

Fractional exhaled nitric oxide as a determinant for the clinical course of asthma: a systematic review

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ABSTRACT

Background: Precision medicine means linking the right patient to the right management strategy including best possible pharmacological therapy, considering the individual variability of the disease characteristics, type of inflammation, genes, environment, and lifestyle. For heterogenous diseases such as asthma, reliable biomarkers are needed to facilitate the best possible disease control and reduce the risk of side effects. The present review examines fractional exhaled nitric oxide (FeNO) as a guide for the management strategy of asthma and predictor of its clinical course.

Method: The literature included was identified by searching the PubMed database using specific key words and MeSH terms. Studies were not excluded based on their design alone. The search resulted in 212 hits, of which 35 articles were included in this review.

Results: Several studies support a potential role for high FeNO levels as a prognostic biomarker for accelerated lung function decline in adults with newly diagnosed asthma. Furthermore, studies report an association between high FeNO levels and excess decline in FEV₁ in adults with long-standing moderate to severe asthma despite optimised therapy, whereas the findings for patients with less severe disease are conflicting. Applying a FeNO-based management algorithm reduces the exacerbation rate in adults with asthma. Similar observations are seen in children, though based on fewer studies. The available studies provide evidence that the level of FeNO may be useful as a predictor of subsequent loss of asthma control in adults, though the evidence is somewhat conflicting in children and young adults.

Conclusion: The present review provides evidence of the prognostic value of FeNO as a surrogate biomarker for type 2 inflammation in the airways. FeNO is likely to emerge as an important biomarker in monitoring and tailoring modern asthma treatment, either alone or in combination with other biomarkers.

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Introduction

Asthma is a chronic heterogenous inflammatory disease of the airways affecting more than 300 million individuals globally [1] and 5–10% of the population in Europe [2]. Most asthma patients can be successfully treated with standard therapy. However, some individuals have difficult-to-treat or severe asthma, which remains partially controlled or uncontrolled, even with intensive treatment. Asthma is characterised by the type of predominant inflammatory cells that are increased (eosinophils, neutrophils, or both), or indeed a paucity of such cells [3,4]. Asthma immunological phenotypes are complex with differing clinical and inflammatory characteristics [5,6]. Thus, there is an increasing need for biomarkers with predictive and prognostic value for

the progression of asthma, and their association with available treatments [7]. One phenotype of severe asthma is related to type 2 inflammation, found in approximately half the people with severe asthma [8]. Type 2 inflammation is characterised by the release of cytokines such as interleukin (IL)-4, IL-5 and IL-13 from cells of both the innate and adaptive immune systems acting on respiratory epithelium and other stromal cells, often on recognition of allergens [3,9]. Type 2 inflammation is also characterised by the presence of eosinophils [10]. Biomarkers that reflect the pathophysiological mechanisms involved in type 2 inflammation-driven asthma include fractional exhaled nitric oxide (FeNO), serum IgE, serum periostin, and blood and sputum eosinophils [3].

The production of nitric oxide (NO) in bronchial epithelial cells helps to regulate pulmonary blood flow, ciliary activity, mucus secretion and mucosal inflammation [11]. In asthma, levels of NO in exhaled breath (FeNO) are increased due to the activation of inducible NO synthase (iNOS) by inflammatory cytokines in the airway epithelial cells, suggesting a role for NO in asthma pathogenesis [12,13]. NO can be measured in exhaled air as FeNO (Figure 1) that is used to support the diagnosis and management of asthma. The level of FeNO appears to increase in proportion to the severity of bronchial wall inflammation [14].

FeNO is measured from a single breath exhalation, usually using online techniques [15]. The standard measurement is at an exhalation flow-rate of 50 mL/s. Exhalation against positive pressure excludes the possibility of contamination from nasal NO, which can affect the FeNO value. Other factors influencing FeNO values include the exhalation flow rate and the individual's age, sex, height, smoking

habits, allergies and anti-inflammatory medications used [16–18].

FeNO is probably the most widely used biomarker in clinical practice today for the assessment of airway inflammation [7,16]. FeNO can facilitate the identification of patients with type 2 inflammation [19], and has the potential to identify those patients who will respond to anti-inflammatory treatment, particularly inhaled corticosteroids (ICS). Identifying patients at the greatest risk of future exacerbations and the ability to control inflammation and thereby prevent exacerbations and decline in lung function is key in achieving good asthma control [19].

In this review, we aim to address the following clinical question: Does the level of FeNO have prognostic value in the clinical course of asthma, in terms of (a) decline in lung function, (b) risk of exacerbations and (c) loss of control in adults and children with mild, moderate or severe asthma?

