used ICS medication. In this study the proportional difference was lowest among all the six studies (37.7% (95% CI 28.1 to 46.9)).

*4b Smoking asthmatics on ICS-treatment vs. smoking healthy subjects*

Four studies (32,37,39,50) provided data for this comparison and they all concluded that FeNO levels in smoking steroid-treated asthmatics did not differ from those in smoking healthy subjects (Online Supplement 7).

5. Can FeNO be used as aid in diagnosing asthma in smoking subjects?

*Summary: There is incomplete information, but based on three studies with unclear risk of bias and different setups and divergent results (two with positive and one with negative finding) there is some indication that FeNO may be useful as aid in diagnosing asthma in smoking subjects.*

The results of the three studies providing data for this research question were divergent. The study populations in these studies were also different between each other. One study assessed the performance of FeNO to differentiate asthmatics from non-asthmatics in a population sample of subjects with asthma-like symptoms (38), while another study assessed the ability of FeNO to differentiate symptomatic asthmatics from symptom free healthy subjects (49). The third study assessed the ability of FeNO to differentiate

asthmatic subjects from a control group consisting of both symptomatic subjects without asthma and symptom free healthy subjects (51).

Malinovschi and colleagues (38) concluded that FeNO could differentiate asthmatic subjects from non-asthmatic subjects with asthma-like symptoms equally well in both never- and current smokers within a random population sample (among smokers AUC = 0.70 (95% CI 0.59 to 0.82) for the optimum cut-point of FeNO of 17 ppb, with sensitivity of 56.3%, specificity of 82.5%, PPV of 57% and NPV of 82%).

On the contrary, the study by Kostikas and colleagues (51) with heterogeneous sample of subjects concluded that FeNO is not a good marker for the diagnosis of asthma in smokers (AUC = 0.648 (95% CI 0.53 to 0.76) for the optimum cut-point of FeNO of 19 ppb). However, FeNO values over 25 ppb were characterized by specificity over 90% also in smokers (specificity of 92.0% [95% CI 80.7 to 97.7] and sensitivity of 6.2% [95% CI 3.7 to 21.2]).

Matsunaga and colleagues (49) found that FeNO could differentiate symptomatic asthmatics from symptom free healthy subjects, but the result was to some extent dependent on rhinitis status of subjects (among smoking subjects without rhinitis: AUC = 0.935 for the optimum cut-point of FeNO of 18 ppb, with sensitivity of 100% and specificity of 87%; among smoking subjects with rhinitis: AUC = 0.865 for the optimum cut-point of FeNO of 22 ppb, with sensitivity of 80% and specificity of 86%).

We assessed all the three studies as having unclear risk of bias. In addition to some unclear information, all the studies evaluated the diagnostic accuracy of FeNO against an optimal cut-off value derived from their own data, but the study populations were rather small for adequate analyses. Description of the studies, detailed results and risk of bias assessments are presented in Online Supplement 9.


Research questions 6-9

We found no studies providing data for these research questions.

Discussion

To our knowledge this is the first systematic review to assess basic characteristics and clinical value of FeNO in smokers with asthma. We found that there is some evidence on the basic relations between asthma and FeNO in smoking subjects but lack of data to assess the clinical value of FeNO in diagnosis and treatment of smoking subjects with asthma.

Basic characteristics of exhaled nitric oxide in smoking asthmatics

Although smoking decreases FeNO, we found that many of the basic relations of asthma and FeNO may hold true also in active smokers. There is indication that FeNO may be associated with eosinophilic airway inflammation also in smokers with asthma, FeNO is increased in untreated asthma but normal in ICS-treated asthma as compared with healthy smokers. These are basically the same findings as in non-smokers but absolute FeNO levels are lower.

The information on association of FeNO with eosinophilic airway inflammation in smoking asthmatics is incomplete as there were only three studies focusing on this topic. Based on two studies there is an association between FeNO and eosinophilic airway inflammation, but one study did not support this. The study by Hillas and colleagues (32) in smoking ICS-treated asthmatics found a clear association between FeNO and induced sputum eosinophil count. Also the study by Schleich and colleagues (33) found a reasonable accuracy for FeNO to identify airway eosinophilia. However, the study included steroid-naïve asthmatics and those receiving ICS in low to high doses, but there was no detailed information on ICS use among smoking asthmatics specifically. On the contrary, the study by Berry (31) with heterogeneous population of adult asthmatics, did not find a correlation between FeNO and sputum eosinophils. In this study, it remained unclear how many of the 113 smoking asthmatics used ICS treatment (56% of all study used inhaled steroids). Thus, the conclusions in the two studies with heterogeneous populations were divergent. In the study by Hillas 2011 with homogenous population, the number of smoking asthmatics (n= 40) was rather small for adequate analysis (analyses were based on use of optimal cut-off values of FeNO derived from their own data, in small sample size this may be a risk of bias (52)). The excluded study in this research question by Nagasaki (41) considered the relationship between FeNO and eosinophilic inflammation in never- and ex-smokers but not in current smokers.

Two cross-sectional studies evaluated whether single FeNO value is associated with asthma control and concluded that FeNO reflects rather poorly asthma control in smoking asthmatics (34,35). This is

understandable as FeNO reflects activity of eosinophilic inflammation while symptom control is determined also by lung function and possibly activity of non-eosinophilic inflammation. However, one of the two studies found that changes in FeNO between control visits are related to simultaneous changes in asthma control in smokers. However, they did not study whether changes in FeNO would predict future changes in asthma control, which would be clinically more valuable.

Based on 15 cross-sectional studies FeNO levels in smoking asthmatics are lower than in non-smoking asthmatics similarly as smoking decreases FeNO in healthy subjects. However, the magnitude of the difference in FeNO values is unclear. There was marked variation between studies regarding inclusion criteria of subjects, asthma severity and use of anti-inflammatory medication, proportion of atopics, and technical details of FeNO measurement (e.g. exhalation flow rate). In many studies, there was incomplete or missing information on these factors influencing FeNO. However, in most studies that reported these factors, they were balanced between smoking and non-smoking groups. Important reasons for variation in the proportional differences in FeNO values are probably differences in smoking habits of smokers. Our sensitivity analysis showed that inclusions of ex-smokers (having quit at least 6 months earlier) did not affect the results.

We found quite reliable data to show that FeNO is increased in smoking subjects with untreated asthma but similar in smoking asthmatics on ICS-treatment, when compared to smoking healthy subjects. These findings were both based on several studies (6 and 4, respectively) with similar results. This is an important finding suggesting that FeNO might be useful in finding asthma also among symptomatic smokers similarly as FeNO is recommended to be used in diagnostic workout of asthma in non-smokers (53).

Clinical value of FeNO in smoking asthmatics

This review found data for only one of the five research questions evaluating clinical value of FeNO in smokers with asthma. Based on three studies with unclear risk of bias there is some indication that FeNO may be useful as aid in diagnosing asthma in smoking subjects. However, the studies had different kinds of study populations, different setups and somewhat divergent results.

The study by Malinovschi and colleagues had clinically most relevant setting of the three studies included, as they evaluated the ability of FeNO to differentiate asthmatics from non-asthmatics among subjects with

symptoms suggestive of asthma (38). The other two studies used either only asymptomatic healthy subjects (49) or a combination of symptomatic non-asthmatics and healthy subjects (51) as the reference group.

We assessed all the three studies as having unclear risk of bias. In one study, it remained unclear whether asthma diagnosis of all asthmatics fulfilled the criteria of this review (i.e. based on objective measures of lung function) (38). In one study, it remained unclear how patients and controls were recruited (49). In addition, all the three studies evaluated the diagnostic accuracy of FeNO against an optimal cut-off value derived from data. It has, however, been stated that dichotomising continuous variables based on the data may lead to overly optimistic measures of diagnostic accuracy (sensitivity and specificity) if the sample size is under 200 (52). Therefore, we decided to judge studies with under 200 participants (100 individuals without asthma and 100 with asthma) as having unclear risk of bias. The sample sizes of the included studies varied from 49 to 112 subjects.

Clinical implications and guidance for future research

Although there is lack of studies on the role of FeNO in smokers with asthma, we found indication that FeNO may be associated with eosinophilic airway inflammation also in smokers, FeNO is higher in asthmatic than healthy smokers, and FeNO may be a useful aid in diagnosing asthma among smokers. However, cut-off values of FeNO for possible clinical decision making probably need to be lower for smokers than for non-smokers. In non-smoking adults, FeNO above 50 ppb suggests that eosinophilic inflammation and responsiveness to corticosteroids are likely (54), while FeNO above 35 ppb is recommended to be considered a positive test result in diagnostic workout of asthma (53). In the present review, the cut-off values of FeNO in the included studies with smoking asthmatics were clearly lower than these presented for non-smoking asthmatics: the suggested cut-off points in the two studies with positive results to detect airway eosinophilia varied between 14 and 33 ppb (32,33) and the suggested cut-off points in two studies with positive results in diagnosing asthma were 17 and 18 ppb (38,49). These cut-off values should, however be interpreted with caution because the scarce and methodologically somewhat incomplete data does not allow making explicit conclusions on cut-off values since most analyses were based on use of optimal cut-off values derived from own data with relatively small sample size. Future analyses should be based on larger study populations (52).

Another important factor to bear in mind is different phenotypes of asthma and their relation to FeNO. Asthma is a heterogeneous disease and eosinophilic airway inflammation is not present in all subjects with asthma. As NO production and FeNO increase as a reaction to interleukin-13 (55), secreted in both allergic

and non-allergic eosinophilic asthma, FeNO is more a marker of type 2 inflammation characterized by mucosal eosinophilia than a marker of asthma or any other diagnostic label per se. However, most of the research concerning FeNO in both smokers and non-smokers with asthma has been conducted in asthma in general rather than specifically focusing on eosinophilic phenotypes of asthma. This causes variation and inaccuracy and may cause false negative results on the ability of FeNO to provide clinically useful information. Future research on FeNO in smokers should focus on the ability of FeNO to detect airway eosinophilia and to predict ICS responsiveness in smokers with asthma. Clinical trials assessing the usability of FeNO in ICS dose titration in individual subjects should optimally recruit only those smoking asthmatics who have eosinophilic airway inflammation present in their asthma.

## Potential sources of bias in the review process

We excluded studies reported only as abstracts and therefore some published data may have been left out from the review. However, it has been shown that discrepancies occur between data reported as abstracts or full reports and quality of data may not be sufficient if the results are reported as abstract only (26,27). Further, although we had no restrictions on language or date of publication in literature search, we restricted the selection of reports to studies published in English, German, Scandinavian languages or Hungarian. Therefore, four potential reports (published in Polish, Japanese and Russian) were not evaluated for possible inclusion in this review.

## Conclusions

In conclusion, at the moment there is insufficient data to give specific guidelines on using FeNO in diagnosing or guiding treatment of asthma in smoking subjects, but although smoking decreases FeNO it does not seem to make FeNO redundant. According to current systematic review, there is some indication that FeNO may be associated with eosinophilic inflammation also in smokers and FeNO may be useful in diagnosing asthma among smoking subjects. As smoking is unfortunately common in subjects with asthma we encourage researchers to conduct further trials on the clinical value of FeNO in this population.

## Acknowledgements

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Criteria for anti-inflammatory medication

The criteria for anti-inflammatory medication depended on the study question as follows. In questions 1 and 2: no restrictions on ICS treatment. Question 3: both groups in a single study had to have similar status regarding ICS treatment (i.e. both smoking and non-smoking asthmatics either on ICS or off ICSA). Question 4: no criteria for anti-inflammatory medication but we collected and reported the studies with ICS-treated and non-treated subjects separately. Question 5: no restrictions on ICS treatment but use of medication was taken into account in the assessment of risk of bias. Question 6: subjects had to be steroid-naïve; question 7: subjects had to be on regular stable ICS and additional oral glucocorticoids were allowed; and questions 8 and 9: subjects had to be on regular stable glucocorticoid treatment at baseline.

Comparisons

Requirements for comparisons in the included studies depended on the research questions as follows:

Question 1: comparison of FeNO against direct measures of eosinophilic airway inflammation (eosinophils in sputum, bronchial biopsies or broncho-alveolar lavage) in smoking asthmatics.

Question 2: comparison of FeNO against conventional methods to assess asthma control or severity (questionnaires, symptoms, lung function, exacerbations/hospitalisations, emergency visits, per oral glucocorticoid use, need for add-on therapy) in smoking asthmatics.

Question 3: FeNO among smoking asthmatics compared to non-smoking asthmatics.

Question 4: FeNO among smoking asthmatics compared to healthy smoking individuals.

Question 5: diagnostic accuracy of FeNO measurement to identify asthma in comparison with lung function measures.

Question 6 to 8: in smoking asthmatics, prognostic accuracy of: a) high FeNO value compared to low FeNO value or, b) FeNO measurement compared to other clinical measurement (symptoms, lung function, blood tests). Cut-off values for high and low FeNO taken from each original study instead of setting predetermined values.

Question 9: tailoring of ICS treatment based on FeNO compared to usual treatment strategy.

Outcome measures and outcomes

Requirements for outcomes and outcome measures depended on the research questions as follows.

Questions 1 and 2: performance of FeNO described e.g. as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), or area under receiver-operating characteristics (AUC of ROC) curve. Questions 3 and 4: we accepted only studies in which FeNO was analysed as continuous variable (not categorised or dichotomous variables).

Question 5: measures of diagnostic accuracy such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), the area under receiver-operating characteristics curve (AUC of ROC), or likelihood ratios.

Questions 6 to 8:

- if the prognostic accuracy of high FeNO value was compared to low FeNO value, the following clinical outcomes had to be reported:
  - o asthma symptoms, lung function or exacerbations of asthma in questions 6 and 8
  - o exacerbations of asthma in question 7
- if the prognostic accuracy of FeNO measurement was compared to other clinical measurement (symptoms, lung function, blood tests), accuracy of FeNO should be described e.g. as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), or area under receiver-operating characteristics curve (AUC of ROC).

Question 9: exacerbations of asthma, asthma symptoms, lung function, dose of inhaled steroids.

*Quality assessment of the included studies*

Assessment of risk of bias

In the research question 5, we assessed the risk of bias of included studies on FeNO's diagnostic accuracy by using the quality assessment tool of QUADAS-2 (1). It consists of four key domains covering patient selection, index test, reference standard, and flow of patients through the study and timing of the index test(s) and reference standard ("flow and timing"). The assessment tool was expanded by additional signaling questions relevant to this review. To judge the study as having low risk of bias FeNO measurement

had to be performed before spirometry and the proportion of atopics had to be reported. In case FeNO could not differentiate asthmatics from non-asthmatics, the proportion of subjects using ICS medication had to be under 20 %. Further, FeNO should be analysed as a continuous variable or, when dichotomised, this was based on reasonable cut-off values. If diagnostic accuracy of FeNO was evaluated e.g. against an optimal cut-off value derived from data, we decided to judge the study having as unclear risk of bias if the study population was under 200 (100 individuals without asthma and 100 with asthma).

To draw conclusions about the overall risk of bias within a study, we decided to classify the studies into three categories: studies with low, unclear or high risk of bias. If all the domains within a study were graded as having low risk of bias, the overall judgement was low risk of bias. If even one of the domains was assessed as having high or unclear risk of bias, the overall risk of bias for a study was graded as high or unclear, respectively.

Our intention was to assess risk of bias of included studies also in the other clinical research questions 6 to 9 with appropriate risk of bias assessment tool for each design (with the Quality In Prognosis Studies tool QUIPS (2) in prognostic questions 6 to 8; and Cochrane risk of bias tool (3) in question 9, but there were no included studies in these questions.

