Clinical utility of fractional exhaled nitric oxide in severe asthma management

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The optimisation of F_{eNO} testing methods in a variety of clinical settings, as a non-invasive, readily available, and affordable technology, could play an important role in advancing effective asthma control http://bit.ly/2FN6P3j

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ABSTRACT Asthma is a chronic inflammatory disease of the airways, affecting over 350 million people worldwide and placing a significant burden on healthcare providers and wider society. Approximately 5–10% of asthma patients are diagnosed with severe asthma and typically are associated with increased risk of hospitalisation from exacerbations, increased morbidity, mortality and higher asthma-associated healthcare costs. Nitric oxide (NO) is an important regulator of immune responses and is a product of inflammation in the airways that is over-produced in asthma. Fractional exhaled NO (F_{eNO}) is predominantly used as a predictor of response to inhaled corticosteroids (ICSs), to monitor adherence and as a diagnostic tool in ICS-naïve patients. In the UK, the National Institute for Health and Care Excellence (NICE) guidelines recommend the use of F_{eNO} for the initial diagnosis of patients with suspected asthma. In the USA, American Thoracic Society (ATS) guidelines recommend F_{eNO} as part of the initial diagnosis of asthma and for monitoring of airway inflammation. F_{eNO} has also been shown to be a predictive factor for asthma exacerbations, with higher levels being associated with a greater number of exacerbations. In addition, higher levels of F_{eNO} have been shown to be associated with a decline in lung function. F_{eNO} testing is a cost-effective procedure and has been shown to improve patient management when combined with standard assessment methods. Recent evidence suggests that F_{eNO} may also be useful as a surrogate biomarker for the assessment and management of severe asthma and to predict responsiveness to some biological therapies.

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Introduction

Asthma is the most common chronic respiratory disease worldwide, with over 350 million people affected [1], resulting in significant economic and societal burdens [2, 3]. Severe asthma, which is associated with increased morbidity, risk of hospitalisation from exacerbations and increased risk of mortality, affects approximately 5–10% of asthma patients [4–6], and it generates greater healthcare costs than mild or moderate asthma [7–9].

The international European Respiratory Society (ERS)/American Thoracic Society (ATS) guidelines define severe asthma as "asthma that requires treatment with high-dose inhaled corticosteroids (ICSs) plus a second controller and/or systemic corticosteroids to prevent it from becoming "uncontrolled" or that remains "uncontrolled" despite this therapy once the diagnosis of asthma has been confirmed and any comorbidities have been addressed [4]. Poor adherence to treatment, persistent triggers and comorbidities (*e.g.* chronic rhinosinusitis, gastro-oesophageal reflux disease and obesity) often contribute to severe asthma [10].

Although heterogeneous in nature, type 2 inflammation-driven asthma (type 2 asthma) is prevalent, affecting a high proportion of children and approximately 50% of adults with asthma overall and up to 80% of corticosteroid-naïve patients [11–14]. Indeed, these figures may underestimate the true prevalence of type 2 asthma due to the suppressive effects of corticosteroid treatment on type 2 biomarkers [11, 13], and there is some evidence suggesting that almost all patients with asthma will have an element of type 2 disease [14].

Type 2 cytokines such as interleukin (IL)-4, IL-5 and IL-13 play an important role in type 2 asthma. These cytokines are often produced in response to the recognition of allergens by the adaptive immune system but may also be activated by bacteria, viruses and allergens through the innate immune system [15]. Severe type 2 asthma is often associated with increased eosinophilic infiltration, raised serum immunoglobulin E (IgE) and raised fractional exhaled nitric oxide (F_{eNO}) levels [16]. The peripheral blood eosinophil (PBE) count is frequently used as a biomarker to predict the response to treatment in patients with type 2 asthma. In the UK, the Medical Research Council (MRC) is funding the Refractory Asthma Stratification Programme (RASP-UK), which will explore novel biomarker stratification strategies in severe asthma, with the aims of improving the clinical management of patients and accelerating the development of new therapies [17].

Nitric oxide and type 2 inflammation

There is increasing evidence that nitric oxide (NO) plays a key role in modulating type 2 inflammation and in regulating type 2 immune responses [18]. NO is derived endogenously from the amino acid L-arginine in a synthesis catalysed by three forms of the enzyme NO synthase (NOS); two constitutive NO synthases (cNOS) (generally expressed in platelets, neuronal, epithelial and endothelial cells) are involved in physiological regulation of airway function. An inducible form of the enzyme (iNOS) (predominantly expressed in macrophages, neutrophils, hepatocytes and epithelial, mesangial, endothelial and vascular smooth muscle cells) is typically produced in response to airway inflammation and in host defence against infection (figure 1) [19, 20]. iNOS expression can be induced by proinflammatory cytokines, such as tumour necrosis factor α, interferon γ and IL-1β [20]. In addition, it has been suggested that IL-13 upregulates the iNOS gene and protein expression in epithelial cells, leading to increased levels of F_{eNO} [21, 22].