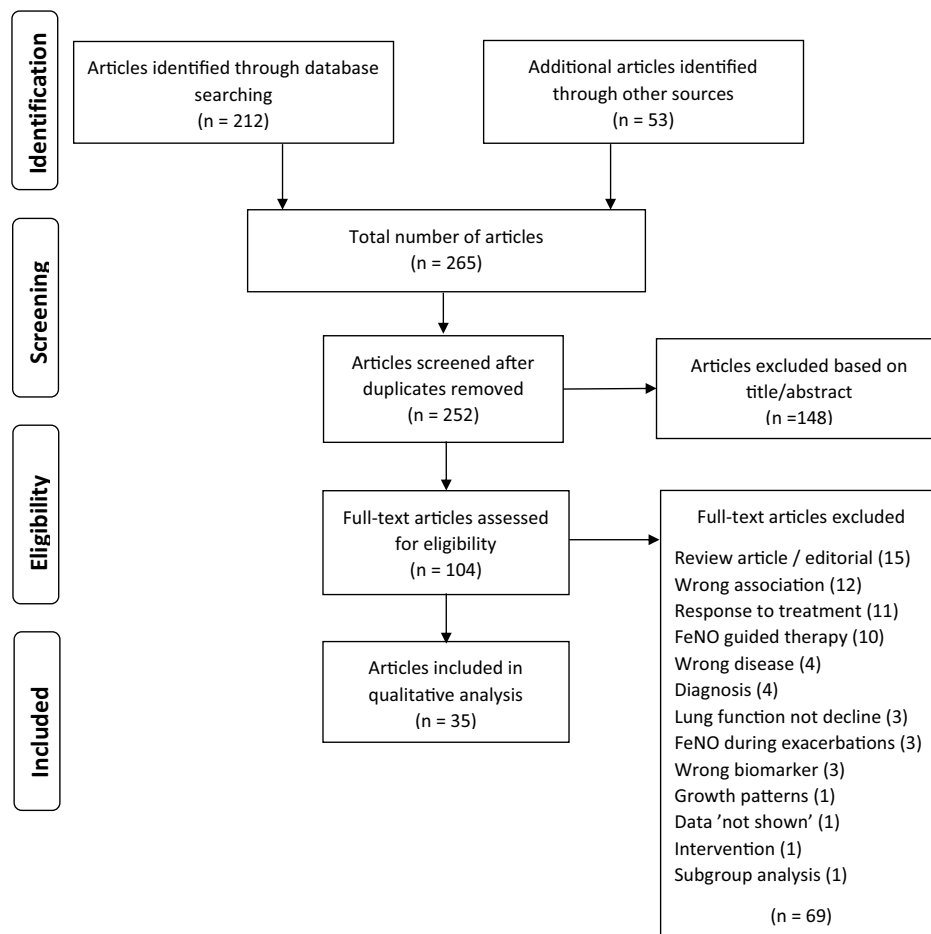


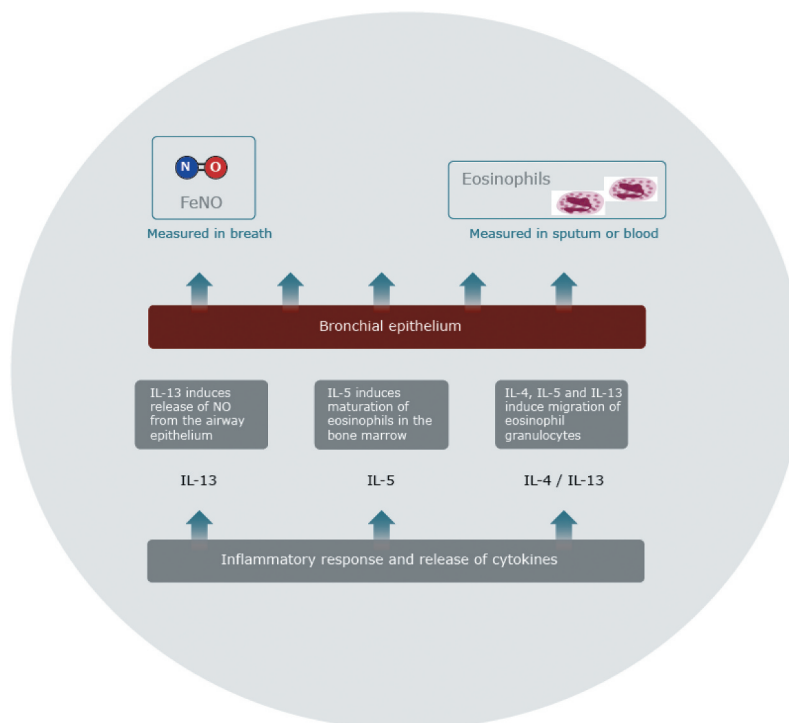
Figure 1. Type 2 inflammation. FeNO, fractional exhaled nitric oxide; IL, interleukin; NO, nitric oxide.

Methods

This is a review of the current literature on the prognostic and predictive value of FeNO in the clinical course of asthma in adults and children. A systematic literature search of the PubMed database was done on 2 April 2020 using the following key words or MeSH terms: 'FeNO [Title/Abstract] OR fractionated exhaled nitric oxide [Title/Abstract] OR (FeNO OR fractionated exhaled nitric oxide [MeSH Terms]) AND prognosis [Title/Abstract] OR prognosis [MeSH Terms] AND asthma [Title/Abstract] OR asthma [MeSH Terms] OR airway inflammation [Title/Abstract] OR airway inflammation [MeSH Terms] OR lung function decline [Title/Abstract] OR exacerbation [Title/Abstract] OR chronic airway disease [Title/Abstract] OR chronic airway disease [MeSH Terms]'. We did not exclude any studies based on the study design, i.e. we included randomised, controlled trials as well as prospective and retrospective observational studies.

The search resulted in 212 articles (Figure 2). A further 53 articles were found by other means (known by the authors or mentioned in the retrieved articles). Of the 265 total articles, 252 were screened after duplicates were removed. Of these, 148 were excluded based on the abstract due to the following

reasons: the article did not answer the clinical question, concerned a different disease than asthma (for example, allergic rhinitis or chronic obstructive pulmonary disease), was related to asthma diagnosis, was a methodology article or was not in the English language. Of the remaining 104 articles, 69 were excluded based on read of the full text for the following reasons: the article was a review article or editorial and contained no new information (15), concerned prediction of asthma, asthma phenotype or blood eosinophilia (12), concerned response to a type of treatment rather than prognosis for long-term asthma outcome (11), evaluated FeNO guided treatment versus conventional or symptom-based therapy (10), concerned a different disease or condition than asthma per se (bronchial wall thickening, lung cancer, nasal polyps, irreversible airway obstruction) (4), was related to asthma diagnosis (4), assessed lung function not decline in lung function (3), assessed FeNO before and after an exacerbation (3), focussed on a different biomarker(s) (3), examined the association between childhood growth patterns and FeNO (1), the FeNO data were 'not shown' (1), concerned an intervention (1), or was a subgroup analysis of an original article (1). The remaining 35 articles were included in this review.