References:

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1    Nitric Oxide/ (81416)

2    Exhalation/ (3179)

3    1 and 2 (919)

4    ((fraction$ or exhal$ or expir$) adj3 (nitric oxide or nitrogen oxide or nitrogen monoxide or no)).ti,ab,kf. (7682)

5    eno.ti,ab,kf. (1002)

6    feno.ti,ab,kf. (1494)

7    or/3-6 (8493)

8    Breath Tests/ (13553)

9    ((breath or breathing) adj3 test$).ti,ab,kf. (8838)

10    or/7-9 (24602)

11    Smoking/ (138215)

12    (smoking or smoker$ or tobacco or cigarette$ or cigar$1 or pipe).ti,ab,kf. (282070)

13    or/11-12 (312572)

14    exp Asthma/ (117763)

15    (asthma or asthmatic$).ti,ab,kf. (141446)

16    Respiratory Hypersensitivity/ (9220)

17    Bronchial Hyperreactivity/ (7172)

18    ((airway$1 or bronchus or bronchi or bronchial or respiratory) adj3 (hyperreact$ or hyper-react$ or hypersensitiv$ or hyperrespons$)).ti,ab,kf. (13059)

19    (airway$1 adj3 inflammat$).ti,ab,kf. (16834)

20    or/14-19 (173605)

21    10 and 13 and 20 (477)

22    exp Animals/ not Humans/ (4403818)

23    (news or comment or letter or editorial or case reports).pt. or case report.ti. (3482171)

24    21 not (22 or 23) (470)

25    remove duplicates from 24 (459)

**1.1 Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>**
**Service provider: OvidSP**
**Date of search: 4 September 2019**
**Retrieved records: 61**
**Note: this is the strategy for the update search. New 2018 MeSH terms were added to line 11.**

1    Nitric Oxide/ (83851)

2    Exhalation/ (3486)

3    1 and 2 (1038)

4    ((fraction$ or exhal$ or expir$) adj3 (nitric oxide or nitrogen oxide or nitrogen monoxide or no)).ti,ab,kf. (8006)

5    eno.ti,ab,kf. (1026)

6    feno.ti,ab,kf. (1672)

7    or/3-6 (8882)

8    Breath Tests/ (13881)

9    ((breath or breathing) adj3 test$).ti,ab,kf. (9058)

10    or/7-9 (25379)

11    Smoking/ or exp Tobacco Smoking/ or Pipe Smoking/ (134019)

12    (smoking or smoker$ or tobacco or cigarette$ or cigar$1 or pipe).ti,ab,kf. (294701)

13    or/11-12 (323382)

14    exp Asthma/ (120328)

15    (asthma or asthmatic$).ti,ab,kf. (146648)

16    Respiratory Hypersensitivity/ (9289)

17    Bronchial Hyperreactivity/ (7212)

18    ((airway$1 or bronchus or bronchi or bronchial or respiratory) adj3 (hyperreact$ or hyper-react$ or hypersensitiv$ or hyperrespons$)).ti,ab,kf. (13377)

19    (airway$1 adj3 inflammat$).ti,ab,kf. (17776)

20    or/14-19 (179076)

21    10 and 13 and 20 (514)

22    exp Animals/ not Humans/ (4493304)

23    (news or comment or letter or editorial or case reports).pt. or case report.ti. (3579100)

24    21 not (22 or 23) (506)

25    limit 24 to yr="2017 -Current" (64)

26     remove duplicates from 25 (61)

1     Nitric Oxide/ (138333)

2     exp Exhalation/ (22132)

3     1 and 2 (1598)

4     ((fraction$ or exhal$ or expir$) adj3 (nitric oxide or nitrogen oxide or nitrogen monoxide or no)).ti,ab,kw. (10826)

5     eno.ti,ab,kw. (1484)

6     feno.ti,ab,kw. (3276)

7     or/3-6 (12358)

8     breath analysis/ (12530)

9     ((breath or breathing) adj3 test$).ti,ab,kw. (12815)

10     or/7-9 (31454)

11     exp smoking/ (274225)

12     (smoking or smoker$ or tobacco or cigarette$ or cigar$1 or pipe).ti,ab,kw. (369366)

13     or/11-12 (431572)

14     respiratory tract allergy/ (9433)

15     allergic airway inflammation/ (1684)

16     bronchus hyperreactivity/ (11913)

17     exp asthma/ (226992)

18     (asthma or asthmatic$).ti,ab,kw. (197067)

19     ((airway$1 or bronchi or bronchus or bronchial or respiratory) adj3 (hyperreact$ or hyper-react$ or hypersensitiv$ or hyperrespons$)).ti,ab,kw. (17838)

20     (airway$1 adj3 inflammat$).ti,ab,kw. (24450)

21     or/14-20 (271377)

22     10 and 13 and 21 (933)

23     (animal/ or animal experiment/ or animal model/ or animal tissue/ or nonhuman/) not exp human/ (5635016)

24     (conference abstract or conference paper or conference proceeding or conference review or letter or editorial).pt. or case report.ti. (5026392)

25    22 not (23 or 24) (534)

26    remove duplicates from 25 (517)


**2.1. Embase <1974 to 2018 August 31>**
**Service provider: OvidSP**
**Date of Search: 4 September 2018**
**Retrieved records: 71**
**Note: this is the strategy for the update search.**


1    Nitric Oxide/ (145634)

2    exp Exhalation/ (23524)

3    1 and 2 (1667)

4    ((fraction$ or exhal$ or expir$) adj3 (nitric oxide or nitrogen oxide or nitrogen monoxide or no)).ti,ab,kw. (11715)

5    eno.ti,ab,kw. (1535)

6    feno.ti,ab,kw. (3719)

7    or/3-6 (13393)

8    breath analysis/ (12972)

9    ((breath or breathing) adj3 test$).ti,ab,kw. (13864)

10    or/7-9 (33570)

11    exp smoking/ (339517)

12    (smoking or smoker$ or tobacco or cigarette$ or cigar$1 or pipe).ti,ab,kw. (401016)

13    or/11-12 (477510)

14    respiratory tract allergy/ (8793)

15    allergic airway inflammation/ (2006)

16    bronchus hyperreactivity/ (12082)

17    exp asthma/ (231724)

18    (asthma or asthmatic$).ti,ab,kw. (204604)

19    ((airway$1 or bronchi or bronchus or bronchial or respiratory) adj3 (hyperreact$ or hyper-react$ or hypersensitiv$ or hyperrespons$)).ti,ab,kw. (18599)

20    (airway$1 adj3 inflammat$).ti,ab,kw. (26315)

21    or/14-20 (278150)

22    10 and 13 and 21 (1039)

23    (animal/ or animal experiment/ or animal model/ or animal tissue/ or nonhuman/) not exp human/ (5524967)

24    (conference abstract or conference paper or conference proceeding or conference review or letter or editorial).pt. or case report.ti. (5711493)

25    22 not (23 or 24) (598)

26    limit 25 to yr="2017 -Current" (74)

27    remove duplicates from 26 (71)


**3. Cochrane Database of Systematic Reviews (CDSR), Issue 5 of 12, May 2017**
**Service provider: Cochrane Library, Wiley**
**Date of search: 25 May 2017**
**Retrieved records: 1**


#1        [mh ^"Nitric Oxide"]        1728

#2        [mh ^Exhalation]        174

#3        #1 and #2    61

#4        ((fraction* or exhal* or expir*) near/3 ("nitric oxide" or "nitrogen oxide" or "nitrogen monoxide" or no)):ti,ab,kw        1148

#5        eno:ti,ab,kw  132

#6        feno:ti,ab,kw 289

#7        {or #3-#6}    1220

#8        [mh ^"Breath Tests"]        1470

#9        ((breath or breathing) near/3 test*):ti,ab,kw        2926

#10       {or #7-#9}    3934

#11       [mh ^Smoking]        6224

#12       (smoking or smoker* or tobacco or cigarette* or cigar? or pipe):ti,ab,kw        22348

#13       {or #11-#12} 22348

#14       [mh Asthma] 10008

#15       (asthma or asthmatic*):ti,ab,kw        26459

#16       [mh ^"Respiratory Hypersensitivity"]        217

#17       [mh ^"Bronchial Hyperreactivity"]        567

#18       ((airway? or bronchi or bronchial or respiratory) near/3 (hyperreact* or hyper-react* or hypersensitiv* or hyperrespons*)):ti,ab,kw        1457

#19       (airway? near/3 inflammat*):ti,ab,kw        170

#20       {or #14-#19} 26803

#21       #10 and #13 and #20 in Cochrane Reviews (Reviews and Protocols)     1

**3.1. Cochrane Database of Systematic Reviews (CDSR), Issue 9 of 12, September 2018**
**Service provider: Cochrane Library, Wiley**
**Date of search: 4 September 2018**
**Retrieved records: no records**
**Note:** this is the strategy for the update search. New 2018 MeSH terms were added to line 11. The Cochrane Library has gone through a revision during summer 2018 and DARE, NHS EED and HTA databases are no longer included. Therefore, individual result numbers on lines 1-20 cannot be compared against the result numbers in the original search.

| | | |
|---|---|---|
| 1 | [mh ^"Nitric Oxide"] | 1923 |
| #2 | [mh ^Exhalation] | 195 |
| #3 | #1 and #2 | 70 |
| #4 | ((fraction* or exhal* or expir*) near/3 ("nitric oxide" or "nitrogen oxide" or "nitrogen monoxide" or no)):ti,ab,kw | 624 |
| #5 | eno:ti,ab,kw | 177 |
| #6 | feno:ti,ab,kw | 484 |
| #7 | {or #3-#6} | 1190 |
| #8 | [mh ^"Breath Tests"] | 1476 |
| #9 | ((breath or breathing) near/3 test*):ti,ab,kw | 3293 |
| #10 | {or #7-#9} | 4297 |
| #11 | [mh ^Smoking] or [mh "Tobacco Smoking"] or [mh ^"Pipe Smoking"] | 133 |
| #12 | (smoking or smoker* or tobacco or cigarette* or cigar? or pipe):ti,ab,kw | 27344 |
| #13 | {or #11-#12} | 27380 |
| #14 | [mh Asthma] | 10843 |
| #15 | (asthma or asthmatic*):ti,ab,kw | 28951 |
| #16 | [mh ^"Respiratory Hypersensitivity"] | 220 |
| #17 | [mh ^"Bronchial Hyperreactivity"] | 578 |
| #18 | ((airway? or bronchi or bronchial or respiratory) near/3 (hyperreact* or hyper-react* or hypersensitiv* or hyperrespons*)):ti,ab,kw | 1562 |
| #19 | (airway? near/3 inflammat*):ti,ab,kw | 219 |
| #20 | {or #14-#19} | 29332 |
| #21 | #10 and #13 and #20 with Cochrane Library publication date between Jan 2017 and Sep 2018, in Cochrane Reviews, Cochrane Protocols | 0 |

| #1 | [mh ^"Nitric Oxide"] | 1728 |
|----|----|----|
| #2 | [mh ^Exhalation] | 174 |
| #3 | #1 and #2 | 61 |
| #4 | ((fraction* or exhal* or expir*) near/3 ("nitric oxide" or "nitrogen oxide" or "nitrogen monoxide" or no)) | 1291 |
| #5 | eno | 157 |
| #6 | feno | 319 |
| #7 | {or #3-#6} | 1378 |
| #8 | [mh ^"Breath Tests"] | 1470 |
| #9 | ((breath or breathing) near/3 test*) | 3094 |
| #10 | {or #7-#9} | 4257 |
| #11 | [mh ^Smoking] | 6224 |
| #12 | (smoking or smoker* or tobacco or cigarette* or cigar? or pipe) | 25288 |
| #13 | {or #11-#12} | 25288 |
| #14 | [mh Asthma] | 10008 |
| #15 | (asthma or asthmatic*) | 28917 |
| #16 | [mh ^"Respiratory Hypersensitivity"] | 217 |
| #17 | [mh ^"Bronchial Hyperreactivity"] | 567 |
| #18 | ((airway? or bronchi or bronchial or respiratory) near/3 (hyperreact* or hyper-react* or hypersensitiv* or hyperrespons*)) | 1640 |
| #19 | (airway? near/3 inflammat*) | 219 |
| #20 | {or #14-#19} | 29270 |
| #21 | #10 and #13 and #20 in Trials | 49 |

**4.1. Cochrane Central Register of Controlled Trials (CENTRAL), Issue 8 of 12, August 2018**
**Service provider: Cochrane Library, Wiley**
**Date of search: 4 September 2018**
**Retrieved records: 3**
**Note**: this is the strategy for the update search. New 2018 MeSH terms were added to line 11. The Cochrane Library has gone through a revision during summer 2018 and DARE, NHS EED and HTA databases are no longer included. Therefore, individual result numbers on lines 1-20 cannot be compared against the result numbers in the original search.

| | | |
|---|---|---|
| #1 | [mh ^"Nitric Oxide"] | 1923 |
| #2 | [mh ^Exhalation] | 195 |
| #3 | #1 and #2 | 70 |
| #4 | ((fraction* or exhal* or expir*) near/3 ("nitric oxide" or "nitrogen oxide" or "nitrogen monoxide" or no)) | 682 |
| #5 | eno | 207 |
| #6 | feno | 501 |
| #7 | {or #3-#6} | 1280 |
| #8 | [mh ^"Breath Tests"] | 1476 |
| #9 | ((breath or breathing) near/3 test*) 3363 | |
| #10 | {or #7-#9} | 4456 |
| #11 | [mh ^Smoking] or [mh "Tobacco Smoking"] or [mh ^"Pipe Smoking"] 133 | |
| #12 | (smoking or smoker* or tobacco or cigarette* or cigar? or pipe) 29168 | |
| #13 | {or #11-#12} | 29204 |
| #14 | [mh Asthma] | 10843 |
| #15 | (asthma or asthmatic*) | 31012 |
| #16 | [mh ^"Respiratory Hypersensitivity"] | 220 |
| #17 | [mh ^"Bronchial Hyperreactivity"] | 578 |
| #18 | ((airway? or bronchi or bronchial or respiratory) near/3 (hyperreact* or hyper-react* or hypersensitiv* or hyperrespons*)) 1752 | |
| #19 | (airway? near/3 inflammat*) | 262 |
| #20 | {or #14-#19} | 31400 |
| #21 | #10 and #13 and #20 with Publication year from 2017 to 2018, in Trials | 3 |

**5. Database of Abstracts of Reviews of Effects (DARE)**
**Service provider: Centre for Reviews and Dissemination**, https://www.crd.york.ac.uk/CRDWeb/
**Date of search: 25 May 2017**
**Retrieved records: no records**

| | | |
|---|---|---|
| 1 | MeSH DESCRIPTOR nitric oxide | 58 |
| 2 | MeSH DESCRIPTOR exhalation | 12 |
| 3 | #1 AND #2 | 5 |
| 4 | ((fraction* or exhal* or expir*) NEAR3 (nitric oxide or nitrogen oxide or nitrogen monoxide or no)) | 41 |
| 5 | (eno OR feno) | 18 |
| 6 | #3 OR #4 OR #5 | 45 |
| 7 | MeSH DESCRIPTOR breath tests | 68 |
| 8 | ((breath or breathing) NEAR3 test*) | 167 |
| 9 | #6 OR #7 OR #8 | 202 |
| 10 | MeSH DESCRIPTOR smoking | 360 |
| 11 | (smoking or smoker* or tobacco or cigarette* or cigar or cigars or pipe) | 1602 |
| 12 | #10 OR #11 | 1602 |
| 13 | MeSH DESCRIPTOR asthma EXPLODE ALL TREES | |
| 14 | (asthma or asthmatic*) | 1232 |
| 15 | MeSH DESCRIPTOR respiratory hypersensitivity | 11 |
| 16 | MeSH DESCRIPTOR bronchial hyperreactivity | 10 |
| 17 | ((airway* or bronchi or bronchial or respiratory) NEAR3 (hyperreact* or hyper-react* or hypersensitiv* or hyperrespons*)) | 33 |
| 18 | (airway* NEAR3 inflammat*) | 34 |
| 19 | #13 OR #14 OR #15 OR #16 OR #17 OR #18 | 1245 |
| 20 | #9 AND #12 AND #19 | 2 |
| 21 | * IN DARE | 45418 |
| 22 | #20 AND #21 | 0 |

**5.1. Database of Abstracts of Reviews of Effects (DARE)**
**Service provider: Centre for Reviews and Dissemination**, https://www.crd.york.ac.uk/CRDWeb/
**Date of search: 4 September 2018**
**Retrieved records: no records**
**Note:** this is the strategy for the update search. New 2018 MeSH terms were included in lines 11-12.

| | | |
|---|---|---|
| 1 | (MeSH DESCRIPTOR nitric oxide) | 59 |
| 2 | (MeSH DESCRIPTOR exhalation) | 13 |
| 3 | (#1 AND #2) 6 | |
| 4 | ((fraction* or exhal* or expir*) NEAR3 (nitric oxide or nitrogen oxide or nitrogen monoxide or no)) | 42 |
| 5 | ((eno OR feno)) | 19 |
| 6 | (#3 OR #4 OR #5) | 46 |
| 7 | (MeSH DESCRIPTOR breath tests) | 71 |
| 8 | (((breath or breathing) NEAR3 test*)) | 170 |
| 9 | (#6 OR #7 OR #8) | 205 |
| 10 | (MeSH DESCRIPTOR smoking) | 360 |
| 11 | (MeSH DESCRIPTOR tobacco smoking EXPLODE ALL TREES) 0 Delete | |
| 12 | (MeSH DESCRIPTOR pipe smoking ) 0 | |
| 13 | (smoking or smoker* or tobacco or cigarette* or cigar or cigars or pipe) | |
| 14 | #10 OR #11 OR #12 OR #13 | 1603 |
| 15 | (MeSH DESCRIPTOR asthma EXPLODE ALL TREES) | 676 |
| 16 | ((asthma or asthmatic*)) | 1235 |
| 17 | (MeSH DESCRIPTOR respiratory hypersensitivity) | 11 |
| 18 | (MeSH DESCRIPTOR bronchial hyperreactivity) | 10 |
| 19 | (((airway* or bronchi or bronchial or respiratory) NEAR3 (hyperreact* or hyper-react* or hypersensitiv* or hyperrespons*))) | 33 |
| 20 | ((airway* NEAR3 inflammat*)) | 34 |
| 21 | #15 OR #16 OR #17 OR #18 OR #19 OR #20 | 1248 |
| 22 | #9 AND #14 AND #21 | 2 |
| 23 | * IN DARE | 45418 |
| 24 | #22 AND #23 | 0 |