FeNO, fractional exhaled nitric oxide; IL, interleukin; NO, nitric oxide.

Figure 2. Flow of literature selection.

Results

There is increasing evidence potentially supporting FeNO as a prognostic biomarker for disease progression in asthma. Results from studies investigating the association between FeNO and long-term asthma clinical course and outcome, that is, decline in lung function, exacerbations, and loss of disease control are presented in the following.

Decline in lung function

In the general adult asthma population, factors known to contribute to accelerated lung function decline include smoking [20], recurrent exacerbations [21] and low forced expiratory volume in 1 s (FEV₁) [22]. Another important contributor to excess lung function decline may be the severity and persistence of airway inflammation [23–25], possibly by facilitating airway remodelling [26]. Accelerated decline in FEV₁ has also been shown to be associated with severe asthma exacerbations [21] and the presence of CD8-positive T-cells in the bronchial epithelium [27]. In a recent study, adults with severe asthma had a decrease of 50 mL in FEV₁ for each severe exacerbation [28]. However, another study showed no association between exacerbations and accelerated decline in lung function [29].

High FeNO levels have been shown to be associated with an excess decline in lung function both in long-standing disease and in adults with newly-diagnosed asthma (Table 1). Adult-onset asthma accounts for approximately half of new asthma diagnoses [30,31]. A prospective 5-year study in 200 individuals with adult-onset asthma measured several potential predictors for a decline in lung function at baseline and subsequent visits [29]. The study found that higher FeNO levels and lower body mass index (BMI) were independently associated with lung function decline, as defined by a decline of >54.2 mL/year in post-bronchodilator FEV₁, and all patients with a combination of FeNO ≥57 ppb ($p = 0.015$) and BMI ≤23.05 kg/m² ($p = 0.002$) showed an accelerated decline in FEV₁. The results suggest that, for new-onset asthma, individuals of normal weight and with relatively severe airway inflammation are at highest risk of subsequent lung function decline.

Other studies have reported an association between high FeNO levels and accelerated lung function decline in patients with long-term asthma. In a prospective 3-year study in 140 patients with controlled long-standing (~10 years) asthma, a persistently high

FeNO level of ≥40 ppb was independently associated with an accelerated decline in FEV₁ ($p < 0.05$), whereas there was no association between lung function decline and age, BMI, blood eosinophil numbers, Asthma Control Test (ACT) score or airflow limitation [32]. High levels of FeNO were also associated with a decrease in bronchodilator response.

Similarly, in a 5-year prospective follow-up of 136 adults with difficult-to-treat long-standing (18.5 years) asthma, high baseline FeNO levels predicted accelerated lung function decline, with no influence of other potential predicting factors, apart from baseline FEV₁ [33]. Individuals with a FeNO level ≥20 ppb, despite high doses of ICS with or without oral corticosteroids (OCS), had an increased risk of accelerated lung function decline (defined as an annual decline of ≥25 mL/year) over 5 years compared to those with FeNO <20 ppb (relative risk [RR] 1.9 [95% confidence interval (CI), 1.1; 2.6]). In individuals with both FeNO ≥20 ppb and FEV₁ within normal levels (≥80% of predicted values), the risk of accelerated lung function decline was even greater (RR 3.1 [95% CI, 1.7; 3.4]).

In contrast, in a 5-year longitudinal observational study of 30 patients with well-controlled, stable asthma (duration ~10 years), only the severity of airway hyperresponsiveness and not FeNO was associated with a decline in FEV₁ over 5 years (Table 1) [34]. In this study, FeNO was measured every 6 months during the first 2 years of the study. Furthermore, in a follow-up study of 212 patients from two previous cohorts in a broad asthma population (cohort 1 enrolled patients with intermittent mild to moderate asthma; cohort 2 enrolled those with stable moderate to severe asthma), no association between baseline FeNO level and lung function decline was noted [35]. Instead an association between lung function decline and baseline blood eosinophils was observed ($p < 0.001$). The authors suggested that the lack of an association between FeNO and decline in lung function could have been due to the population assessed, consisting predominantly of patients with mild to moderate asthma of whom only 74% were on ICS therapy.

In general, the above studies tend to support the use of FeNO as a prognostic biomarker for accelerated lung function decline in adults with newly diagnosed asthma, uncontrolled asthma or difficult-to-treat asthma, though the evidence for an association in patients with mild to moderate disease is less compelling. FeNO-guided therapy is currently recommended for individuals with frequent exacerbations only [36]. However, the guidelines are based on studies that have not taken the rate of lung

Table 1. Summary of studies investigating the association between FeNO and lung function decline in adults.

Reference	Type of study	Number of patients	Age and FeNO at baseline	Findings
Coumou et al 2018 [29]	Prospective 5-year study of initial ADONIS study	200 patients with adult-onset asthma; BL data based on 141 with repeated lung function tests. 84% were on ICS.	Mean (SD) age 49 (15) years. Mean FeNO 22 (range 13–47) ppb.	Association of FeNO Multivariate mixed effect model showed that FeNO ≥ 57 ppb ($p = 0.015$) and BMI ≤ 23.05 kg/m ² ($p = 0.002$) were independently associated with lung function decline (post-bronchodilator FEV ₁ of >54.2 mL/year). No effect of blood or sputum eosinophils, sex, or presence of nasal polyps.
Matsunaga et al 2016 [32]	Prospective 3-year study	140 patients with controlled asthma on ICS with/without LABA, leukotriene modifier or theophylline; BL data based on 122 analysed.	Mean (SD) age 42.4 (13.4) years in low FeNO group (20.2 (7.4) ppb), 47.0 (14.6) years in high FeNO group (56.5 (11.2) ppb).	Multivariate logistic regression showed that FeNO ≥ 40 ppb was independently associated with lung function decline (FEV ₁ of >40 mL/year), OR 4.42 (95% CI, 1.34; 14.66); $p < 0.05$. No effect of age, obesity, eosinophilia, asthma control or airflow limitation.
Van Veen et al 2008 [33]	Prospective 5–6 year study	136 patients with difficult-to-treat asthma on high doses of ICS with long-acting bronchodilators; BL data based on 98 reassessed after 5–6 years.	Mean (SD) age 45.1 (13.1) years. Mean FeNO 9.6 (range 2–123.8) ppb.	Logistic regression showed that FeNO ≥ 20 ppb was associated with a RR for lung function decline (FEV ₁ of ≥ 25 mL/year) of 1.9 (95% CI, 1.1; 2.6) vs. FeNO < 20 ppb. No effect of other factors, apart from baseline FEV ₁ . For FeNO ≥ 20 ppb and FEV ₁ within normal levels, the RR was 3.1 (95% CI, 1.7; 3.4).
Ohkura et al 2009 [34]	Longitudinal 5-year observational study	30 patients with well-controlled, stable asthma on ICS.	Mean (SEM) age 54.2 (2.6). Mean FeNO at baseline not stated.	No association of FeNO Single regression analysis showed that the PC20 ($r = 0.58$, $p = 0.001$) but not FeNO ($r = -0.16$, $p = 0.39$) was associated with a decline in lung function (deltaFEV ₁ /year).
Semprini et al 2018 [35]	Prospective observational 5-year study	212 patients from 2 previous studies with mild, moderate or severe asthma. 74% were on ICS, and 43% were on ICS/LABA.	Mean (SD) age 50.5 (13.2) years. Median FeNO 22.5 (range 13.6–44.4) ppb.	Linear regression model showed no association between FeNO and lung function decline (FEV ₁ over time), whereas a baseline peripheral blood eosinophil count of $<0.21 \times 10^9$ was associated with lung function decline ($p < 0.001$).

ADONIS, Adult-Onset Asthma and Inflammatory Subphenotypes; BL, baseline; BMI, body mass index; CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; ICS, inhaled corticosteroids; LABA, long-acting beta agonist; PC20, provocative dose causing a 20% fall in FEV₁; OR, odds ratio; ppb, parts per billion; RR, relative risk; SD, standard deviation; SEM, standard error of the mean.

function decline into account [29]. It might be useful to include FeNO measurements in the clinical assessment of asthma patients, in order to identify those who are at risk of poor asthma outcome and who might be eligible for novel asthma treatments or personalised treatment strategies [33,37].

Data are not sufficient to determine the prognostic value of FeNO for the course of lung function in children [38]. However, a 5-year follow-up study in 193 Chinese children with asthma (mean age 9.7 years) showed that a high baseline FeNO value was associated with decreased lung function development [39]. In a longitudinal cohort study of 42 infants and toddlers with wheezing (mean age 15.6 months), high baseline FeNO values were associated with a decline in lung function by 3 years of age and also bronchodilator response [40].

Risk of exacerbations

Exacerbations in adults

Several studies support a role for FeNO as a prognostic biomarker for the risk of future exacerbations (Table 2). In a 3-year observational study of 105 adults with severe long-standing (~20 years) asthma on high-dose

ICS with or without OCS, the patients were split into 3 groups: those who did not experience exacerbations, those with ≥ 2 exacerbations over each of the 3 years, and those who did not fulfil either of these criteria. A multivariate analysis was conducted with several type 2-related biomarkers measured annually over 3 years, including blood/sputum eosinophil levels, total IgE, serum periostin and FeNO, as well as exacerbation history [41]. Of the biomarkers assessed, only FeNO was associated with exacerbation status ($p = 0.013$), and this association remained significant even when considering past exacerbation status, which is usually the strongest predictor of future exacerbations. In a 1-year study of 93 patients with severe asthma and 76 with mild to moderate asthma (asthma duration ~20 years), biomarkers including FeNO were measured at baseline and at subsequent visits [42]. Patients in the severe group had 104 exacerbations and those in the mild to moderate group had 18 exacerbations. FeNO >45 ppb (odds ratio 4.32, $p = 0.047$) and a history of smoking (odds ratio 2.90, $p = 0.025$) were associated with an increased risk of 2 or more exacerbations per year (Table 2).

Similar associations between FeNO and asthma exacerbations were observed in other observational studies of adults with severe, moderate or mild asthma

(Table 2). A prospective 22-week study in 1800 adults with severe asthma prescribed high-dose ICS plus a second controller demonstrated that FeNO (measured regularly over the 22 weeks) was a valuable prognostic biomarker for exacerbations, together with FEV₁ [43]. Similarly, a strong correlation with asthma exacerbations was shown for FeNO measured longitudinally in a real-life retrospective study (duration not stated) of 115 adults with severe asthma on high-dose ICS plus OCS [44]. The correlation between FeNO and exacerbations ($r = 0.42$, $p = 0.0008$) was stronger than for peripheral blood eosinophils or perostin. Similar results were shown in a prospective study of 44 non-smoking adults with stable mild to severe asthma who had received treatment for at least 3 years [45]. Independent of baseline FEV₁, baseline FeNO values of ≥ 28 ppb were associated with an increased relative risk for exacerbations of 3.4 (95% CI, 1.3; 9.1; Mantel-Haenszel, $p = 0.007$). Combining baseline FeNO of ≥ 28 ppb and FEV₁ of $\leq 76\%$ identified 13 stable adults with asthma with 85% probability for future exacerbations, whereas 9 adults with FeNO < 28 ppb and FEV₁ $> 76\%$ had a 0% probability of exacerbations. In the follow-up study of 212 patients from two previous cohorts in a broad asthma population (mild, moderate or severe) in which no association between baseline FeNO and lung function decline was noted (mentioned above), a significant association between baseline log FeNO and time to severe exacerbation was observed (hazard ratio 0.65 [95% CI, 0.52; 0.81] per 0.693 log FeNO increase), $p < 0.001$ [35].