**6. Health Technology Assessment Database (HTA)**
**Service provider: Centre for Reviews and Dissemination**, https://www.crd.york.ac.uk/CRDWeb/
**Date of search: 25 May 2017**
**Retrieved records: no records**

| | | |
|---|---|---|
| 1 | MeSH DESCRIPTOR nitric oxide | 58 |
| 2 | MeSH DESCRIPTOR exhalation | 12 |
| 3 | #1 AND #2 | 5 |
| 4 | ((fraction* or exhal* or expir*) NEAR3 (nitric oxide or nitrogen oxide or nitrogen monoxide or no)) | 41 |
| 5 | (eno OR feno) | 18 |
| 6 | #3 OR #4 OR #5 | 45 |
| 7 | MeSH DESCRIPTOR breath tests | 68 |
| 8 | ((breath or breathing) NEAR3 test*) | 167 |
| 9 | #6 OR #7 OR #8 | 202 |
| 10 | MeSH DESCRIPTOR smoking | 360 |
| 11 | (smoking or smoker* or tobacco or cigarette* or cigar or cigars or pipe) | 1602 |
| 12 | #10 OR #11 | 1602 |
| 13 | MeSH DESCRIPTOR asthma EXPLODE ALL TREES | |
| 14 | (asthma or asthmatic*) | 1232 |
| 15 | MeSH DESCRIPTOR respiratory hypersensitivity | 11 |
| 16 | MeSH DESCRIPTOR bronchial hyperreactivity | 10 |
| 17 | ((airway* or bronchi or bronchial or respiratory) NEAR3 (hyperreact* or hyper-react* or hypersensitiv* or hyperrespons*)) | 33 |
| 18 | (airway* NEAR3 inflammat*) | 34 |
| 19 | #13 OR #14 OR #15 OR #16 OR #17 OR #18 | 1245 |
| 20 | #9 AND #12 AND #19 | 2 |
| 21 | * IN HTA | 16941 |
| 22 | #20 AND #21 | 0 |

**6.1. Health Technology Assessment Database (HTA)**
**Service provider: Centre for Reviews and Dissemination**, https://www.crd.york.ac.uk/CRDWeb/
**Date of search: 4 September 2018**
**Retrieved records: no records**
**Note:** this is the strategy for the update search. New 2018 MeSH terms were included in lines 11-12. The database was closed for new records in 31 March 2018 and is no longer updated.

| | | |
|---|---|---|
| 1 | (MeSH DESCRIPTOR nitric oxide) | 59 |
| 2 | (MeSH DESCRIPTOR exhalation) | 13 |
| 3 | (#1 AND #2) 6 | |
| 4 | ((fraction* or exhal* or expir*) NEAR3 (nitric oxide or nitrogen oxide or nitrogen monoxide or no)) | 42 |
| 5 | ((eno OR feno)) | 19 |
| 6 | (#3 OR #4 OR #5) | 46 |
| 7 | (MeSH DESCRIPTOR breath tests) | 71 |
| 8 | (((breath or breathing) NEAR3 test*)) | 170 |
| 9 | (#6 OR #7 OR #8) | 205 |
| 10 | (MeSH DESCRIPTOR smoking) | 360 |
| 11 | (MeSH DESCRIPTOR tobacco smoking EXPLODE ALL TREES) 0 Delete | |
| 12 | (MeSH DESCRIPTOR pipe smoking ) 0 | |
| 13 | (smoking or smoker* or tobacco or cigarette* or cigar or cigars or pipe) | |
| 14 | #10 OR #11 OR #12 OR #13 | 1603 |
| 15 | (MeSH DESCRIPTOR asthma EXPLODE ALL TREES) | 676 |
| 16 | ((asthma or asthmatic*)) 1235 | |
| 17 | (MeSH DESCRIPTOR respiratory hypersensitivity) | 11 |
| 18 | (MeSH DESCRIPTOR bronchial hyperreactivity) | 10 |
| 19 | (((airway* or bronchi or bronchial or respiratory) NEAR3 (hyperreact* or hyper-react* or hypersensitiv* or hyperrespons*))) | 33 |
| 20 | ((airway* NEAR3 inflammat*)) | 34 |
| 21 | #15 OR #16 OR #17 OR #18 OR #19 OR #20 | 1248 |
| 22 | #9 AND #14 AND #21 | 2 |
| 23 | * IN HTA FROM 2017 TO 2018 | 506 |
| 24 | #22 AND #23 | 0 |

**7. NHS Economic Evaluation Database (NHS EED)**
**Service provider: Centre for Reviews and Dissemination**, https://www.crd.york.ac.uk/CRDWeb/
**Date of search: 25 May 2017**
**Retrieved records: 2**

| | | |
|---|---|---|
| 1 | MeSH DESCRIPTOR nitric oxide | 58 |
| 2 | MeSH DESCRIPTOR exhalation | 12 |
| 3 | #1 AND #2   5 | |
| 4 | ((fraction* or exhal* or expir*) NEAR3 (nitric oxide or nitrogen oxide or nitrogen monoxide or no))      41 | |
| 5 | (eno OR feno)      18 | |
| 6 | #3 OR #4 OR #5      45 | |
| 7 | MeSH DESCRIPTOR breath tests | 68 |
| 8 | ((breath or breathing) NEAR3 test*)      167 | |
| 9 | #6 OR #7 OR #8      202 | |
| 10 | MeSH DESCRIPTOR smoking | 360 |
| 11 | (smoking or smoker* or tobacco or cigarette* or cigar or cigars or pipe) 1602 | |
| 12 | #10 OR #11  1602 | |
| 13 | MeSH DESCRIPTOR asthma EXPLODE ALL TREES | |
| 14 | (asthma or asthmatic*)      1232 | |
| 15 | MeSH DESCRIPTOR respiratory hypersensitivity      11 | |
| 16 | MeSH DESCRIPTOR bronchial hyperreactivity      10 | |
| 17 | ((airway* or bronchi or bronchial or respiratory) NEAR3 (hyperreact* or hyper-react* or hypersensitiv* or hyperrespons*))      33 | |
| 18 | (airway* NEAR3 inflammat*)      34 | |
| 19 | #13 OR #14 OR #15 OR #16 OR #17 OR #18      1245 | |
| 20 | #9 AND #12 AND #19      2 | |
| 21 | * IN NHSEED      17613 | |
| 22 | #20 AND #21      2 | |

**7.1. NHS Economic Evaluation Database (NHS EED)**
**Service provider: Centre for Reviews and Dissemination**, https://www.crd.york.ac.uk/CRDWeb/
**Date of search: 4 September 2018**
**Retrieved records: no records**
**Note:** this is the strategy for the update search. New 2018 MeSH terms were included in lines 11-12.

| | | |
|---|---|---|
| 1 | (MeSH DESCRIPTOR nitric oxide) | 59 |
| 2 | (MeSH DESCRIPTOR exhalation) | 13 |
| 3 | (#1 AND #2) 6 | |
| 4 | ((fraction* or exhal* or expir*) NEAR3 (nitric oxide or nitrogen oxide or nitrogen monoxide or no)) | 42 |
| 5 | ((eno OR feno)) | 19 |
| 6 | (#3 OR #4 OR #5) | 46 |
| 7 | (MeSH DESCRIPTOR breath tests) | 71 |
| 8 | (((breath or breathing) NEAR3 test*)) | 170 |
| 9 | (#6 OR #7 OR #8) | 205 |
| 10 | (MeSH DESCRIPTOR smoking) | 360 |
| 11 | (MeSH DESCRIPTOR tobacco smoking EXPLODE ALL TREES)   0 Delete | |
| 12 | (MeSH DESCRIPTOR pipe smoking ) 0 | |
| 13 | (smoking or smoker* or tobacco or cigarette* or cigar or cigars or pipe) | |
| 14 | #10 OR #11 OR #12 OR #13 | 1603 |
| 15 | (MeSH DESCRIPTOR asthma EXPLODE ALL TREES) | 676 |
| 16 | ((asthma or asthmatic*)) 1235 | |
| 17 | (MeSH DESCRIPTOR respiratory hypersensitivity) | 11 |
| 18 | (MeSH DESCRIPTOR bronchial hyperreactivity) | 10 |
| 19 | (((airway* or bronchi or bronchial or respiratory) NEAR3 (hyperreact* or hyper-react* or hypersensitiv* or hyperrespons*))) | 33 |
| 20 | ((airway* NEAR3 inflammat*)) | 34 |
| 21 | #15 OR #16 OR #17 OR #18 OR #19 OR #20 | 1248 |
| 22 | #9 AND #14 AND #21 | 2 |
| 23 | * IN NHSEED FROM 2017 TO 2018 | 0 |
| 24 | #22 AND #23 | 0 |

**8. ClinicalTrials.gov**
**Service provider:** https://www.clinicaltrials.gov
**Date of search: 25 May 2017**
**Retrieved records: 24**

Advanced search was used to run the search.

(exhaled nitric oxide OR feno OR eno) AND (asthma OR respiratory hypersensitivity OR broncial hyperreactivity OR airway inflammation) AND smoking

**8.1. ClinicalTrials.gov**
**Service provider:** https://www.clinicaltrials.gov
**Date of search: 4 September 2018**
**Retrieved records: 2**
**Note: this is the strategy for the update search.**

Advanced search was used to run the search.

(exhaled nitric oxide OR feno OR eno) AND (asthma OR respiratory hypersensitivity OR broncial hyperreactivity OR airway inflammation) AND smoking
First posted from 05/25/2017 to 09/04/2018

**9. WHO International Clinical Trials Registry Platform (WHO ICTRP)**
**Service provider:** http://apps.who.int/trialsearch/Default.aspx
**Date of search: 25 May 2017**
**Retrieved records: 6**

The search was run using the default search option.

exhaled nitric oxide AND asthma AND smok* OR feno AND asthma AND smok* OR eno AND asthma AND smok* OR

exhaled nitric oxide AND respiratory hypersensitivity AND smoking OR feno AND respiratory hypersensitivity AND smok* OR eno AND respiratory hypersensitivity AND smok* OR

exhaled nitric oxide AND bronchial hyperreactivity AND smoking OR feno AND bronchial hyperreactivity AND smok* OR eno AND bronchial hyperreactivity AND smok* OR

exhaled nitric oxide AND airway inflammation AND smoking OR feno AND airway inflammation AND smok* OR eno AND airway inflammation AND smok*

**9.1. WHO International Clinical Trials Registry Platform (WHO ICTRP)**
**Service provider:** http://apps.who.int/trialsearch/Default.aspx
**Date of search: 4 September 2018**
**Retrieved records: 1**
**Note: this is the strategy for the update search.**

The search was run using the default search option.

exhaled nitric oxide AND asthma AND smok* OR feno AND asthma AND smok* OR eno AND asthma AND smok* OR

exhaled nitric oxide AND respiratory hypersensitivity AND smoking OR feno AND respiratory hypersensitivity AND smok* OR eno AND respiratory hypersensitivity AND smok* OR

exhaled nitric oxide AND bronchial hyperreactivity AND smoking OR feno AND bronchial hyperreactivity AND smok* OR eno AND bronchial hyperreactivity AND smok* OR

exhaled nitric oxide AND airway inflammation AND smoking OR feno AND airway inflammation AND smok* OR eno AND airway inflammation AND smok*


The search retrieved seven records. The results were browsed for studies registered after the original search in May 2017. One study was downloaded.

Online Supplement 3

Characteristics of the 16 studies excluded at final stage

| First author, Publication year | Reason for exclusion |
|---|---|
| Al-shamkhi 2016 | Criteria of asthma diagnosis of subjects included in the study did not fulfill the criteria of the asthma diagnosis assessed in this review (asthma was not diagnosed based on reversible or variable airway obstruction but defined as self-reported diagnosis of asthma and either asthma symptoms or asthma treatment). |
| Ewald-Kleimeier 2013 | It remains unclear whether all subjects were asthmatics. Only eight smokers had positive SIT (and were thus confirmed asthmatics). |
| Giovannelli 2016 | Criteria of asthma diagnosis of the subjects included in the study did not fulfill the criteria of the asthma diagnosis assessed in this review (asthma was not diagnosed based on reversible or variable airway obstruction but defined as self-reported diagnosis of asthma and either asthma symptoms or asthma treatment). |
| Grarup 2014 | Criteria of smoking status of subjects included in the study did not fulfil the criteria of smoking status assessed in this review. (The proportion of ex-smokers of all subjects was 23% and thus exceeding the limit of 20% assessed in this review. Subjects were defined as ex-smokers if they had stopped smoking before or when the current pregnancy became known. Data of current smokers and ex-smokers were combined in the analyses.) |
| Lappas 2016 | Criteria of asthma diagnosis of the subjects included in the study did not fulfill the criteria of the asthma diagnosis assessed in this review; the report did not state criteria of asthma diagnosis at all. (Subjects had sporadically symptoms and used sporadically short-acting ß2-agonists). |
| Malinovschi 2009 | Criteria of asthma diagnosis of subjects included in the study did not fulfill the criteria of the asthma diagnosis assessed in this review (asthma was not diagnosed based on reversible or variable airway obstruction but defined as self-reported physician-diagnosis of asthma and at least one asthma symptom or one asthma attack during the 12 months preceding the study). |
| Nadif 2010 | Criteria of asthma diagnosis of subjects included in the study did not fulfill the criteria of the asthma diagnosis assessed in this review (asthma was not diagnosed based on reversible or variable airway obstruction but asthma diagnosis was based on self-reported respiratory symptoms in the past 12 months or the use of inhaled and/or oral medicines because of breathing problems). |
| Nguyen 2016 | Criteria of asthma diagnosis of the subjects included in the study did not fulfill the criteria of the asthma diagnosis assessed in this review (asthma was not diagnosed based on reversible or variable airway obstruction but defined as self-reported diagnosis of asthma).<br>FeNO value was not measured as continuous measure (but dichotomized). |
| Olin 2006 | Criteria of asthma diagnosis of subjects included in the study did not fulfill the criteria of the asthma diagnosis assessed in this review (asthma was not diagnosed based on reversible or variable airway obstruction but defined as self-reported diagnosis of asthma). |
| Rossios 2017 | Criteria of smoking status of subjects included in the study did not fulfil the criteria of smoking status of subjects assessed in this review (data of current smokers and ex-smokers were combined in the analyses; there was no information on proportion of ex-smokers. Data of 11 European countries were combined in the analyses). |
| Sastre 2013 | Incomplete information on smoking asthmatic subjects. (The study was mainly focused to investigate the usefulness of FeNO measurements for monitoring airway |

| | response to SIC (specific inhalation challenge) with occupational agents.) |
|---|---|
| Taylor 2007 | Criteria of asthma diagnosis of subjects included in the study did not fulfill the criteria of the asthma diagnosis assessed in this review (asthma was not diagnosed based on reversible or variable airway obstruction but defined as reported diagnosed asthma with symptoms in the last 12 months). |
| Thorhallsdottir 2016 | Criteria of asthma diagnosis of subjects included in the study did not fulfill the criteria of the diagnosis asthma assessed in this review (asthma was not diagnosed based on reversible or variable airway obstruction but defined as reported asthma confirmed by a doctor and at least one respiratory symptom or at least one asthma attack or use of medicines because of breathing problems in the 12 months at the clinical interview). Criteria of smoking status of subjects did not fulfil the criteria of smoking assessed in this review (data of ex-smokers and never-smokers were combined in the analyses; proportion of ex-smokers about 45% from all subjects). |
| Torre 2008 | Criteria of smoking status of subjects included in the study did not fulfil the criteria of smoking status of subjects assessed in this review (ex-smokers 34 % of subjects but no detailed information when the subjects had quit smoking or criteria of ex-smokers. In FeNO results, data of current smokers and ex-smokers were combined). Additional information was inquired from the authors to assess the adequacy of the study for this review (no response). |
| Yap 2013 | Criteria of asthma diagnosis of subjects included in the study did not fulfill the criteria of the asthma diagnosis assessed in this review (asthma was not diagnosed based on reversible or variable airway obstruction but was frequently made by the family physician). Criteria of smoking status of subjects included in the study did not fulfil the criteria of smoking status of subjects assessed in this review (data of current smokers and ex-smokers were combined). |
| Westerhof 2015 | Criteria of smoking status of subjects included in the study did not fulfil the criteria of smoking status of subjects assessed in this review (data of current smokers and ex-smokers were combined in the analyses. There was no information on the proportion of ex-smokers, the total amount of current and ex-smokers was 54 % of subjects). Additional information was inquired from the authors to assess the adequacy of the study for this review (no response). |