In contrast to the above studies, a 12-month study of different prediction models (history of previous exacerbations; history plus spirometry; and history plus spirometry plus FeNO, assessed at baseline and every 3 months) in 611 adults diagnosed with asthma (82% on ICS with or without a LABA or short-acting beta agonist) found that the model of exacerbation history plus spirometry identified those adults prone to severe exacerbations and the additional prognostic value of FeNO was modest (Table 2) [46]. Furthermore, in a recent subgroup analysis of a 1-year open-label randomised, controlled trial in 675 patients with mild asthma, elevated FeNO (measured at baseline, week 12 and week 52) showed no value as a prognostic biomarker for exacerbation rate [47].

In general, the above studies tend to support the clinical use of FeNO for the prediction of future exacerbation risk in adults with moderate to severe asthma, in addition to the individual's exacerbation history, though the evidence in those with mild asthma is less conclusive. Monitoring of airway inflammation as reflected by FeNO may enable early detection of an

exacerbation in a subclinical form and the potential to adjust anti-inflammatory treatment to reduce the risk of future exacerbations.

Exacerbations in children and young adults

There is also evidence to support the use of FeNO as a prognostic biomarker for exacerbations in children. In a prospective 1-year cohort study of 70 Thai children and young adults (median age 12.6 years (range 7.2 to 19.8 years)) and atopic asthma, median baseline FeNO levels were significantly higher in individuals having an exacerbation compared to those who did not (35.6 vs. 16.5 ppb; $p = 0.012$), and previous 12-month exacerbations were also predictive [48]. In a single-centre longitudinal study of 45 infants and toddlers with wheezing (mean age 15.7 months), high baseline FeNO values had a prognostic value for future exacerbations and were superior to both bronchodilator responsiveness and a positive asthma predictive index [40].

Overall, the monitoring of FeNO levels in children holds some promise as a prognostic biomarker for exacerbations in children. Assessing asthma status at an early age using an objective test such as FeNO may facilitate more targeted use of asthma therapies, prevent under- and over-treatment with ICS, and reduce preventable emergency department visits and hospitalisation for asthma exacerbations.

Loss of asthma control

Asthma control is usually evaluated by symptoms and lung function with the aim to reduce future risk [49,50].

Loss of control in adults

Several studies have shown that increased FeNO levels are associated with a deterioration in asthma control in adults (Table 3). In a prospective 2-year study in 90 adults with moderate or severe asthma who were on ICS and without clinical symptoms for ≥ 6 months, patients had their ICS dose halved while remaining on other asthma medication (theophylline, LABA, leukotriene receptor antagonists, and/or anticholinergic agents) [51]. Asthma control was defined by a reduction in ICS dose with no increase in symptoms. In a multivariate logistic regression model, baseline FeNO level was predictive of successfully maintaining control over the second year of the study ($p = 0.028$), and 39 of 50 patients continued to maintain asthma control for ≥ 2 years after ICS reduction. In

Table 2. Summary of studies investigating the association between FeNO and exacerbation risk in adults.

	Type of study	Number of patients Age and FeNO at baseline	Association of FeNO
Kimura et al 2018 [41]	Prospective observational 3-year study	105 patients with severe asthma on high doses of ICS or OCS. Mean (SD) age 58.5 (12.1) years. Mean (SD) FeNO 30.2 (0.36) ppb.	Multivariate analysis showed that FeNO concentration was an independent factor associated with exacerbation status ($p = 0.013$), with a tendency for an effect for blood eosinophils ($p = 0.063$) and no effect for other biomarkers (blood neutrophils, serum IgE, sputum eosinophils or periostin).
Kupczyk et al 2014 [42]	Prospective 1-year subgroup study	169 patients: 93 with severe asthma (high dose ICS with/without OCS), 76 with mild to moderate asthma (low to medium ICS dose). Mean (SD) age 46.5 (1.04) years. Mean (SD) FeNO 43.5 (3.9) ppb.	In a multivariate logistic regression model, FeNO >45 ppb was associated with an increased risk of frequent exacerbations (≥ 2 events/year), OR 4.32 (95% CI, 1.02; 18.31, $p = 0.047$) after adjusting for age, sex, smoking, asthma severity and atopy. Similar results were observed in the severe group alone, OR 5.98 (1.28; 27.99, $p = 0.023$).
Abdelrahman et al 2019 [43]	Prospective longitudinal 22-week study	1800 patients with severe asthma on high doses of ICS plus a second control drug. 70% were aged 18–27 years. Mean baseline FeNO not stated.	In a logistic deterioration model, FeNO and reduced FEV ₁ were found to be prognostic biomarkers for asthma exacerbations. Also, increased eosinophils was associated with a reduced risk of exacerbations in females, but not males.
Mansur et al 2018 [44]	Retrospective real-life study	115 patients with severe asthma on high-dose ICS and OCS. Mean age 45.4 (range 18–70) years. Mean FeNO 34.8 (range 5–169.0) ppb.	In a rank correlation coefficient analysis, FeNO was correlated with asthma exacerbations to a greater extent ($r = 0.42$, $p = 0.0008$) than blood eosinophils or periostin, with no added benefit of combining the 3 biomarkers.
Gelb et al 2006 [45]	Prospective 18-month study	44 non-smoking patients with stable mild to severe asthma on ICS and LABA for ≥ 3 years. Mean (SD) age 51 (21) years. Mean baseline FeNO not stated.	Independent of baseline FEV ₁ , FeNO ≥ 28 ppb increased the RR for exacerbation by 3.4 (95% CI, 1.3; 9.1, Mantel-Haenszel, $p = 0.007$). Independent of baseline FeNO, FEV ₁ $\leq 76\%$ predicted increased RR by 1.7 (1.0; 2.7, $p = 0.02$). Combined baseline FeNO ≥ 28 ppb and FEV ₁ $\leq 76\%$ of predicted identified 13 stable asthmatics with 85% probability for future exacerbations.
Semprini et al 2018 [35]	Prospective observational 5-year study	212 patients from 2 previous studies with mild, moderate or severe asthma. 74% were on ICS, and 43% were on ICS/LABA. Mean (SD) age 50.5 (13.2) years. Median FeNO 22.5 (range 13.6–44.4) ppb.	A multivariate model showed an association between baseline log FeNO and time to severe exacerbation, with a hazard ratio of 0.65 (95% CI, 0.52; 0.81) per 0.693 log FeNO increase ($p < 0.001$).
Loymans et al 2016 [46]	Primary care pragmatic 12-month study	611 patients with diagnosed asthma. 82% were on ICS and 49% were on LABA. Mean (SD) age 39.4 (9.1) years. Mean FeNO 26 (range 5–297) ppb.	Modest effect of FeNO Multivariate model showed that addition of FeNO to predictors based on medical history had modest additional effect to predict patients at risk of severe exacerbations.
Pavord et al 2020 [47]	Subgroup analysis of a randomised controlled 1-year trial	675 patients with mild asthma. Baseline characteristics by baseline biomarker group. Mean age range: 31.2 to 38.1 years. Mean FeNO range: 12.9 to 107.3 ppb.	No effect of FeNO In a logistic regression model, there was no significant interaction between FeNO level (<20, 20–50 or >50 ppb) and asthma exacerbations in any treatment group: as-needed salbutamol (SABA), low-dose budesonide (ICS) plus as-needed salbutamol, or low-dose budesonide-formoterol (ICS with fast-onset LABA).

CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; ICS, inhaled corticosteroids; IQR, interquartile range; LABA, long-acting beta agonist; OCS, oral corticosteroids; OR, odds ratio; ppb, parts per billion; RR, relative risk; SABA, short-acting beta agonists; SD, standard deviation.

a prospective study of 78 patients with mild to moderate asthma on ICS for ≥ 6 months, ICS therapy was stopped and patients followed weekly for up to 6 weeks or until loss of control occurred [52]. Sixty patients (77.9%) experienced loss of control. Different FeNO measurements, in terms of varying cut-off values, were all associated with a positive predictive value of between 80 and 90% for predicting loss of asthma control. In a prospective observational study of 250 patients with stable asthma on ICS with or without LABA and/or other therapies, poor asthma control was defined as an ACT score <20, or FEV₁ < 80% or peak expiratory flow variability <80% [53]. After 12-weeks, 229 patients who maintained high or low FeNO

were selected and the study found that a FeNO level >39.5 ppb gave 67% sensitivity and 76% specificity for identifying the patients with poorly controlled asthma.

The prognostic value of FeNO was assessed in a regular clinic setting in a prospective longitudinal 3-month study of 341 patients with mild, moderate or severe asthma [54]. The patients were either newly diagnosed and ICS naïve or had chronic asthma and were on ICS with or without LABA and/or other therapies. FeNO was measured once or on several visits for each patient. In the whole population, FeNO >45 ppb was associated with asthma that was not well controlled in terms of Asthma Control Questionnaire (ACQ) >0.75 (negative predictive value 88%),

$p < 0.001$. FeNO values >45 ppb were less effective for predicting asthma control as ICS dose increased: negative predictive values of 92%, 76% and 85% with ICS naïve, medium-dose or high-dose ICS, respectively. The study also suggested that sequential FeNO measurements may be beneficial for predicting improvement in asthma control over time.

In a prospective 6-month study of 90 patients with severe or non-severe asthma, asthma control was evaluated according to the ACQ (ACQ ≥ 1.5 denoted uncontrolled asthma) [55]. FeNO (the number of measurements not stated) was significantly increased in uncontrolled as compared to controlled asthmatics using both a chemiluminescent device (the 'gold standard' recommended technique) and a portable electrochemical device for the measurement. FeNO levels were able to predict maintenance of control in well-controlled asthma patients, and with cut-off values of 31 and 40 ppb, the negative predictive values were 95 and 97% for the two devices. In this study, the authors were able to predict loss of control from a single FeNO measurement using the electrochemical device, which could be helpful in clinical practice.

A retrospective 1-year study of 71 adults with mild newly-diagnosed asthma not on ICS found that full asthma control based on the ACT score was associated with lower FeNO levels ($p < 0.01$), shorter duration until first clinic visit ($p < 0.01$), lower sputum eosinophil count ($p < 0.05$) and lower initial ICS dose ($p < 0.05$) (Table 3) [56]. In a multivariate logistic regression analysis comparing findings in patients with controlled versus uncontrolled asthma, inclusion of FeNO levels at the first visit in the model significantly improved the prediction of asthma control (odds ratio [OR] 0.9459 (95% CI, 0.9024; 0.9915); $p = 0.021$). A cohort study of 170 patients with mild to moderate (72%) or severe (28%) asthma on ICS and/or other treatments found that sputum IL-13 was superior to both FeNO and sputum eosinophils with respect to predicting well-controlled asthma at 6 months [57]. Nevertheless, FeNO levels differed significantly between patients with well-controlled asthma (ACT ≥ 20) and those with uncontrolled asthma: median (interquartile range [IQR]) 21 (14–28) vs. 45 (19–67), $p < 0.001$. A baseline FeNO value <43 ppb was a modest predictor of well-controlled asthma for the whole population (negative predictive value 50%) compared to an IL-13 value <156 pg/mL (negative predictive value 76%), indicating the superiority of IL-13, though the methodology for this biomarker can be semi-invasive and time-consuming.

In contrast to the above studies, a cross-sectional outpatient study of 81 women, 41 of whom were obese,

with mild (84%), moderate (12%) or severe (4%) stable asthma and on at least one asthma controller for at least 6 months, found that only high BMI was associated with poor asthma control (in terms of ACT score <20) [58]. In the obese group, no significant difference was observed with respect to median FeNO levels between those with uncontrolled asthma ($n = 24$; 21.0 [range 15–51] ppb FeNO) and those with controlled asthma ($n = 17$; 20.5 [11–52] ppb), $p = 0.799$. Similar results were observed for the nonobese group, with median FeNO 26.0 (10.0–297.0) ppb for those with uncontrolled asthma ($n = 15$) and 19.5 (6.0–171.0) ppb for those with controlled asthma ($n = 25$), $p = 0.194$.

Loss of control in children and young adults

In children and young adults, some evidence supporting the prognostic value of FeNO to predict loss of asthma control is available; however, the evidence is somewhat conflicting. A study of the association between the loss of asthma control and longitudinal FeNO measurements in 178 children and adolescents (aged between 8 and 16 years) with atopic asthma found that only a high mean FeNO value and the frequency of FeNO values above 21 ppb (participants had FeNO measured at least 10 times) were independently associated with a loss of control ($p = 0.001$ and 0.021, respectively) [59]. When different cut-off values of each were investigated, the best combination of sensitivity and specificity for predicting a future loss of asthma control was observed with a mean FeNO value >47 ppb (70% sensitivity and 96% specificity) as well as FeNO levels >21 ppb achieved at a frequency of $>41\%$ (72% sensitivity and 88% specificity). Also, in a study of 201 children aged 8–16 years with atopic intermittent or mild persistent asthma, pulmonary function tests including FeNO and bronchodilator response were performed ≥ 10 times over 2 years and loss of control was assessed by the ACQ (ACQ ≥ 1.5 denoted uncontrolled asthma) after an additional year [60]. The study showed that the risk for loss of control in the FeNO >35 ppb group was 2.58 times that in the FeNO ≤ 35 ppb group (95% CI 1.60; 4.15; $p < 0.001$), and the prognostic value of both FeNO >35 ppb and bronchodilator response $\geq 12\%$ was greater than including each measurement separately (hazard ratio 7.08; 95% CI 2.57; 19.49; $p < 0.001$).

With regard to older children and young adults, a large population-based cohort study of FeNO levels and blood eosinophil counts in 406 children and young adults aged 10–35 years (~80% of patients had atopic asthma) showed that a combination of increased

Table 3. Summary of studies investigating the association between FeNO and loss of asthma control in adults.

Loss of control		Association of FeNO	
Tsurikisawa et al 2012 [51]	Prospective 2-year study	90 patients with moderate or severe asthma on ICS and without clinical symptoms for ≥ 6 months. Mean (SD) age: 49.1 (14.6) years in exacerbation-free group (n = 50), 50.9 (15.9) years in exacerbation group (n = 40). Mean (SD) FeNO: 25.6 (12.0) ppb in exacerbation-free group, 43.4 (27.3) ppb in exacerbation group.	Multivariate logistic regression showed a rank order of predictors of successful ICS reduction while retaining asthma control (exacerbations): acetylcholine PC20 ($p < 0.01$); length of time with no clinical symptoms before ICS reduction ($p < 0.01$); FeNO ($p = 0.028$); and FEV ₁ (% predicted) ($p = 0.03$).
Jones et al 2001 [52]	Prospective 6-week study	78 patients with mild to moderate asthma on ICS for ≥ 6 months. Mean age 42.9 (range 18–74) years. Mean FeNO 9.38 (95% CI, 2.72; 32.35) ppb.	By regression analysis, single measurements and different FeNO cut-off values (>10 ppb, >15 ppb, or an increase of >60% above baseline) had positive predictive values ranging from 80 to 90% for predicting loss of control.
Matsunaga et al 2012 [53]	Prospective observational 12-week study	250 patients with stable asthma on ICS with/without LABA and/or other therapies. Mean (SD) age 46.6 (14.7) years. Mean (SD) FeNO 34.6 (22.0) ppb.	Multivariate logistic regression analysis showed that a FeNO level >39.5 ppb gave 67% sensitivity and 76% specificity for identifying the patients with poorly controlled asthma.
Michils et al 2008 [54]	Prospective longitudinal 3-month study	341 patients with mild, moderate or severe asthma, ICS naïve, or on ICS with/without LABA and/or other therapies. Mean (SD) age 41 (16) years. Geometric mean (range) FeNO 32.9 (13.8–78.1) ppb.	In the whole population, FeNO >45 ppb was not associated with well-controlled asthma (negative predictive value 88%), $p < 0.001$. The use of FeNO for predicting asthma control was less effective as ICS dose increased.
Ozier et al 2011 [55]	Prospective 6-month study	90 patients with severe or non-severe asthma, 72% on ICS, 50% on LABA and 6.7% on OCS. Mean (SD) age: 38.5 (2.1) years in controlled group (n = 62), 44.8 (3.2) years in uncontrolled group (n = 28). Mean baseline FeNO not stated.	A correlation analysis was used to determine that a low baseline FeNO <31 ppb was a good predictor of not losing good asthma control (negative predictive value $\geq 95\%$), but high FeNO >31 ppb was only a modest predictor of loss of asthma control (positive predictive value 67%).
Yamashita et al 2016 [56]	Retrospective 1-year study	71 patients with mild asthma newly diagnosed and not on ICS. 37 patients continued clinic visits for 1 year. Mean (SD) age 50.9 (15.6) years. Mean (SD) FeNO 42.2 (31.9) ppb.	In a multivariate logistic regression, FeNO levels at the first visit were identified as a possible predictor of asthma control (OR 0.9459 (95% CI, 0.9024; 0.9915); $p = 0.021$).
Tsilogianni et al 2016 [57]	Ongoing cohort 6-month study	170 patients with mild to moderate (72%) or severe (28%) asthma on ICS and/or LABA, LTRA, OCS or omalizumab. Median (range) age 53 (42–62) years. Median (range) FeNO 22 (16–35) ppb.	FeNO levels differed significantly between patients with well-controlled asthma and those with uncontrolled asthma, median (IQR) 21 (14–28) vs. 45 (19–67), $p < 0.001$. Receiver operating characteristic analysis for the whole study population showed that FeNO <43 ppb was only a modest predictor of well-controlled asthma (negative predictive value 50%).
Kilic et al [58]	Cross-sectional outpatient study	81 women (41 obese) with mild (84%), moderate (12%) or severe (4%) stable asthma on ≥ 1 asthma therapy for ≥ 6 months. Mean (SD) age: 53.9 (10.2) in obese group, 47.3 (15.7) in nonobese group. Median (range) FeNO: 21.0 (11.0–52.0) in obese group, 22.5 (6.0–297.0) in nonobese group.	No effect of FeNO By regression analysis, high BMI was found to be the only significant factor that contributed to poor asthma control, with no effect of FeNO ($p = 0.799$ in the obese group, $p = 0.194$ in the nonobese group).