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**Research question 1    Descriptions and results of studies**

| Study reference, country, study design | Characteristics of subjects | FeNO measurement and reference standard | Methods | Results | Funding and Notes |
|---|---|---|---|---|---|
| **Berry 2005**, UK<br><br>Cross-sectional study design.<br><br>Consecutive patients who were seen at the hospital outpatients with stable asthma were recruited. | n = 566 asthmatics, of them current smokers 113, males 44%<br><br>Demographics for all subjects (not stated separately for the 113 smoking asthmatics):<br><br>Age (yr), mean (range): 49 (16-82)<br><br>Severity of asthma: no information<br><br>FEV$_1$, % pred: no information<br><br>Steroid medication:<br>On ICS medication were 56% of subjects, with average beclomethasone equivalent dose (µg) of ICS, median (range): 800 (200-4000).<br>On oral corticosteroid were 6%.<br><br>Atopy/allergy: atopics 51%<br><br>Smoking habits, median (range): | FeNO measurement:<br>Device: Chemilumiscence analyser (model LR2000, UK).<br>Flow rate: 250 mL/s<br><br>Reference standard:<br>Induced sputum eosinophil count > 3%.<br><br>(Eosinophil count was expressed as a percentage of non-squamous cells based on a count of 400 inflammory cells.) | Percentage eosinophil count and FeNO concentrations were log transformed for analyses (to achieve normal distributions of values).<br><br>Correlation coefficients were calculated using Pearson's product-moment correlation co-efficient.<br>Multiple independent regression was used to examine the relationship between FeNO and sputum eosinophil counts and the effect of smoking, gender, ICS, age and FEV1 % predicted on this relationship.<br><br>ROC (receiver operating characteristic) curves were created to assess the performance of FeNO to identify sputum eosinophil counts > 3% (separately for smokers and non-smokers). AUCs (areas under the ROC curves) with 95% confidence intervals and their differences from 0.5 were calculated. | FeNO values:<br>In smoking asthmatics: geometric mean of 2.8 ppb (no information on SD).<br>In the whole study group: geometric mean of 6.7 (0.65) ppb.<br><br>Sputum eosinophil count, % (for the whole study group): geometric mean (log standard deviation): 2.3 (0.73).<br><br>Relationship of FeNO and eosinophil count:<br>In smoking asthmatics, significant positive correlation (R$^2$ = 0.15, p < 0.001).<br><br>AUC of ROC in smoking asthmatics:<br>The area under ROC (receiver operating characteristic) curve was not significantly different from 0.5 for identifying a sputum eosinophil count > 3% (AUC = 0.63, 95% CI 0.48 to 0.78, p = 0.10)).<br><br>Reporting conclusion:<br>In smoking asthmatics, FeNO concentration did not relate closely to sputum eosinophil count and did not predict the presence of a sputum eosinophilia. | Funging and conflict of interests:<br>Two authors were supported by research grants from Asthma UK.<br><br>Notes:<br>No detailed information on confounding factors of smoking asthmatics (e.g. on asthma severity and use of steroids) and on sputum eosinophil count of smokers.<br><br>No information on blinding of observers. |

| Hillas 2011, Greece | n = 40 smoking asthmatics, males 45% | FeNO measurement: Device: NIOX MINO (Aerocrine, Sweden). Flow rate: 50 ml/s | ROC (receiver operating characteristic) curve was created to assess the performance of FeNO to identify purely eosinophilic phenotype (sputum eosinophil counts ≥ 3% and neutrophil count <60 %). | FeNO values in smoking asthmatics: median (interquartile range (IQR)): 12 (10-16). | Funging and conflict of interests: No information provided. |
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| Cross-sectional study design. No detailed information how and where the patients were recruited. | Age (yr), mean (± SD): 48.7 ± 11.2 | | AUCs (areas under the ROC curves) with 95% confidence intervals and their differences from 0.5 were calculated. | Sputum eosinophil count, %: median (IQR): 2.0 (1.0-5.0). | Notes: ROC curves were used to analyse the ability of FeNO to detect purely eosinophilic phenotype (sputum eos ≥ 3 % and neutrophils < 60 %) instead of sputum eos ≥ 3 %. |
| | Severity of asthma: mild to moderate, well-controlled asthma | Reference standard: Induced sputum eosinophil count ≥ 3% (without sputum neutrophilia) | | Correlation of FeNO and eosinophil count: Significant positive correlation (r = 0.71, p < 0.001). Analysed by using Spearman's rank correlation coefficient. | |
| | $FEV_1$, % pred, mean (± SD): 87.8 ± 11.6 | | Sensitivies, specificities, positive (PPV) and negative (NPV) predictive values were calculated for the optimal cut-points. The optimal cut-off points were selected as those points on the ROC curve that represented the best combination of sensitivity and specifity. | Sputum phenotype was purely eosinophilic in 14/40 (35%) of smoking asthmatics. | Analyses were based on use of optimal cutoff values of FeNO derived from data. The sample size (40) was rather small to adequate analysis (Leeflang 2008). |
| | Steroid medication: all asthmatics were receiving ICS. ICS, n/mean budesonide equivalent dose: 10/400 ICS/LABA, n/mean budesonide equivalent dose: 30/640 | Sputum induction was performed according to the European Respiratory society guidelines. A sample was considered adequate when the patient was able to expectorate at least 2 ml of sputum. | | AUC of ROC: The area under ROC (receiver operating characteristic) curve was significantly different from 0.5 for identifying a sputum eosinophil count ≥ 3% with a optimal FeNO cut-off value > 14 ppb (AUC = 0.880, 95% CI 0.74 to 0.96, p < 0.0001)). | |
| | Atopy/allergy: atopics 60% (based on skin prick test) | Sputum cell counting was performed by an observer who was blind to the clinical | | Sensitivity, specificity, PPV, and NPV for the optimal FeNO cut-off value of 14 ppb: Sensitivity: 71% Specificity: 96% | |
| | Smoking habits, mean (± SD): 45.1 ± 20.2 pack-years | characteristics of the subjects. | | PPV: 91% NPV: 86% | |
| | | | | Reporting conclusion: The predictive performance of FeNO for eosinophilic phenotype | |
| Schleich 2010, Belgium | n = 295, current smokers 58, males of smoking asthmatics 53 % | FeNO measurement: Device: Chemilumiscence analyser (NIOX, Aerocrine, Sweden). Flow rate: 50 mL/s | Logistic regression analysis was used to assess the relationship between the binary outcome (sputum eosinophil count ≥ 3%) and a set of covariates, individually or in combination. Covariates included FeNO (log-transformed), age, gender, smokinfg, ICS and atopy. | FeNO values in smoking asthmatics: median (IQR): 17 (12-37) ppb. | Funging and conflict of interests: Supported by Interuniversity Attraction Poles (IAP) project P6/35 and by unrestricted grants from GSK, Astra-Zeneca and Novartis. |
| Cross-sectional study design. | Characteristics of smoking asthmatics: Age (yr), mean (range): 45.7 (23-81) | | Cut-off points on the FeNO scale were determined in each case so that there was a ≥ 50% probability of a sputum eosinophil count ≥ 3%. | Sputum eosinophil count, %: median (IQR): 1.7 (0.2-8). Simple logistic regression analysis assessing the effect of smoking on the FeNO threshold for identifying sputum eosinophil count ≥ 3%: | |
| Retrospective study of unselected patients with asthma, who had undergone both FeNO measurement and succesfull sputum induction, recruited from a university asthma clinic. | Severity of asthma: no information | Reference standard: Induced sputum eosinophil count ≥ 3%. | Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were also calculated for each cut-off point from the data. | sensitivity 76%, specificity 62%, PPV 59%, NPV 78%, for FeNO cut-off value of 28 ppb, p-value 0.066 (information from online supplement) | Notes: No detailed information on confounding factors of smoking asthmatics (e.g. on asthma severity and use of steroids). |
| | $FEV_1$, % pred, mean (± SD): 81 ± 19 | | | Multiple logistic regression analyses with covariates: smoking, atopy and high dose of ICS: PPVs ranging from 52% to 62% and NPVs ranging from 76% to 89%, depending on the covariates included in the analyses (the FeNO thresholds ranged depending on the analysis). | Analyses were based on retrospective data. |
| | Steroid medication: no information Atopy/allergy: atopics 71%. Smoking habits: no information | | | Multiple logistic regression analysis including smoking and atopy but no high dose of ICS in the analysis: sensitivity 70%, specificity 68%, PPV 62%, NPV 76%, for FeNO cut-off value of 33 ppb. | |
| | | | | Reporting conclusion: FeNO is able to identify the presence of sputum eosinophilia in unselected patients with asthma with reasonable accuracy as long as thresholds are adjusted for high doses of ICS, atopy and | |

**Research question 2    Descriptions of studies and results**

**Cross-sectional study designs**

| Study reference, country, study design | Characteristics of subjects | FeNO measurement and reference standard | Methods | Results | Notes |
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| **Kostikas 2011**, Greece<br><br>Cross-sectional study design.<br><br>Patients with a previously established diagnosis of asthma that were evaluated in the outpatient asthma clinics of two tertiary university hospitals were included in the study. | n = 274 asthmatics, of them current smokers 82 (ICS-untreated smokers 32, ICS-treated smokers 50), males 40%.<br><br>Demographics for all subjects (not stated separately for the 83 smoking asthmatics):<br><br>Age (yr), mean (± SD): 50 ± 17<br><br>FEV$_1$, % pred, mean (± SD): 85 ± 19<br><br>Severity of asthma: no information<br><br>Steroid medication: ICS-treated smokers 50 and ICS-untreated smokers 32. No information on dose.<br><br>Atopy/allergy: no information provided<br><br>Smoking habits of smokers: no information | FeNO measurement: Device: NIOX MINO, Aerocrine, Sweden. Flow rate: 50 mL/s<br><br>Reference standard: Asthma control according to Global Initiative for Asthma (GINA) guidelines.<br><br>Classification was evaluated by two asthma specialists blinded to FeNO. | Comparison of FeNO measurement for the identification of patients with not well-controlled (i.e. partly controlled or uncontrolled) asthma against asthma control status defined to GINA guidelines.<br><br>ROC (receiver operating characteristic) curve was created to assess the performance of FeNO to identify of not well-controlled (i.e.partly and uncontrolled) asthma. AUCs (areas under the ROC curves) with 95% confidence intervals and their differences from 0.5 were calculated.<br><br>Sensitivies, specificities, positive (PPV) and negative (NPV) predictive values were calculated for the optimal cut-points.<br><br>The diagnostic performance of FeNO for the identification of not well-controlled asthma at different cut-off points was also evaluated. | FeNO values: Smoking ICS-untreated patients with uncontrolled and partly controlled asthma had statistically significantly higher FeNO values compared to those with well-controlled asthma (p < 0.05), median (IQR):<br>uncontrolled 22 (21-108)<br>partly controlled 21(15-38)<br>well controlled 16 (12-19).<br>In ICS-treated smoking asthmatics the differences in FeNO values between groups with different asthma control levels were not statistically significantly different.<br><br>AUC of ROC: In ICS-untreated smokers, AUC (95%) for the optimum cut-point of FeNO > 19 ppb: 0.680 (0.492-0.833), p-value 0.059; indicating poor diagnostic performance to identify not well-controlled asthma. In ICS-treated smokers, AUC (95%) for the optimum cut-point of FeNO > 23 ppb: 0.597 (0.449-0.733), p-value 0.256; indicating poor diagnostic performance to identify not well-controlled asthma.<br><br>PPV: FeNO values > 30 ppb presented a PPV of 83% with ICS-treated smokers, suggesting that high FeNO values are indicative of poor asthma control even in ICS-treated smoking asthmatics.<br><br>Reporting conclusion: | Funding and conflicts of interests: No information on funding provided. One of the authors has received two lecture fees and an unrestricted research grant from Aerocrine.<br><br>Notes: No information from smoking asthmatics on asthma severity, dose of ICS in steroid-treated subjects, proportion of atopics and smoking habits. Only information that all subjects had stable asthma.<br><br>Analyses were based on use of optimal cutoff values of FeNO derived from data.The sample sizes of smokers (32 and 50) were rather small to adequate analysis (Leeflang 2008). |
| **Michils 2009**, Belgium<br><br>Cross-sectional study design in this study question.<br><br>Post hoc analysis of database that was continuously updated. | n = 59 smoking asthmatics , males 58%<br><br>Age (yr), mean (± SD): 38 ± 11<br><br>FEV$_1$, % pred, mean (± SD): 86.2 ± 17.9<br><br>Asthma severity: no information<br><br>Steroid medication: At baseline: ICS dose in µg equivalents beclomethasone diproprionate per day$^{-1}$, median (range): 500 (0-2000). Asthma treatment was adjusted according to the GINA guidelines, regardless of FeNO value. No information how many used ICS.<br><br>Atopy/allergy: atopics 92%<br><br>Smoking habit: no detailed information reported. Additional information obtained from the authors: smoking at least one cigarette per day for at least 1 | FeNO measurement: Device: Chemilumiscence analyser (model LR2000, UK). Flow rate: 50 mL/s<br><br>Reference standard: ACQ (asthma control questionnaire): patients subjectively evaluate the degree of impairment caused by their asthma during the preceding 7 days by responding to six questions using a seven-point scale (Juniper). | Ability of FeNO measurement to identify patients with controlled asthma defined as ACQ scores < 1.5 was evaluated.<br><br>ROC (receiver operating characteristic) curve was created and AUCs (areas under the ROC curves) with 95% confidence intervals and their differences from 0.5 were calculated.<br><br>Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values and accuracy were stated for the optimal cut-off of FeNO value (value which best combined sensitivity and specifity in the data). | In cross-sectional assessment, FeNO was unable to assess asthma control in smoking asthma patients (cross-sectional part of the study, one single FeNO measurement).<br><br>Sensitivity, specificity, PPV, NPV and accuracy for the optimal FeNO cut-off value of 25 ppb: Sensitivity: 66% Specificity: 48% PPV: 30% NPV: 81% Accuracy: 53%. P-value: 0.39.<br><br>At baseline: 25% (15/59) of the smoking subjects had controlled asthma (ACQ score < 1.5). | Funging and conflict of interests: AstraZeneca provided a grant for the exhaled biomarker laboratory.<br><br>Notes: No detailed information on asthma severity of subjects and smoking habits.<br><br>No reporting whether the results differed between subjects treated with low or high ICS doses, although in methods section of the report it is stated that patients treated with low and high-to-moderate ICS doses were considered separately.<br><br>Analyses were based on use of optimal cutoff values of FeNO derived from data. The sample size of smokers (59) was perhaps too small to adequate analysis (Leeflang 2008). |

**Follow-up data**

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| **Michils 2009**, Belgium<br><br>Post hoc analysis of database that was continuously updated.<br><br>Follow-up time, median (IQR), days: 93 (49-182), (range: 7-525 days). | n = 51 smoking asthmatics, males 51%<br><br>Age (yr), mean (± SD): 39 ± 11<br><br>FEV$_1$, % pred, mean (± SD): 86.5 ± 18.0<br><br>Asthma severity: no information<br><br>Steroid medication:<br>Asthma treatment was adjusted according to the GINA guidelines, regardless of FeNO value.<br>At baseline: ICS dose in μg equivalents beclomethasone diproprionate per day$^{-1}$, median (range): 500 (0-2000).<br>No information how many used.<br><br>Atopy: atopics 92%<br><br>Smoking habit: no detailed information reported. Additional information obtained from the authors: smoking at least one cigarette per day for at least 1 year | FeNO measurement:<br>Device: Chemilumiscence analyser (model LR2000, UK).<br>Flow rate: 50 mL/s<br><br>Reference standard:<br>ACQ (asthma control questionnaire): patients subjectively evaluate the degree of impairment caused by their asthma during the preceding 7 days by responding to six questions using a seven-point scale (Juniper). | ROC curves were used to analyse the ability of change in FeNO between a pair of visits to identify:<br>a) change from uncontrolled (ACQ score of ≥ 1.5) to controlled (ACQ score of < 1.5) asthma or vice versa.<br>b) significant improvement or worsening of asthma control defined as a decrease or increase in ACQ of ≥ 0.5.<br><br>Sensitivies, specificities, positive (PPV) and negative (NPV) predictive values and accuracy were stated for the optimal cut-off of FeNO values. | Change from uncontrolled to controlled asthma between two consecutive visits:<br>prevalence 33% (17/52), the highest NPV of 82% for cut-off value in change of FeNO -20%, accuracy 67%, p-value 0.016.<br><br>Change from controlled to uncontrolled asthma between two consecutive visits:<br>prevalence 25% (10/40), the highest NPV of 89% for cut-off value in change of FeNO +50%, accuracy 83%, p-value 0.017.<br><br>Improvement of asthma control between two consecutive visits:<br>prevalence 43% (40/92), NPV of 70%, accuracy 66% and p-value < 0.001 for cut-off value in change of FeNO -20%.<br>When subjects were treated with a high ICS dose (>500 μg equivalents of beclometasone diproprionate day$^{-1}$, FeNO lost its ability to assess a control improvement (p=0.07).<br><br>Worsening of asthma control between two consecutive visits, assessed to FeNO cut-off value of +30%:<br>prevalence 28% (26/92), NPV of 86%, accuracy 74% and p-value < 0.001 for cut-off value in change of FeNO +30%.<br>When subjects were treated with a high ICS dose (>500 μg equivalents of beclometasone diproprionate day$^{-1}$, FeNO ability to detect a worsening of control was somewhat reduced (p=0.037).<br><br>Reporting conclusion:<br>Sequential changes in FeNO are related to asthma control in smokers.<br>An FeNO reduction of < 20% would indicate that asthma remains uncontrolled in most smoking asthmatics. When asthma is controlled, and FeNO increase of < 50% would indicate that asthma | Funding and conflict of interests:<br>AstraZeneca provided a grant for the exhaled biomarker laboratory.<br><br><br>Notes:<br>No detailed information on asthma severity of subjects and smoking habits.<br><br>Analyses were based on use of optimal cutoff values of FeNO derived from data. The sample size of smokers (51) was perhaps too small to adequate analysis (Leeflang 2008). |

## Research question 3      Descriptions and results of studies

| Study design | Characteristics of subjects | | FeNO measurement | Results | Notes |
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| Study reference, country, study design | Characteristics of smoking asthmatics | Characteristics of non-smoking asthmatics | FeNO measurement: flow rate, device | Results | Funding and notes |
| **Cahn 2015**, Belgium and UK<br><br>Cross-sectional study design related to baseline FeNO values.<br><br>The study was a randomized cross-over trial evaluating asthma medications, on allergen-induced asthmatic responses. | n = 17, males 29%<br><br>Age (yr), mean (range): 30 (19-48)<br><br>Severity of asthma: no information<br><br>FEV$_1$, % pred, mean (range): 98.9 (73.9 -121.0).<br><br>Steroid medication: steroid-naive<br><br>Atopy/allergy: atopics 100%<br><br>Smoking habits, median (range): 8 (5-18) pack-years | n = 18, males 67%<br><br>Age (yr), mean (range): 30 (21-47)<br><br>Severity of asthma: no information<br><br>FEV$_1$, % pred, mean (range): 95.2 (78.3 - 114.1)<br><br>Steroid medication: steroid-naive<br><br>Atopy/allergy: atopics 100%<br><br>Non-smokers included never smokers and ex-smokers who had stopped smoking more than 6 months prior and never exceeded 5 pack-years at any time | Flow rate: 50 ml/s<br>Device: Aerocrine Niox Flex, Sweden | FeNO levels were significanty lower in smoking steroid-naive asthmatics than in non-smoking steroid-naive asthmatics.<br><br>FeNO values:<br>In smoking steroid-naive asthmatics, geometric mean (95% CI): 16.72 (11.74, 23.82) ppb, measured pre-dose on Day 1 of placebo treatment (summary of log-transformed data)<br><br>In non-smoking steroid-naive asthmatics, geometric mean (95% CI): 53.40 (42.73, 66.75) ppb, measured pre-dose on Day 1 of placebo treatment (summary of log-transformed data).<br><br>The FeNO levels were log-transformed before summarizing results. | Funging and conflict of interest:<br>Funding provided by GSK (GlaxoSmithKline)<br><br>Notes:<br>Gender imbalance in the smoking and non-smoking groups, with a predominance of males in the non-smoking group and females in the smoking group.<br><br>Sputum eosinophil counts were similar in both groups measured pre-dose on Day 1 of placebo treatment (data stated only graphically, not possible to extract exact figures) |
| **Hillas 2011**, Greece<br><br>Cross-sectional study desing.<br><br>No information how and where the patients were recruited. | n = 40, males 45%<br><br>Age (yr), mean (± SD): 48.7 ± 11.2<br><br>Severity of asthma: mild to moderate<br><br>FEV$_1$, % pred, mean (± SD): 87.8 ± 11.6<br><br>Steroid medication:<br>all were receiving ICS.<br>ICS, n/mean budesonide equivalent dose: 10/400<br>ICS/LABA, n/mean budesonide equivalent dose: 30/640<br><br>Sputum eosinophil count, %, median (IQR): 2.0 (1.0-5.0)<br><br>Atopy/allergy:<br>atopics 60%,<br>no acute rhinitis<br><br>Smoking habits, mean (± SD): 45.1 ± 20.2 pack-years | n = 43, males 40%<br><br>Age (yr), mean (± SD): 52.9 ± 15.2<br><br>Severity of asthma: mild to moderate<br><br>FEV$_1$, % pred, mean (± SD): 88.3 ± 12.4<br><br>Steroid medication:<br>all were receiving ICS.<br>ICS, n/mean budesonide equivalent dose: 9/400<br>ICS/LABA, n/mean budesonide equivalent dose: 34/640<br><br>Sputum eosinophil count, %, median (IQR): 3.0 (1.0-6.0)<br><br>Atopy/allergy:<br>atopics 58%,<br>no acute rhinitis<br><br>Non-smokers had never smoked. | Flow rate: 50 ml/s<br>Device: NIOX MINO, Aerocrine, Sweden | Smoking steroid-treated asthmatic group had significantly lower FeNO levels compared to non-smoking steroid-treated asthmatic group (p < 0.001).<br><br>FeNO values:<br>In smoking asthmatics, median (interquartile range): 12 (10-16) ppb<br><br>In non-smoking asthmatics, median (interquartile range): 19 (14-25) ppb<br><br>Distributions of FeNO levels were skewed.<br>Statistical comparison of FeNO levels between smoking asthmatic and non-smoking asthmatic groups was performed using Mann-Whitney U-test. | Funging and conflict of interest:<br>No information provided |

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| **Horvath 2004**, UK<br><br>Cross-sectional study desing.<br><br>No information how and where the patients were recruited. | n = 22, males 55%<br><br>Age (yr), mean (± SEM): 30 ± 2<br><br>Severity of asthma: no information<br><br>$FEV_1$, % pred, mean (± SEM): 94 ± 2<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: no information<br><br>Smoking habits, mean (± SEM):<br>13 ± 2 pack-years | n = 30, males 53%<br><br>Age (yr), mean (± SEM): 29 ± 1<br><br>Severity of asthma: no information<br><br>$FEV_1$, % pred, mean (± SEM): 93 ± 1<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: no information<br><br>No detailed information on non-smoking criteria but all non-smokers were tested by NicCheck I which determined the levels of nicotine and its metabolites, to ensure non-smoking status. | Flow rate: 83-100 ml/s<br>Device: chemiluminescence analyser, R2000, Logan Research, UK | FeNO levels were lower in smoking steroid-naive asthmatic subjects than in non-smoking steroid-naive asthmatics.<br><br>FeNO values:<br>In smoking steroid-naive asthmatics, median (range):<br>7.7 (3.4 - 32.5) ppb<br><br>In non-smoking steroid-naive asthmatics, median (range):<br>25.0 (9.7 - 92.8) ppb<br><br>Data were analysed non-parametrically. Comparisons between groups were performed by Dunn's test. | <u>Funging and conflict of interest:</u><br>Supported by a joint grant of the British Countil and and Hungarian OMFB, a NATO Scientic Fellowship Programme, the Hungarian National Scientific Foundation and the Hungarian Ministry of Health Care. |
| **Malinovschi 2012**, Denmark<br><br>Cross-sectional study desing related to baseline FeNO values.<br><br>The aim of the study was to assess the value of FeNO to diagnose asthma.<br><br>A random population sample of 10,400 subjects was drawn from the civil registration list. Subjects were mailed with a validated self-administered asthma and rhinitis screening questionnaire. Of the 47% that responded, 686 subjects recorded two or more respiratory symptoms and were examined. | n = in total 96 asthmatics: current smokers 32, never-smokers 45, ex-smokers 19, males 41%<br><br><u>Demographics for all asthmatic subjects (not stated separately for the 32 smoking or 45 never-smoking asthmatics):</u><br><br>Age (yr), mean (SD): 32.7 (SD 8.7)<br><br>Severity of asthma: no information<br><br>$FEV_1$, % pred, mean (SD): 94.4 (SD 15.2)<br><br>Steroid medication:<br>25% of asthmatics used ICS (no information on dose)<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy:<br>Rhinitis 65.6% in all asthmatics.<br>Atopics among all smokers 22% (not detailed for smoking asthmatics)<br><br>Smoking habits, median (range): 10 (0-30) pack-years.<br>This is information for all smoking subjects (n = 112)<br><br>Never-smoker group used as control group | | Flow rate: 50 ml/s<br>Device: NIOX Mino, Aerocrine, Sweden | FeNO values:<br>In smoking asthmatics, geometrical mean (95% CI):<br>16.7 (12.8, 21.7) ppb.<br><br>In non-smoking asthmatics, geometrical mean (95% CI):<br>24.4 (19.0, 31.3) ppb.<br><br>FeNO levels were not normally distributed and FeNO values were log-transformed in the analyses. The comparison of smoking asthmatics vs. non-smoking asthmatics was not tested. | <u>Funging and conflict of interest:</u><br>Funded by an unrestricted grant from AstraZeneca. A. Malinovschi was funded by Bror Hjerpsteds Stiftelse and Uppsala University Hospital.<br>None of the authors have any conflcts of interest to declare.<br><br><u>Notes:</u><br>Asthma diagnosis of all included asthmatics perhaps do not fulfil the asthma diagnosis criteria of this review. We, however, assumed that ≥ 80% of asthmatics fulfilled the criteria. |

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| **McSharry 2005**, UK<br><br>Cross-sectional study desing related to baseline FeNO values.<br><br>The aim of the study was to identify and model short-term and long-term influences of cigarette smoking on FeNO.<br><br>Subjects were recruited from respiratory outpatient clinics and hospital staff. | n = 17, males 53%<br><br>Age (yr), mean (95% CI): 40.0 (35.5-52.0)<br><br>Severity of asthma: no information<br><br>FEV$_1$, % pred, mean (95% CI): 75.4 (70.3-89.1)<br><br>Steroid medication: 81% of all asthmatics used ICS (no information how many smoking asthmatics used ICS).<br>Dose: ICS (ug/d): 800 (100-900).<br>No oral steroids.<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: 76% atopics<br><br>Smoking habits:<br>20.0 (17.5-27.5) cigarettes per day,<br>27.5 (16.0-35.7) pack-years | n = 23, males 74%<br><br>Age (yr), mean (95% CI): 39.0 (33.0-52.0)<br><br>Severity of asthma: no information<br><br>FEV$_1$, % pred, mean (95% CI): 81.4 (72.6-86.9)<br><br>Steroid medication: 81% of all asthmatics used ICS, (no information how many non-smoking asthmatics used ICS).<br>Dose: ICS (ug/d): 400 (0-600).<br>No oral steroids.<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: 96% atopics<br><br>Never-smoker group used as control group | Flow rate: 250 mL/s<br>Device: chemilumiscence analyser (model LR2000, UK) | Smoking asthmatic group had significantly lower FeNO levels compared with non-smoking asthmatic group (p < 0.01).<br><br>FeNO values:<br>In smoking asthmatics, mean (95% CI):<br>4.03 (2.96-6.47) ppb<br><br>In non-smoking asthmatics, mean (95% CI):<br>14.30 (10.63-27.86) ppb<br><br>Difference between groups were tested by using the rank-sum test. | <br><br>Notes:<br>No detailed information how many smoking and non-smoking asthmatics used ICS. Only information that 43 out of all 53 asthmatics used ICS (including ex-smokers). |
| **Michils 2009**, Belgium<br><br>Cross-sectional study design related to baseline FeNO values.<br><br>The aim of study was to investigate whether changes in FeNO might be related to changes in asthma control in smoking asthmatics.<br><br>Post hoc analysis of database that was continuously updated. | n = 59, males 58%<br><br>Age (yr), mean (± SD): 38 ± 11<br><br>Severity of asthma: no detailed information<br><br>FEV$_1$, % pred, mean (± SD): 86.2 ± 17.9<br><br>At baseline: ICS dose in ug equivalents beclomethasone diproprionate per day, median (range): 500 (0-2000).<br>No information on how many used.<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: atopics 92%<br><br>Smoking habit: no detailed information reported. Additional information obtained from the authors: smoking at least one cigarette per day for at least 1 year | n = 411, males 47%<br><br>Age (yr), mean (± SD): 41 ± 16<br><br>Severity of asthma: no detailed information<br><br>FEV$_1$, % pred, mean (± SD): 85.6 ± 15.7<br><br>At baseline: ICS dose in ug equivalents beclomethasone diproprionate per day, median (range): 250 (0-2000).<br>No information on how many used.<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: atopics 85%<br><br>Additional information obtained from the authors that the criteria of non-smoking of this review are complied. | Flow rate: 50 mL/s<br>Device: Chemilumiscence analyser (model LR2000, UK), | FeNO levels were significantly lower in smoking asthmatic subjects than in non-smoking asthmatics (p < 0.001).<br><br>FeNO values:<br>In smoking asthmatics, geometric mean (geometrical interval): 18.1 (6.9-47.5).<br><br>In non-smoking asthmatics, geometric mean (geometrical interval): 33.7 (14.3-79.2).<br><br>Despite similar mean ACQ scores (1.5 in smoking asthmatics versus 1.7 in non-smoking asthmatics at baseline), FeNO was reduced in smoking asthmatics.<br><br>Unpaired t-test were used when considering log-transformed FeNO values. | <br><br>Notes:<br>No detailed information on asthma severity but it was stated that:<br>25% (15/59) of the smoking subjects and 48% (197/411) of the non-smoking subjects, at baseline, were defined to have controlled asthma (classified according to ACQ score < 1.5).<br><br>No detailed information on smoking habits. |

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| **Murphy 2010**, Australia<br><br>Cross-sectional study design related to baseline FeNO values.<br><br>The aim of the study was to assess clinical implications of smoking on asthma exacerbations in pregnancy.<br><br>Patients were consecutively recruited through the hospital antenatal clinics and the community. | n = 27, study with pregnant women<br><br>Age (yr), mean (± SD): 26.4 ± 5.9 (range 18-43)<br><br>Severity of asthma: severe asthma in 26%<br><br>$FEV_1$, % pred, mean (± SD): 92.3 ± 15.1<br><br>Steroid medication, ICS (ug/day), median (IQR): 0 (0, 1000). No information on how many used.<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: no information<br><br>Pack-years, median (IQR): 4 (2.3-7.9) (8.5 years total smoking); 5-6 cigarettes per day | n = 26, study with pregnant women<br><br>Age (yr), mean (± SD: 28.7 ± 4.2 (range 18-43)<br><br>Severity of asthma: severe asthma in 15.4%<br><br>$FEV_1$, % pred, mean (± SD): 96.0 ± 14.0<br><br>Steroid medication: ICS (ug/day), median (IQR): 0 (0, 1000). No information on how many used.<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: no information<br><br>Never-smoker group used as control group | Flow rate: 50 ml/s<br>Device: NIOX, Aerocrine, Sweden | FeNO values:<br>In smoking asthmatics, median (IQR): lowest FeNO: 8.3 (6.6.-16.9) ppb.<br><br>In non-smoking asthmatics, median (IQR): lowest FeNO: 13 (10.1.-19.6) (in never-smokers group).<br><br>It remained unclear whether the FeNO levels were normally distributed or not and which analysis method was used when comparing the FeNO levels between the groups. | |
| **Nagasaki 2013**, Japan<br><br>Cross-sectional study design related to FeNO values.<br><br>The aim of the study was to determine the effects of smoking and age on serum IgE levels and eosinophilic inflammation in patients with asthma.<br><br>Participants were recruited at asthma clinic of university hospital. Asthma of patients were newly diagnosed. | n = 46, males 63%<br><br>Age (yr), mean (± SD): 47 ± 13 (range 24-74)<br><br>Severity of asthma: no information<br><br>$FEV_1$, % pred, mean (± SD): 91.3 ± 17.4<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil count, %, mean (± SD): 17.0 (± 18.4) (obtained from 23 subjects)<br><br>Atopy/allergy: atopics 87%, allergic rhinitis: 45%<br><br>Smoking habits, mean (± SD): 30 ± 19 pack-years, | n = 196, males 28%<br><br>Age (yr), mean (± SD): 49 ± 20 (range 16-95)<br><br>Severity of asthma: no information<br><br>$FEV_1$, % pred, mean (± SD): 94.2 ± 22.1<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil count, %, mean (± SD): 11.5 (± 20.0) (obtained from 101 subjects)<br><br>Atopy/allergy: atopics 75%, allergic rhinitis: 43%<br><br>Never-smoker group used as control group | Flow rate: 50 mL/s<br>Device: chemiluminescence analyser, NOA 280, Sievers, USA | FeNO levels in smoking asthmatic group did not differ from those in non-smoking asthmatic subjects.<br><br>FeNO values:<br>In smoking asthmatics, mean (± SD): 55 ± 56<br><br>In non-smoking asthmatics, mean (± SD): 57 ± 60<br><br>FeNO levels were log-transformed to achieve normal distributions in analyses. | |

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| **Rouhos 2010**, Finland<br><br>Cross-sectional study design.<br><br>Subjects were army conscripts referred to the military hospital because of respiratory symptoms and diagnosed with current symptomatic asthma. | n = 46 (atopic 30), males 96%<br><br>Age (yr), mean (range): 19.5 (18-23)<br><br>Severity of asthma: symptomatic asthma, mild<br><br>$FEV_1$, % pred, mean (range):<br>atopics: 86 (60-119),<br>nonatopics: 92 (75-108)<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy:<br>atopics 65% (based on skin prick test)<br><br>Smoking habit, pack-years, mean (range):<br>atopics: 2.5 (0.2-9.0),<br>nonatopics: 4.2 (0.6-11) | n = 70 (atopic 54), males 86%<br><br>Age (yr), mean (range): 21 (18-30)<br><br>Severity of asthma: symptomatic asthma, mild<br><br>$FEV_1$, % pred, mean (range):<br>atopics: 94 (74-118),<br>nonatopics: 90 (68-111)<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy:<br>atopics 77% (based on skin prick test)<br><br>Additional information was obtained from the authors that the non-smokers had not smoked for at least 6 months previously and that they had smoked a maximum 99 cigarettes in their entire lives. | Flow rate: 90-120 mL/s<br>Device: chemiluminescence analyser, Sievers 270B, USA | Median FeNO levels were significantly lower in steroid-naive asthmatic smokers than in steroid-naive asthmatic non-smokers (p = 0.002).<br><br>FeNO values:<br>In smoking asthmatics, median (25-75% interquartile range) for all smoking asthmatics:<br>13.5 (9.1 - 30.1) (not detailed for atopics/non-atopics). (Detailed result obtained from author.)<br><br>In non-smoking asthmatics, median (25-75% interquartile range) for all non-smoking asthmatics:<br>24.0 (14.9 - 40.1) (not detailed for atopics/non-atopics). (Detailed result obtained from author.)<br><br>Because FeNO was not normally distributed, $log_{10}$-transformed FeNO values were used in the analyses.<br>Comparison between groups were performed by one-way variance analysis, and p-values were corrected for multiple comparisons by the least significant difference method. | |
| **Rutgers 1998**, The Netherlands<br><br>Cross-sectional study design related to baseline FeNO values.<br><br>The aim of the study was to investigate whether there is a difference between NO values measured with single-breath and tidal-breathing methods.<br><br>No information how and where the patients were recruited. | n = 16, males 38%<br><br>Age (yr), mean (range): 30 (21-45)<br><br>Severity of asthma: no information<br><br>$FEV_1$, % pred, mean (range): 81(41-107)<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: atopics 100%<br><br>Smoking habits, mean (range):<br>9 (0-35) pack-years | n = 16, males 31%<br><br>Age (yr), mean (range): 28 (18-44)<br><br>Severity of asthma: no information<br><br>$FEV_1$, % pred, mean (range): 77 (25-113)<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: atopics 100%<br><br>No detailed information on non-smoking criteria but the way of reporting gives an impression that non-smoking group included never-smokers (the study had also an ex-smoker group) | Flow rate: 150 mL/s<br>Device: chemiluminescence analyser CLD 700 AL, ECO physics, Switzerland | Smoking asthmatics tended to have lower FeNO consentrations than non-smoking asthmatics, as measured with single-breath method (p=0.08)<br><br>FeNO values based on single-breath method:<br>In smoking asthmatics, mean (± SD):<br>16.1 (10.1) ppb<br><br>In non-smoking asthmatic subjects, mean (± SD):<br>22.5 (14.5) ppb<br><br>Differences between groups in FeNO values with single-breath were tested using analysis of variance (ANOVA). | |