BMI, body mass index; CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; ICS, inhaled corticosteroids; IQR, interquartile range; LABA, long-acting beta agonist; LTRA, leukotriene receptor antagonists; n, number of patients; PC20, provocative dose causing a 20% fall in FEV₁; OR, odds ratio; ppb, parts per billion; SD, standard deviation.

baseline FeNO levels (≥ 20 –25 ppb) and blood eosinophil counts (≥ 300 cells/ μ L) was able to predict uncontrolled asthma (ACT score <20) at 3 months better than either biomarker alone [7]. A prospective non-interventional study in 304 children and adults aged 12–56 years with persistent asthma found that high baseline FeNO levels predicted future uncontrolled asthma at 1 year [61]. In a study of 30 children aged 7–17 years with mild (n = 8), moderate (n = 17) or severe (n = 5) asthma, baseline FeNO was significantly correlated with asthma control ($r = -0.51$, $p = 0.001$), which was based on overall evaluation of symptoms,

FEV₁ measurements, and the frequency of beta-agonist use [62].

Other studies have shown that elevated FeNO may predict asthma relapse after cessation of ICS treatment. In a study of 40 children with asymptomatic asthma (mean age 12.2 years) who had discontinued use of ICS, FeNO was measured at baseline and at 2, 4, 12 and 24 weeks after discontinuation of ICS use. Geometric mean FeNO values in children who were about to relapse were higher than in those who did not relapse: 35.3 ppb vs. 15.7 ppb at 2 weeks (ratio 2.3; 95% CI 1.2; 4.1; $p = 0.01$) and 40.8 ppb vs. 15.9 ppb at

4 weeks (ratio 2.6; 95% CI 1.3; 5.1; $p = 0.009$) [63]. Similarly, in a study of 40 children aged 6–17 years with stable asthma whose ICS dose was halved if clinically indicated, increased FeNO (OR 6.3; CI, 3.75; 10.58) and percentage of sputum eosinophils (OR 1.38; CI, 1.06; 1.81) were significant predictors of a failed reduction in terms of loss of asthma control [64]. In this study, biomarkers were assessed every 8 weeks.

In contrast to the above results, a prospective observational study of 28 children aged 6–14 years with controlled asthma found no association between baseline FeNO levels (≥ 49 ppb or < 49 ppb) and loss of control, in terms of recurrence of asthma attacks, at 6 months [65]. In fact, 5 patients in the low FeNO group experienced loss of control, as did only 1 in the high FeNO group. A 1-year observational study of 96 children aged 6–18 years with persistent asthma found no association between exhaled inflammatory markers measured every 2 months, including FeNO, and asthma control in children, as assessed by the ACQ [66]. Another study investigated associations between inflammatory biomarkers and asthma control in a prospective cohort study of 40 children and adolescents aged between 6 and 18 years with severe asthma (all on ICS and LABA) [67]. No significant differences in FeNO levels between the two groups (those who achieved control and those who did not) were observed, either at baseline or after a 3-month follow-up period. In a cross-sectional clinical study in 114 children aged ≥ 7 years (mean age 12.1 years) with atopic asthma, the median baseline FeNO level in the uncontrolled group of 39.15 (range 2.4–192.3) ppb was higher than in the partly controlled group (24.9 [2.2–85.7] ppb), and the controlled group (19.2 [5.1–108.9] ppb), though the differences were not statistically significant ($p = 0.238$) [68].

Overall, the above results indicate the potential benefit of using FeNO as a prognostic marker of asthma control and relapse in adults, although the evidence for a role of FeNO as a robust prognostic indicator of asthma control in children and young adults is less compelling. There is some indication that combining the FeNO assessment with another biomarker, such as blood eosinophil counts [7] or bronchodilator response [60], may help to fully obtain the status of a patient with asthma and to optimise the type and intensity of treatment. Also, sequential FeNO measurements may be better than a single assessment [54].

Conclusions

The present review has clearly demonstrated the prognostic value of measurement of FeNO as a surrogate biomarker for type 2 inflammation in the airways. High

FeNO levels measured at the time of asthma diagnosis may reflect ongoing airway inflammation and indicate a poorer prognosis, with long-term impairment of lung function and increased exacerbation risk and loss of control. In order to determine individual disease characteristics and optimise treatment, a combination of the FeNO assessment with other biomarkers could be useful.

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