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| **Shimoda 2016**, Japan<br><br>Cross-sectional study design related to baseline FeNO values.<br><br>The aim of the study was to evaluate influence of cigarette smoking on airway inflammation and inhaled corticosteroid treatment in patients with asthma.<br><br>Patients with newly diagnosed asthma who visited hospital were recruited. | n = 52, males 63%<br><br>Age (yr), mean ($\pm$ SD): 42 $\pm$ 13<br><br>Severity of asthma:<br>mild 73%; moderate 27%<br><br>$FEV_1$, % pred, mean ($\pm$ SD): 81 $\pm$ 20<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil, %: 21 $\pm$ 18<br><br>Atopy/allergy: atopics 75%<br><br>Smoking habits, mean ($\pm$ SD), 28 $\pm$ 14 pack-years | n = 81, males 25%<br><br>Age (yr), mean ($\pm$ SD): 41 $\pm$ 12<br><br>Severity of asthma:<br>mild 86%; moderate 14%<br><br>$FEV_1$, % pred, mean ($\pm$ SD): 84 $\pm$ 17<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil, %: 32 $\pm$ 24<br><br>Atopy/allergy: atopics 62%<br><br>Non-smokers were never-smokers | Flow rate: 50 mL/s<br>Device: chemiluminescence analyser NOA 280, Sievers Instruments Inc. Boulder, CO | FeNO levels were significantly lower in steroid-naive asthmatic smokers than in non-smoking steroid-naive asthmatics ($p < 0.01$).<br><br><u>FeNO values:</u><br>In smoking asthmatics, mean ($\pm$SD):<br>77 $\pm$ 55<br><br>In non-smoking asthmatics, mean ($\pm$SD):<br>108 $\pm$ 88<br><br>t-test was used to compare baseline FeNO levels between groups. | <u>Funding and conflicts of interest:</u><br>No external funding sources.<br>The authors have no conflicts of interest. |
| **Spears 2011**, UK<br><br>Cross-sectional study design related to baseline FeNO values.<br><br>The study aimed to test the hypotheses that $C_{alv}$ is raised and $J'_{aw}$ is reduced in smokers with asthma compared to non-smoking asthmatics.<br><br>No information how and where the patients were recruited. | n = 22, males 45%<br><br>Age (yr), mean (SD): 46.6 (6.7)<br><br>Severity of asthma: mild to moderate<br><br>$FEV_1$, % pred, mean (SD): 73.6 (18.5)<br><br>Steroid medication, ICS (mcg/day), mean (SD): 1046 (611)<br><br>Sputum eosinophil count, %: 0.4 (0.0, 1.0); median (IQR) ($10^4$): 2.0 (0.0, 4.0)<br><br>Atopy/allergy: no information<br><br>Smoking habits, mean (SD):<br>27.6 (15.7) pack-years | n = 21, males 48%<br><br>Age (yr), mean (SD): 42.5 (10.0)<br><br>Severity of asthma: mild to moderate<br><br>$FEV_1$, % pred, mean (SD): 73.3 (15.3)<br><br>Steroid medication, ICS (mcg/day), mean (SD): 679 (419)<br><br>Sputum eosinophil count, %: 0.3 (0.0, 2.0); median (IQR) ($10^4$): 1.0 (0.0, 7.0)<br><br>Atopy/allergy: no information<br><br>Non-smokers were never-smokers | Flow rate: 50 ml/s<br>Device: Niox-Flex, Aerocrine Sweden | FeNO values were significantly reduced in smoking asthmatics compared to non-smoking asthmatics ($p < 0.001$).<br><br><u>FeNO values:</u><br>In smoking asthmatics, median (IQR): 11.1 (3.6, 13.5)<br><br>In non-smoking asthmatics, median (IQR): 32.8 (17.7, 73.2)<br><br>Comparison made using Mann-Whitney test. | <u>Funging and conflict of interest:</u><br>Supported by the donation of a Niox-Flex machine and educational grant from Aerocrine which covered servicing and maintenance. Aerocrine had no involvement in the study design, performance, analysis, interpretation of data and manuscript preparation.<br><br><u>Notes:</u><br>In non-smoker group, daily ICS dose lower than in smoker group ($p < 0.05$). |

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| **Takahashi 2018**, multicentre prospective cohort study recruiting from 16 clinical centres in 11 European countries (U-BIOPRED study)<br><br>Cross-sectional study design related to baseline FeNO values.<br><br>The study population of this article were participants with sputum samples obtained for proteomic analysis (in total 88 participants: 70 asthmatics (11 current smokers, 22 ex-smokers, and 37 nonsmokers) + 18 healthy nonsmokers). Current smoking asthmatics and never-smoking asthmatics considered in this review. | n = 11, males 54.5%<br><br>Age (yr), mean (SD): 50.0 (10.6)<br><br>Severity of asthma: all subjects had severe asthma<br><br>Post bronchodilator FEV1, % pred, mean (SD): 73.7 % (18.2)<br><br>Steroid medication:<br>Maintenance systematic steroids in 30%.<br>Oral steroid dose (mg/day), mean (SD): 2.50 (4.71).<br>Maintenance ICS in 100%, no information on dose.<br><br>Sputum eosinophil count, %: 7.2 ± 15.2<br><br>Atopy/allergy:<br>atopics 89%<br>allergic rhinitis 25%<br><br>Smoking history, mean (SD):<br>29.0 (18.2) pack-years | n = 37, males 40.5%<br><br>Age (yr), mean (SD): 52.6 (13.3)<br><br>Severity of asthma: all subjects had severe asthma<br><br>Post bronchodilator FEV1, % pred, mean (SD): 68.6 % (21.1)<br><br>Steroid medication:<br>Maintenance systematic steroids in 46%.<br>Oral steroid dose (mg/day), mean (SD): 4.18 (6.61).<br>Maintenance ICS in 100%, no information on dose.<br><br>Sputum eosinophil count, %: 18.8 ± 24.6<br><br>Atopy/allergy:<br>atopics 85%<br>allergic rhinitis 55%<br><br>No detailed information on non-smoking criteria but the way of reporting gives an impression that non-smoking group included only never-smokers (the study had also an ex-smoker group) | Flow rate: 50 ml/s<br>Device: no information on device | FeNO values were significantly reduced in smoking asthmatics compared to non-smoking asthmatics (p < 0.052).<br><br>FeNO values:<br>In smoking asthmatics, mean (± SD): 15.2 (16.6)<br><br>In non-smoking asthmatics, mean (± SD): 41.2 (36.3)<br><br>Comparison made using Kruskal-Wallis test. | |

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| **Thomson 2013**, UK<br><br>Cross-sectional study design related to baseline FeNO values.<br><br>The study population were patients with severe refractory asthma recruited to the British Thoracic Society Severe Asthma Registry. | n = 69, males 27%<br><br>Age (yr), mean (SD): 42 (10)<br><br>Severity of asthma: all subjects had severe asthma<br><br>$FEV_1$, % pred, mean (SD): 68 (19) (60 subjects, number used to analyze incomplete data)<br><br>Steroid medication:<br>Maintenance oral steroids 32 %.<br>Oral steroid dose, median (IQR):<br>20 mg (11-30) (20 subjects, number used to analyze incomplete data).<br>ICS: Beclomethasone dipropionate equivalent dose (mg), median (IQR): 2 (1.6-2).<br><br>Sputum eosinophil count, %, median (IQR):<br>1 (0-2) (14 subjects, number used to analyze incomplete data)<br><br>Atopy/allergy:<br>At least 55 % of subjects reported some form of allergic disease<br><br>Smoking habits, median (IQR):<br>19 (10-36) pack-years<br>(52 subjects, number used to analyze incomplete data). | n = 461, males 31%<br><br>Age (yr), mean (SD): 43 (14)<br><br>Severity of asthma: all subjects had severe asthma<br><br>$FEV_1$, % pred, mean (SD): 70 (25) (406 subjects, number used to analyze incomplete data)<br><br>Steroid medication:<br>Maintenance oral steroids 45%.<br>Oral steroid dose, median (IQR):<br>15 mg (10-20) (197 subjects, number used to analyze incomplete data).<br>ICS: Beclomethasone dipropionate equivalent dose (mg), median (IQR): 2 (1.6-2).<br><br>Sputum eosinophil count, %, median (IQR):<br>4 (1-14) (106 subjects, number used to analyze incomplete data)<br><br>Atopy/allergy:<br>At least 64 % of subjects reported some form of allergic disease<br><br>Never-smoker group used as control group | Flow rate: 50 ml/s<br>Device: no information on device | FeNO values in smoking asthmatics were significantly reduced compared to non-smoking asthmatics at baseline (p < 0.001).<br><br>FeNO values:<br>In smoking asthmatics, median (IQR): 14 (8-39)<br>(30 subjects, number used to analyze incomplete data)<br><br>In non-smoking asthmatics, median (IQR): 35 (20-65)<br>(184 subjects, number used to analyze incomplete data).<br><br>FeNO levels were not normally distributed and were logged for analyses. Comparison made using Mann-Whitney U test. | Funging and conflict of interest:<br>no information provided.<br><br><br>Notes:<br>FeNO value available only from 30 out of 69 smoking asthmatics and from 184 out of 461 non-asthmatics.<br><br>Demographics of subjects in this appendix are not based on the 30 and 184 subjects from whom the FeNO value was available. |
| **Verleden 1999**; Belgium<br><br>Cross-sectional study design.<br><br>Subjects were recruited from outpatients at university outpatient asthma clinic. | n = 13, males 62%<br><br>Age (yr), mean (SD): 33.9 (14.5)<br><br>Severity of asthma: mild<br><br>$FEV_1$, % pred, mean (SD): 113.3 (17.2)<br><br>Steroid-medication: steroid-naive<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: no information<br><br>Smoking habits, mean (SD), daily cigarette consumption:<br>17 ± 6 during for 14 ± 6 years | n = 29, males 41 %<br><br>Age (yr), mean (SD): 36.1 (17.4)<br><br>Severity of asthma: mild<br><br>$FEV_1$, % pred, mean (SD): 103.3 (10.6)<br><br>Steroid-medication: steroid-naive<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: no information<br><br>Non-smokers were never-smokers | Flow rate: 200 mL/s<br>Device: chemiluminescence analyser Ecophysics CLD700 AL Med, Switzerland | FeNO levels were significantly lower in steroid-naive asthmatic smokers than in steroid-naive asthmatic non-smokers (p < 0.05).<br><br>FeNO values:<br>In smoking steroid-naive asthmatics, mean (±SD):<br>12.7 ± 5.1<br><br>In non-smoking steroid-naive asthmatics, mean (±SD):<br>21.8 ± 12.7<br><br>The Kruskal-Wallis nonparametric analysis of variance test and Dunn's multiple comparisons test were used to assess significant differences in FeNO levels among the groups.<br>Log-aritmic transformation was done for FeNO values before analysis. | Funging and conflict of interest:<br>No information provided |

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| **Xu 2016**, USA<br><br>Cross-sectional study design related to baseline FeNO values.<br><br>Data from the National Health and Nutrition Eamination Survey (NHANES, 2007-2012). | n = 1500, males 41%, no information on numbers of smokers and non-smokers.<br><br>Demographics for all asthmatic subjects (not stated separately for smoking and non-smoking asthmatics):<br><br>Age (yr), mean (SD): 36.7 (0.76). (FeNO was measured in 6-79 years olds.)<br><br>Severity of asthma: no information<br><br>$FEV_1$, % pred: no information<br><br>Steroid medication: 33% of smokers and 28% of non-smokers reported use of oral/inhaled steroids in the last two days, respectively (the number of non-smokers obtained from the authors).<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: hay fever in the past year in 35%.<br><br>Cigarette smoking was assessed using both self-reported questionnaire and serum cotinine concentrations. Participants with serum cotinine > 10ng/mL were categorized as current smoker, no matter how they responded to the questionnaire.<br>Subjects whose serum cotinine was < 10ng/mL were categorized as current smokers, or former smokers or non-smokers (based on the self-reporting for detailed questionnaire).<br><br>Those responded "no" to the question " Have you smoked at least 100 cigarettes in your entire life?" were categorized as non-smokers. | Flow rate: 50 ml/s<br>Device: NIOX, Aerocrine Sweden | Asthmatic smokers had 45.1% (95% CI 36.9 to 52.8) lower of FeNO compared to non-smoking asthmatics.<br><br>FeNO levels were log-transformed to ensure normal distribution for analyses. | Funding and conflicts of interest:<br>The authors report no conflicts of interest.<br><br><br>Notes:<br>No information on asthma severity but asthma attack in the past year was reported in 49% of the asthmatics and for 64% of subjects were prescribed asthma medication.<br><br><br>Authors of the study commented:<br>Atopy was not considered as a potential confounding factor in analyses.<br><br>Serum cotinine may introduce misclassifications of exposure for smoking because the half-life of serum cotinine averages about 17-20 h. |

**Online Supplement 6**

**Research question 3    Proportional differences in FeNO values and description of confounding factors at study level**

| Study | FeNO in smoking asthmatics | $n_1$ | FeNO in non-smoking asthmatics | $n_2$ | $n_{1+2}$ | Proportional difference | 95% confidence interval of proportional difference | Proportion of asthmatics used ICS medication | Atopy | Severity of asthma |
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| Cahn 2015 | 16.72 | 17 | 53.4 | 18 | 35 | 68,7 | 54.3 to 82.9 | steroid-naïve | 100 % | no information |
| Hillas 2011 | 12 | 40 | 19 | 43 | 83 | 36,8 | 26.5 to 47 | 100% used | 60 % | mild to moderate |
| Horvath 2004 | 7.7 | 22 | 25 | 30 | 52 | 69,2 | 55.8 to 80.8 | steroid-naïve | no information | no information |
| Malinovschi 2012 | 12.8 | 32 | 24.4 | 45 | 77 | 47,5 | 36.4 to 58.4 | 25% used | 22 % | no information |
| McSharry 2005 | 4.03 | 17 | 14.3 | 23 | 40 | 71,8 | 57.5 to 85 | 81% used | 76% of smokers; 96% of non-smokers. | no information |
| Michils 2009 | 18.1 | 59 | 33.7 | 411 | 470 | 46,3 | 41.7 to 50.9 | No information how many used. ICS dose in ug equivalents beclomethasone diproprionate per day, median (range): 500 (0-2000) | 90 % | no information |
| Murphy 2010 | 8.3 | 27 | 13 | 26 | 53 | 36,2 | 22.6 to 49.1 | No information how many used. ICS (ug/day), median (IQR): 0 (0,1000). | no information | Severe asthma: 26% of smokers; 15% of non-smokers. |
| Nagasaki 2013 | 55 | 46 | 57 | 196 | 242 | 3,5 | 1.2 to 5.8 | steroid-naïve | 87% of smokers; 75% of non-smokers. | no information |
| Rouhos 2010 | 13.5 | 46 | 24 | 70 | 116 | 43,8 | 34.5 to 53.4 | steroid-naïve | 65% of smokers; 77% of non-smokers. | mild |
| Rutgers 1998 | 16.1 | 16 | 22.5 | 16 | 32 | 28,4 | 12.5 to 43.8 | steroid-naïve | 100 % | no information |
| Shimoda 2016 | 77 | 52 | 108 | 81 | 133 | 28,7 | 21.1 to 36.1 | steroid-naïve | 75% of smokers; 62% of non-smokers. | mild to moderate |
| Spears 2011 | 11.1 | 22 | 32.8 | 21 | 43 | 66,2 | 51.2 to 79.1 | No information how many used. In non-smoker group, daily ICS dose lower than in smoker group: (mcg/day), mean (SD): 679 (419) vs.1046 (611) (p < 0.05). | no information | mild to moderate |
| Takahashi 2018 | 15.2 | 11 | 41.2 | 37 | 48 | 63,1 | 50.0 to 77.1 | 100% used | 89% of smokers; 85% of non-smokers | 100% had severe |
| Thomson 2013 | 14 | 69 | 35 | 461 | 530 | 60 | 55.8 to 64.2 | 100% used | no information | 100% had severe |
| Verleden 1999 | 12.7 | 13 | 21.8 | 29 | 42 | 41,7 | 26.2 to 57.1 | steroid-naïve | no information | mild |
| Xu 2016 |  |  |  |  | 1500 | 45,1 | 36.9 to 52.8 | 30% used either oral or inhaled steroids | no information | no information |

| Research question 4 A | Smoking asthmatics untreated with ICS vs. smoking healthy subjects | | | | |
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| | | **Description and results of studies** | | | |
| **Study design** | **Characteristics of subjects** | | **FeNO measurement** | **Results** | Notes |
| Study reference, country, study design | **Characteristics of smoking ICS untreated asthmatics** | **Characteristics of smoking healthy subjects** | **FeNO measurement: flow rate, device** | **Results** | **Funding and notes** |
| **Horvath 2004**, UK<br><br>Cross-sectional study desing.<br><br>No information how and where the patients were recruited. | n = 22, males 55%<br><br>Age (yr), mean (± SEM): 30 ± 2<br><br>Severity of asthma: no information<br><br>$FEV_1$, % pred, mean (± SEM): 94 ± 2<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: no information<br><br>Smoking habits, mean (± SEM): 13 ± 2 pack-years | n = 20, males 45%<br><br>Age (yr), mean (± SEM): 33 ± 2<br><br><br><br>$FEV_1$, % pred mean (± SEM): 98 ± 1<br><br><br><br><br><br>Atopy/allergy: no information<br><br>Smoking habits, mean (± SEM): 12 ± 2 pack-year | Flow rate: 83-100 ml/s<br>Device: chemiluminescence analyser, R2000, Logan Research, UK | FeNO levels were significantly higher in steroid-naive asthmatic smokers than in healthy smokers (p < 0.001).<br><br>FeNO values:<br>In smoking asthmatics, median (range):<br>7.7 (3.4 - 32.5)<br><br>In smoking healthy subjects, median (range):<br>3.2 (2.0 - 7.2)<br><br>Data were analysed non-parametrically. Comparisons between groups were performed by Dunn's test. | Funging and conflict of interest:<br>Supported by a joint grant of the British Countil and and Hungarian OMFB, a NATO Scientic Fellowship Programme, the Hungarian National Scientific Foundation and the Hungarian Ministry of Health Care. |
| **Malinovschi 2012**, Denmark<br><br>Cross-sectional study desing related to baseline FeNO values.<br><br>The aim of the study was to assess the value of FeNO to diagnose asthma.<br><br>A random population sample of 10,400 subjects was drawn from the civil registration list. Subjects were mailed with a validated self-administered asthma and rhinitis screening questionnaire. Of the 47% that responded, 686 subjects recorded two or more respiratory symptoms and were examined. | n = 96, current smokers 32, males 41%<br><br>Demographics for all asthmatic subjects (not stated separately for the 32 smoking asthmatics):<br><br>Age (yr), mean (SD): 32.7 (SD 8.7)<br><br>Severity of asthma: no information<br><br><br><br>$FEV_1$, % pred, mean (SD): 94.4 (SD 15.2)<br><br>Steroid medication:<br>ICS use 25% (no information on dose)<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy:<br>rhinitis 65.6%.<br>Atopics among all smokers 22% (not detailed for smoking asthmatics)<br><br>Smoking habits, median (range):<br>10 (0-30) pack-years.<br>This is information for all smoking subjects (n = 112) | n = 186, current smokers 80, males 40%<br><br>Demographics for all subjects with non-specific asthma symptoms (not stated separately for the 80 smoking control subjects):<br>Age (yr), mean (SD): 32.7 (SD 8.9)<br><br>Control group in this study incorporated subjects with non-specific asthma symptoms but no objective signs of asthma in the examination.<br><br>$FEV_1$, % pred, mean (SD): 97.0 (SD 10.8)<br><br>Steroid medication:<br>ICS use 0.5% (no information on dose)<br><br><br><br>Atopy/allergy:<br>rhinitis 46.2%.<br>Atopics among all never-smokers 46% (not detailed for control never-smokers)<br><br>Smoking habits, median (range):<br>10 (0-30) pack-years.<br>This is information for all smoking subjects (n = 112) | Flow rate: 50 ml/s<br>Device: NIOX Mino, Aerocrine, Sweden | Smoking asthmatics had significantly higher FeNO levels than smoking subjects with non-specific asthma symptoms (p < 0.001).<br><br>FeNO values:<br>In smoking asthmatics, geometrical mean (95% CI): 16.7 (12.8, 21.7) ppb.<br><br>In smoking non-asthmatics, geometrical mean (95% CI): 10.4 (9.1, 11.9) ppb.<br><br>In smoking asthmatics, the percentual increase of FeNO was 60% compared to smoking non-asthmatics.<br>After adjusting for sex, the corresponding figure was about 50% (figure taken from a graph).<br><br>FeNO values were log-transformed for analyses (distribution of FeNO levels was skewed to the right and normality assumption was used in analyses).<br><br>Percentual increase of FeNO in asthmatics vs. non-asthmatics was obtained from linear regression models before and after adjusting for sex, height, age, FeV1 (% pred), use of ICSs and pollen season (known determinants of FeNO). | Funging and conflict of interest:<br>Funded by an unrestricted grant from AstraZeneca. A. Malinovschi was funded by Bror Hjerpsteds Stiftelse and Uppsala University Hospital.<br>None of the authors have any conflcts of interest to declare.<br><br><br>Notes:<br>We used never-smokers as control group (including not ex-smokers) because there was no information on e.g. when the ex-smokers had quit smoking.<br><br>Asthma diagnosis of all included asthmatics perhaps do not fulfil the asthma diagnosis criteria of this review. We, however, assumed that ≥ 80% of asthmatics fulfilled the criteria. |

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| **Matsunaga 2011**, Japan<br><br>Cross-sectional study desing related to baseline FeNO values<br><br>This study evaluated FeNO measurement as a diagnostic test for asthma.<br><br>Subjects were recruited from the outpatient clinic of medical university. | n = 142, current smokers 52, non-smokers 90, males 49%<br><br>Demographics for all asthmatic subjects (not separately for smokers and non-smokers):<br><br>Age (yr), mean (±SE): 41.5 ± 1.4<br><br>Severity of asthma: no information<br><br>$FEV_1$, % pred, mean (±SE): 89.1 ± 1.2<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy:<br>rhinitis 66%.<br><br>Smoking habits: no detailed information, only mentioned that subjects were excluded from the study if they had a smoking history with more than 20 pack-years. | n = 224, current smokers 52, non-smokers 172, males 44%<br><br>Demographics for all healthy subjects (not separately for smokers and non-smokers):<br><br>Age (yr), mean (±SE): 39.4 ± 0.9<br><br><br>$FEV_1$, % pred, mean (±SE): 98.7± 0.6<br><br><br>Atopy/allergy:<br>rhinitis 45%.<br><br>Smoking habits: no detailed information, only mentioned that subjects were excluded from the study if they had a smoking history with more than 20 pack-years. | Flow rate: 50 mL/s<br>Device: NIOX MINO (Aerocrine, Sweden) | FeNO levels were significantly higher in steroid-naive asthmatic smokers than in healthy smokers ($p < 0.01$).<br><br>The following FeNO value results are taken from a graph, from which the standard deviations are impossible to assess.<br><br>FeNO values:<br>In smoking asthmatics, mean: 36.3 ppb<br><br>In smoking healthy subjects, mean: 13.4. ppb<br><br>Normality assumptions were achieved by log-transformation of FeNO values, back-transformed values were estimated.<br><br>Comparisons between groups were made by Mann-Whitney U test. Log-transformed FeNO values were used in the analyses. | Funging and conflict of interest:<br>Supported by grant from the Ministry of Health, Labour and Welfare, Japan.<br><br><br>Notes:<br>This study was only included in this comparison smoking asthmatics vs. smoking healthy subjects because there was no information available on proportion of ex-smokers quitting smoking between 3 to 6 months before the study started (ex-smokers quitting smoking during 3 to 6 months before the study started were included in the non-smoker group, issue which did not fulfil the inclusion criteria of this review). |
| **Rouhos 2010**, Finland<br><br>Cross-sectional study design.<br><br>Subjects were army conscripts referred to the military hospital because of respiratory symptoms and diagnosed with current symptomatic asthma. | n = 46 (atopic 30), males 96%<br><br>Age (yr), mean (range): 19.5 (18-23)<br><br>Severity of asthma: symptomatic asthma, mild<br><br>$FEV_1$, % pred, mean (range):<br>atopics: 86 (60-119),<br>nonatopics: 92 (75-108)<br><br>Steroid medication: steroid-naive<br><br>Atopy/allergy: 65% (based on skin prick test)<br><br>Pack-years, mean (range):<br>atopics: 2.5 (0.2-9.0),<br>nonatopics: 4.2 (0.6-11) | n = 10, males 100%<br><br>Age (yr), mean (range): 19 (19-20)<br><br><br><br>$FEV_1$, % pred, mean (range):<br>102 (94-113)<br><br><br><br>Atopy/allergy: no atopy<br><br><br>Pack-years, mean (range):<br>3.7 (1.2 - 9.0) | Flow rate: 90-120 mL/s<br>Device: chemiluminescence analyser, Sievers 270B, USA | FeNO levels were significantly higher in steroid-naive asthmatic smokers than in healthy smokers ($p = 0.001$).<br><br>FeNO values:<br>In smoking asthmatics, median (25-75% interquartile range):<br>all smoking asthmatics:<br>13.5 (9.1 - 30.1) (not detailed for atopics/non-atopics). (Detailed result obtained from author.)<br><br>In smoking healthy subjects, median (25-75% interquartile range):<br>all smoking asthmatics:<br>7.3 (4.6 - 10.7) (not detailed for atopics/non-atopics). (Detailed result obtained from author.)<br><br><br>Because FeNO was not normally distributed, $log_{10}$-transformed FeNO values were used in the analyses.<br>Comparison between groups were performed by one-way variance analysis, and p-values were corrected for multiple comparisons by the least significant difference method. | Funging and conflict of interest:<br>Funded by non-profit foundations and supported by Helsinki University Hospital |
| **Shimoda 2016**, Japan<br><br>Cross-sectional study design related to baseline FeNO values.<br><br>The aim of the study was to evaluate influence of cigarette smoking on airway inflammation and inhaled corticosteroid treatment in patients with asthma.<br><br>Patients with newly diagnosed asthma who visited hospital were recruited. | n = 52, males 63%<br><br>Age (yr), mean (± SD): 42 ± 13<br><br>Severity of asthma: mild to moderate<br><br>$FEV_1$, % pred, mean (± SD): 81 ± 20<br><br>Steroid medication: steroid-naive<br><br>Sputum eosinophil, %: 21 ± 18<br><br>Atopy/allergy: atopics 75%<br><br>Smoking habits, mean (± SD),<br>28 ± 14 pack-years | n = 14, males 50%<br><br>Age (yr), mean (± SD): 35 ± 6<br><br><br>$FEV_1$, % pred, mean (± SD): 97 ± 17<br><br><br>Sputum eosinophil, %: 0.4 ± 0.5<br><br>Atopy/allergy: atopics 0%<br><br>Smoking habits, mean (± SD),<br>25 ± 12 pack-years | Flow rate: 50 mL/s<br>Device: chemiluminescence analyser NOA 280, Sievers Instruments Inc. Boulder, CO | FeNO levels were significantly higher in steroid-naive asthmatic smokers than in healthy smokers ($p < 0.001$).<br><br>FeNO values:<br>In smoking asthmatics, mean (±SD):<br>77 ± 55<br><br>In smoking healthy subjects, mean (±SD):<br>14 ± 4<br><br>t-test was used to compare baseline FeNO levels between groups. | Funding and conflicts of interest:<br>No external funding sources.<br>The authors have no conflicts of interest |

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| **Verleden 1999**, Belgium<br><br>Cross-sectional study design.<br><br>Subjects were recruited from outpatients at university outpatient asthma clinic. | n = 13, males 62%<br><br>Age (yr), mean (SD): 33.9 (14.5)<br><br>Severity of asthma: mild<br><br>$FEV_1$, % pred, mean (SD): 113.3 (17.2)<br><br>Steroid medication: steroid-naive<br><br>Atopy/allergy: no information<br><br>Smoking habits, mean (SD), daily cigarette consumption:<br>17 ± 6 during 14 ± 6 years | n = 16, males 44%<br><br>Age (yr), mean (SD): 34.6 (9.1)<br><br><br>$FEV_1$, % pred, mean (SD): 106.5 (SD 9.7)<br><br><br><br>Atopy/allergy: no information<br><br>Smoking habits, mean (SD), daily cigarette consumption:<br>18 ± 7 during 16 ± 9 years | Flow rate: 200 mL/s<br>Device: chemiluminescence analyser Ecophysics CLD700 AL Med, Switzerland | FeNO levels were significantly higher in steroid-naive asthmatic smokers than in healthy smokers ($p < 0.05$).<br><br>FeNO values:<br>In smoking asthmatics, mean (±SD):<br>12.7 ± 5.1<br><br>In smoking healthy subjects, Mean (±SD):<br>7.4 ± 1.8<br><br>The Kruskal-Wallis nonparametric analysis of variance test and Dunn's multiple comparisons test were used to assess significant differences in FeNO levels among the groups. Log-aritmic transformation was done for FeNO values before analysis. | Funging and conflict of interest:<br>No information provided |

| | | Description and results of studies | | | |
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| Study design | Characteristics of subjects | | FeNO measurement | Results | Notes |
| Study reference, country, study design | Characteristics of smoking ICS treated asthmatics | Characteristics of smoking healthy subjects | FeNO measurement: flow rate, device | Results | Funding and notes |
| **Hillas 2011**, Greece<br><br>Cross-sectional study desing.<br><br>No information how and where the patients were recruited. | n = 40, males 45%<br><br>Age (yr), mean (± SD): 48.7 ± 11.2<br><br>Severity of asthma: mild to moderate, well-controlled.<br><br>FEV$_1$, % pred, mean (± SD): 87.8 ± 11.6<br><br>Steroid medication: all asthmatics were receiving ICS.<br>ICS, n/mean budesonide equivalent dose: 10/400<br>ICS/LABA, n/mean budesonide equivalent dose: 30/640<br><br>Sputum eosinophil count, %, median (IQR): 2.0 (1.0-5.0)<br><br>Atopy/allergy: atopy 60%<br><br>Smoking habits, mean (± SD): 45.1 ± 20.2 | n = 30, males 37%<br><br>Age (yr), mean (± SD): 47.2 ± 8.9<br><br><br><br>FEV$_1$, % pred, mean (± SD): 99.4 ± 10.6<br><br><br><br><br><br><br><br>Atopy/allergy: no atopics<br><br>Smoking habits, mean (± SD: 39.8 ± 16.1 pack-years | Flow rate: 50 ml/s<br>Device: NIOX MINO (Aerocrine, Sweden) | FeNO levels in smoking steroid-treated asthmatic group did not differ from those in smoking healthy subjects (p > 0.05).<br><br>FeNO values:<br>In smoking asthmatics, median (interquartile range): 12 (10-16) ppb<br><br>In smoking healthy subjects, median (interquartile range): 12 (8-14) ppb<br><br>Distributions of FeNO levels were skewed and data are presented as medians (interquartile range).<br>Statistical comparison of FeNO levels between smoking asthmatic and non-smoking asthmatic groups was performed using Mann-Whitney U-test. | Funging and conflict of interest:<br>No information provided |
| **Horvath 2004, UK**<br><br>Cross-sectional study desing.<br><br>No information how and where the patients were recruited. | n = 10, males 50%<br><br>Age (yr), mean (± SEM): 31 ± 3<br><br>Severity of asthma: no information<br><br>FEV$_1$, % pred, mean (± SEM): 89 ± 5<br><br>Steroid medication: all the asthmatics in this comparison were receiving ICS.<br>BDP (beclomethasone diproprionate equivalents per day, ug (range): 968 ± 99<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: no information<br><br>Smoking habits, mean (± SEM): 11 ± 3 pack-years | n = 20, males 45%<br><br>Age (yr), mean (± SEM): 33 ± 2<br><br><br><br>FEV$_1$, % pred, mean (± SEM): 98 ± 1<br><br><br><br><br><br><br><br>Atopy/allergy: no information<br><br>Smoking habits, mean (± SEM): 12 ± 2 pack-years | Flow rate: 83-100 ml/s, Device: chemiluminescence analyser, R2000, Logan Research, UK | There was no significant difference between steroid-treated asthmatic smokers and healthy smokers (p > 0.05).<br><br>FeNO values:<br>In smoking asthmatics, median (range): 5.4 (1.7 - 12.0)<br><br>In smoking healthy subjects, median (range): 3.2 (2.0 - 7.2)<br><br>Data were analysed non-parametrically. Comparisons between groups were performed by Dunn's test. | Funging and conflict of interest:<br>Supported by a joint grant of the British Countil and and Hungarian OMFB, a NATO Scienic Fellowship Programme, the Hungarian National Scientific Foundation and the Hungarian Ministry of Health Care. |

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| **McSharry 2005,** UK<br><br>Cross-sectional study desing related to baseline FeNO values,<br><br>The aim of the study was to identify and model short-term and long-term influences of cigarette smoking on FeNO.<br><br>Subjects were recruited from respiratory outpatient clinics and hospital staff. | n = 17, males 53%<br><br>Age (yr), mean (95% CI): 40.0 (35.5-52.0)<br><br>Severity of asthma: no information<br><br>FEV$_1$, % pred, mean (95% CI): 75.4 (70.3-89.1)<br><br>Steroid medication: 81% of all asthmatics used ICS, no detailed information for smoking asthmatics.<br>ICS (ug/d): 800 (100-900); no oral steroids<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: 76% atopics<br><br>Smoking habits:<br>20.0 (17.5-27.5) cigarettes per day,<br>27.5 (16.0-35.7) pack-years | n = 20, males 45%<br><br>Age (yr), mean (95% CI): 42.5 (36.7-46.0)<br><br><br>FEV$_1$, % pred, mean (95% CI): 95.0 (86.0-105.8)<br><br><br><br><br><br><br><br><br>Atopy/allergy: 45% atopics<br><br>Smoking habits:<br>20.0 (20.0-25.0) cigarettes per day,<br>26.0 (14.5-32.6) pack-years | Flow rate: 250 mL/s<br>Device: chemilumiscence analyser (model LR2000, UK) | There was no difference between steroid-treated asthmatic smokers and healthy smokers.<br><br>FeNO values:<br>In smoking asthmatics, mean (95% CI):<br>4.03 (2.96-6.47) ppb<br><br>In smoking healthy subjects, mean (95% CI):<br>4.71 (3.85-6.82) ppb<br><br>Difference between groups were tested by using the rank-sum test. | Funging and conflict of interest:<br>One of the authors was supported by a research grant from the British National Flying Club. |
| **Papaioannou 2010,** Greece<br><br>Cross-sectional study desing.<br><br>No information how and where the patients were recruited. | n = 10, males 40%<br><br>Age (yr), mean (SD): 34.3 (10.2)<br><br>Severity of asthma: moderate<br><br>FEV$_1$, % pred, mean (SD): 88.9 (12.4)<br><br>All asthmatics were treated with inhaled corticosteroids but detailed information on the medication not stated.<br><br>Sputum eosinophil count: no information<br><br>Atopy/allergy: no information<br><br>Smoking habits, mean (SD):<br>14.8 (8.2) pack-years | n = 10, males 40%<br><br>Age (yr), mean (SD): 35.4 (14.0)<br><br><br>FEV$_1$, % pred, mean (SD): 104.4 (9.4)<br><br><br><br><br><br><br><br><br>Smoking habits, mean (SD):<br>16.2 (21.9) pack-years | Flow rate: 50 ml/s<br>Device: NIOX MINO (Aerocrine, Sweden) | There was no significant difference between steroid-treated asthmatic smokers and healthy smokers (p > 0.05).<br><br>FeNO values:<br>In smoking asthmatics, mean (SD):<br>6.10 (3.10)<br><br>In smoking healthy subjects, mean (SD):<br>9.40 (4.97)<br><br>Independent student t-test was used to compare baseline FeNO levels between groups. | Funging and conflict of interest:<br>No information on funding but no conflict of interest |

**Online Supplement 8**

**Research question 4A    Proportional difference in FeNO values at study level**

| Study | FeNO in smoking asthmatics | $n_1$ | FeNO in smoking healthy subjects | $n_2$ | $n_{1+2}$ | Proportional difference | 95% confidence interval of proportional difference |
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| Horvath 2004 | 7,7 | 22 | 3,2 | 20 | 42 | 58.4 | 42.9 to 73.8 |
| Malinovski 2012 | 16,7 | 32 | 10,4 | 80 | 96 | 37.7 | 28.1 to 46.9 |
| Matsunaga 2011 | 36,3 | 52 | 13,4 | 52 | 104 | 63.1 | 53.8 to 72.1 |
| Rouhos 2010 | 13,5 | 46 | 7,3 | 10 | 56 | 45.9 | 32.1 to 58.9 |
| Shimoda 2016 | 77 | 52 | 14 | 14 | 66 | 81.8 | 72.3 to 90.9 |
| Verleden 1999 | 12,7 | 13 | 7,4 | 16 | 29 | 41.7 | 24.1 to 58.6 |

**Research question 5    Descriptions and results of studies**

| Study reference, country, inclusion process of subjects to the study | FeNO measurement and reference standard | Characteristics of study subjects | Methods | Results | Funding, Notes and Assessment of risk of bias |
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| **Kostikas 2008**, Greece<br><br>Inclusion process of the participants to the study: Subjects giving at least one positive answer to a screening questionnaire of respiratory symptoms related to asthma were considered eligible for the study.<br><br>Subjects were excluded if they had any of the following: (1) previous diagnosis of asthma or rhinitis treated with antiinflammatory medication, (2) history of respiratory tract infection within the past 6 weeks, and (3) recent smoking cessation (< 2 months prior to the study).<br><br>Subjects (students) were recruited from University of Thessally and the Technological Education Institute of Larissa. | FeNO measurement: Device: NIOX MINO (Aerocrine, Sweden) Flow rate: 50 mL/s<br><br>Reference standard: Asthma was defined based on a history of relevant lower respiratory tract symptoms, along with one of the following: significant bronchodilator reversibility, positive methacholine bronchial challenge test, or clinical and spirometric response to a 4-week trial of inhaled corticosteroids, prescribed after FeNO measurements.<br><br>Diagnosis of asthma was established after FeNO measurements, based on evaluation by a resporatory physician blinded to FeNO measurements under prespecified criteria (GINA 2006). | In total 219 subjects (smokers 76), from whom diagnosed asthmatics 63 (smokers 23), subjects with allergic rhinitis 57 (smokers 19), subjects with non-specific respiratory symptoms 29 (smokers 11), and healthy controls 70 (smokers 23) (without no respiratory symptoms).<br><br>No demographics combined for the whole study group at baseline.<br><br>Demographics for diagnosed asthmatics (n=63):<br><br>Males: 54%<br><br>Age (yr), mean (± SD): $21.6 \pm 2.7$<br><br>$FEV_1$, % pred, mean (± SD): $84 \pm 12$ (differing from the corresponding figures among subjects with allergic rhinitis ($107 \pm 11$), subjects with nonspecific symptoms ($104 \pm 12$), and control subjects ($109 \pm 13$).<br><br>Severity of asthma:  majority having mild-to-moderate asthma<br><br>Steroid medication: steroid-naive<br><br>Atopy/allergy: no information<br><br>Smoking habits of smokers: no detailed information | ROC (receiver operating characteristic) curves were created to assess the performance of FeNO to identify asthmatic subjects, and AUCs (areas under the ROC curves) with 95% confidence intervals  were calculated.<br><br>Sensitivies and specifities with 95% confidence intervals were stated for the optimal FeNO cut-off point (point which provided the best combination of sensitivity and specificity for the diagnosis of asthma in the whole population). Diagnostic performance was evaluated separately taking into account factors like allergic rhinitis and smoking.<br><br>Diagnostic performance of FeNO was evaluated also using different cut-off points in the range of 10 to 30 ppb. | FeNO values per diagnosis, presented as median (interquartile range):<br><br>In smoking asthmatics: 16.0 (9.0 - 20.5) ppb In smoking subjects with allergic rhinitis: 16.0 (9.0 - 20.5) ppb In smoking subjects with nonspecific symptoms: 9.0 (7.5 - 14.0) ppb. In smoking control subjects 9.0 (7.0 - 13.0) ppb.<br><br>AUC of ROC: smoking asthmatics vs. other smoking subjects): AUC = 0.648 (95% CI 0.53 to 0.76) for the optimum cut-point of FeNO of 19 ppb (which provided the best combination of sensitivity and specificity for the diagnosis of asthma in the whole population). Sensitivity, and specificity for the FeNO cut-off value of 19 ppb: Sensitivity: 29.2% (95% CI  12.7 to 51.1) Specificity: 86.0% (95% CI  73.3 to 94.2)<br><br>Sensitivity, and specificity for the FeNO cut-off value of > 25 ppb among smokers: Sensitivity: 6.2% (95% CI 3.7 to 21.2) Specificity: 92.0% (95% CI 80.7 to 97.7)<br><br>Reporting conclusion: FeNO was not a good marker for the diagnosis of asthma in smokers. However, FeNO values > 25 ppb were characterized by specificity > 90% also in smokers. | Funging and conflict of interests: No aknowledgements to business companies.<br><br>Notes: Subjects were recruited from population by using symptom questionnaires instead of recruiting subjects seeking medical advice. Control group consisted partly of symptom free healthy subjects, i.e. the focus was not purely in ability of FeNO to differentiate symptomatic asthmatics from symptomatic non-asthmatics.<br><br>Assessment of risk of bias: **Unclear risk of bias.** 1. Analyses were based on use of optimal cut-off value of FeNO derived from the whole data. When analysing performance of FeNO at different cut-off points, the sample size of smokers (76) was rather small to adequate analysis. 2. No information on the proportion of atopics among diagnosed asthmatics. |

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| **Malinovschi 2012**, Denmark<br><br><u>Inclusion process of the participants to the study:</u><br>A random population sample of 10,400 subjects was drawn from the civil registration list. Subjects were mailed with a validated self-administered asthma and rhinitis screening questionnaire. Of the 47% that responded, 686 subjects recorded two or more respiratory symptoms and were examined.<br><br>After examinations the final number of the included subjects was 282. | <u>FeNO measurement:</u><br>Device: NIOX MINO (Aerocrine, Sweden)<br>Flow rate: 50 mL/s<br><br><u>Reference standard:</u><br>Asthma diagnosis was based on presence of symptoms of asthma (i.e. shortness of breath, chest tightness, cough, exercise induced dyspnoea, night-time awakenings, or respiratory symptoms induced by allergen contact) in combination with at least one of the following:<br>1) Airway hyper-responsiveness (AHR) to inhaled methacholine < 8.0 μmol.<br>2) At least 250 ml increase in $FEV_1$ after bronchodilator.<br>3) Daily use of systemic steroid, inhaled steroid, or inhaled beta$_2$-agonist.<br>4) Asthma symptoms during but not outside the pollen season, eventually supported by allergic rhinitis, although no objective signs of asthma outside season were found. | In total 282 subjects, from whom diagnosed asthmatics 96 (32 smokers), and subjects with non-specific asthma symptoms 186 (80 smokers).<br><br>No demographics combined for the whole study group at baseline.<br><br><u>Demographics for diagnosed asthmatics (n=96):</u><br>Males: 41%<br><br>Age (yr), mean (SD): 32.7 (8.7)<br><br>$FEV_1$, % pred, mean (SD): 94.4 (SD 15.2)<br><br>Severity of asthma: no information<br><br>Steroid medication:<br>25% of asthmatics used ICS (no information on dose)<br><br>Atopy/allergy:<br>Rhinitis in 65.6% and IgE-sensitisation in 54.4 % of asthmatics<br>(differing from the corresponding figures among subjects with non-specific symptoms (46.2 % and 22.0 %).<br>Atopics among all smokers 22% (not detailed for smoking asthmatics).<br><br>Smoking habits, median (range): 10 (0-30) pack-years. This is information for all smoking subjects (n = 112) | The optimal cut-off for the ROC-curves was defined as corresponding to the maximum value of Youden's index (sensitivity + specificity-1). In addition to optimal cut-off of FeNO, results are presented to cut-offs for 90% sensitivity and 90% specificity.<br><br>Diagnostic performance of FeNO to identify asthma was evaluated taking into account smoking status. | <u>FeNO values per diagnosis, presented as geometrical mean (95% CI):</u><br><br>In smoking asthmatics: 16.7 (12.8, 21.7) ppb<br>In smoking subjects with non-specific symptoms: 10.4 (9.1, 11.9) ppb<br><br><u>AUC of ROC in the population of smokers:</u><br>AUC = 0.70 (95% CI 0.59 to 0.82) for the optimum cut-point of FeNO of 17 ppb.<br>Sensitivity: 56.3%; Specificity: 82.5%; PPV: 57%; NPV: 82%.<br><br><u>For 90% sensitivity, the cut-off point was 7 ppb:</u><br>At this cut-off point: Specificity: 15.1%; PPV: 32%; NPV: 89%.<br><br><u>For 90% specificity, the cut-off point was 22 ppb:</u><br>At this cut-off point: Sensitivity: 37.5%; PPV: 61%; NPV: 78%.<br><br>(Among never-smokers AUC = 0.72 (95% CI 0.62 to 0.82) for the optimum cut-point of FeNO of 15 ppb, sensitivity of 77.8%, specificity of 63.5%).<br><br><u>Reporting conclusion:</u><br>FeNO could differentiate asthmatic subjects from non-asthmatic subjects with asthma-like symptoms equally well in both never- and current smokers within a random population sample. | <u>Funging and conflict of interests:</u><br>Funded by an unrestricted grant from AstraZeneca. A. Malinovschi was funded by Bror Hjerpsteds Stiftelse and Uppsala University Hospital.<br>None of the authors have any conflcts of interest to declare.<br><br><u>Notes:</u><br>Subjects were recruited from population by using symptom questionnaires instead of recruiting subjects seeking medical advice.<br><br><u>Assessment of risk of bias:</u><br>**Unclear risk of bias.**<br>1. Analyses were based on use of optimal cutoff values of FeNO derived from data. The sample size of smokers (112) was perhaps too small to adequate analysis (Leeflang 2008).<br>2. Asthma diagnosis of all included subjects perhaps do not fulfil the asthma diagnosis criteria of this review (items 3 and 4 under reference standand). |

| Matsunaga 2011, Japan | FeNO measurement: | In total 366 subjects | ROC analysis | FeNO values | Funding and conflict of interests |
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**Matsunaga 2011**, Japan

<u>Inclusion process of the participants to the study:</u>
Adult subjects with and without respiratory symptoms were recruited from the outpatient clinic of medical university.

<u>Subjects were excluded</u> if they had a history of lung diseases except for asthma, had a smoking history with more than 20 pack-years, had had an airway infection or were taking any form of corticosteroids, β2 - agonists, leukotriene modifiers, and H1- antagonists in the 4 weeks preceding the study. Recent quitters were also excluded to stratify the study subjects as either nonsmokers or current smokers (within the past 8 weeks).

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<u>FeNO measurement:</u>
Device: NIOX MINO (Aerocrine, Sweden)
Flow rate: 50 mL/s

<u>Reference standard:</u>
Asthma was diagnosed on the basis of the presence of significant airway reversibility and/or airway hyperresponsiveness during clinical follow up period of 6 months after FeNO measurements.

The diagnosis of asthma was established after the FeNO measurements.

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In total 366 subjects: from whom diagnosed asthmatics 142 (after the follow up period of 6 months) (52/142 smokers; 37%), and control subjects 224 (subjects without respiratory symptoms, no history of asthma and having normal spirometric parameters were included in the control group) (52/224 smokers; 23%).

No demographics combined for the whole study group at baseline.

<u>Demographics for diagnosed asthmatics (n=142):</u>

Males 49%.

Age (yr), mean (±SE): 41.5 ± 1.4

FEV$_1$, % pred, mean (±SE): 89.1 ± 1.2 (differing from the corresponding figures among control subjects (98.7 ± 0.6; p < 0.01).

Severity of asthma: no information

Steroid medication: steroid-naive

Atopy/allergy:
Allergic rhinitis in 66%.

Smoking habits: no detailed information, only mentioned that subjects were excluded from the study if they had a smoking history with more than 20 pack-years.

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Receiver operating characteristic (ROC) curves were plotted in order to estimate the cut-off values for asthma diagnosis. An optimal cut-off value was obtained from the highest sum obtained from adding sensitivity and specificity.

Subjects were divided into four subgroups according to allergic rhinitis and smoking status and the cut-off values for each subgroup were estimated.
Subgroups were: non-smoking subjects without rhinitis, smoking subjects without rhinitis, non-smoking subjects with rhinitis, smoking subjects with rhinitis.
All these subgroups included subjects with asthma and control subjects without respiratory symptoms.

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<u>FeNO values:</u>
In smoking asthmatics, mean: 36.3 ppb.
In smoking healthy subjects, mean: 13.4. ppb.
These FeNO values are taken from a graph, from which the standard deviations are impossible to assess.

<u>AUC of ROC among smoking subjects without rhinitis:</u>
(28 control subjects and 21 patients with asthma):
AUC = 0.935 for the optimum cut-point of FeNO of 18 ppb.
<u>Sensitivity and specificity for the optimal FeNO cut-off value of 18 ppb:</u>
Sensitivity: 100%; Specificity: 87%.

<u>AUC of ROC among smoking subjects with rhinitis:</u>
(24 control subjects and 31 patients with asthma):
AUC = 0.865 for the optimum cut-point of FeNO of 22 ppb.
<u>Sensitivity and specificity for the optimal FeNO cut-off value of 22 ppb:</u>
Sensitivity: 80%; Specificity: 86%.

<u>Reporting conclusion:</u>
The optimal cut-off values of FeNO to discriminate between the subjects with asthma and those without asthma ranged from 18 to 28 ppb depending on rhinitis and smoking status.

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Notes: Control group consisted of symptom free healthy subejcts, i.e. the focus was in ability of FeNO to differentiate symptomatic asthmatics from symptom free healthy subjects.

Assessment of risk of bias:
**Unclear risk of bias.**
1. Subgroup analyses were based on use of optimal cutoff values of FeNO derived from data. The sample sizes of smokers: 49 (smoking subjects without rhinitis) and 55 (smoking subjects with rhinitis) were rather small to adequate analysis of estimating the cut-off values for discrimation of asthmatics and controls (Leeflang 2008).
2. It remains unclear how patients and controls were recruited among hospital patients.