NO is a ubiquitous messenger molecule, the activity of which depends on the level of oxidant stress and the rate of uptake by antioxidant molecules, in addition to the amount and activity of NOS [20]. NO regulates various biological functions, either at low concentrations as a signal in many physiological processes, including platelet reactivity, blood flow, non-adrenergic non-cholinergic neurotransmission and neurological memory, or at high concentrations as cytotoxic and cytostatic defensive mechanisms against tumours and pathogens [23]. NO is also a key inflammatory mediator in the respiratory tract and is produced by a number of cell types, including epithelial cells, mast cells, macrophages, neutrophils and vascular endothelial cells. Evidence highlights several roles for NO in the regulation of pulmonary function and in pulmonary disease, as an endogenous modulator of airway function and as a proinflammatory and immunomodulatory mediator [20].

In the context of asthma, this inflammatory response is deleterious, resulting in increased symptoms and airway obstruction [20, 24]. Increased levels of exhaled NO in asthma, originating mainly from the lower airway, are often associated with airway eosinophilic inflammation and increased expression of corticosteroid-sensitive iNOS. Levels of exhaled NO may also be associated with exacerbations and disease severity [20].

The measurement of exhaled NO has now been standardised for clinical use and, facilitated by the availability of mobile technology and remote monitoring, adoption in general practice has increased in

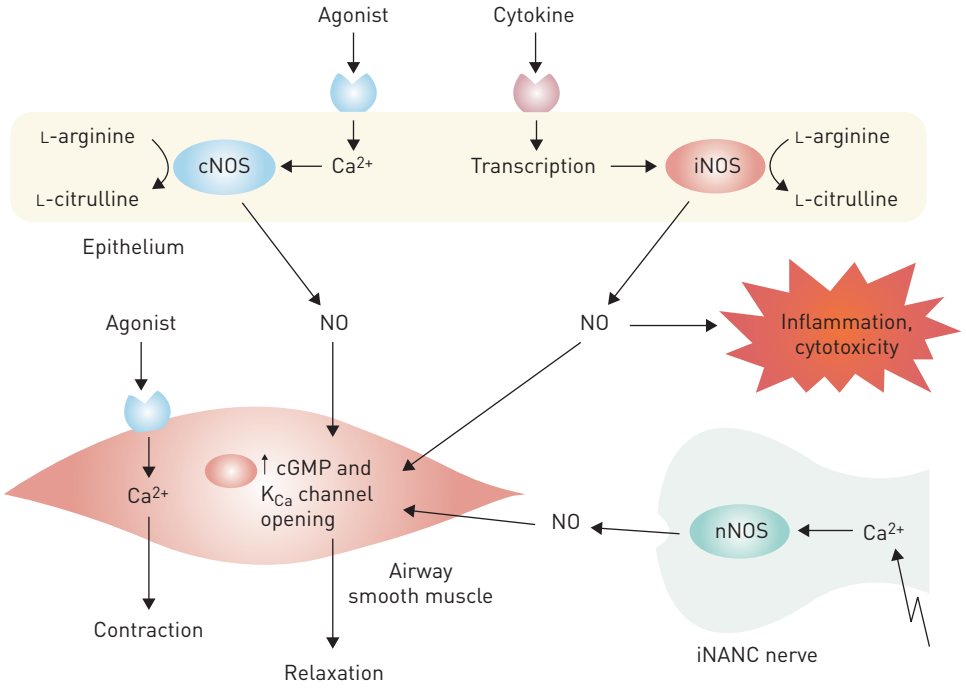

FIGURE 1 Nitric oxide metabolism in asthma pathophysiology. cGMP: cyclic guanosine monophosphate; cNOS: constitutive nitric oxide synthase; iNANC: inhibitory non-adrenergic non-cholinergic; iNOS: inducible nitric oxide synthase; nNOS: neuronal nitric oxide synthase; NO: nitric oxide. Reproduced from [19] with permission from the publisher.

recent years [25–27]. F_{eNO} testing is relatively convenient to perform, with numerous studies providing evidence of the applications of NO measurement in clinical practice [28, 29]. Currently, F_{eNO} measurements are used to predict and document the response to ICSs [30], to monitor adherence [26, 31] and as a diagnostic tool in ICS-naïve patients [28].

In this review, we discuss the current uses of F_{eNO}, its utility in the prediction of future exacerbation risk, the relationship between F_{eNO} and other biomarkers of inflammation in severe type 2 asthma and the potential use of F_{eNO} in patient selection/stratification for personalised treatment.

The association between F_{eNO} and other measures of airways inflammation

Biomarkers of type 2 inflammation include serum IgE, blood or sputum eosinophils, F_{eNO} and serum periostin [16]. Measurement of eosinophil numbers in induced sputum and from bronchial biopsy is considered the "gold standard" for identifying underlying type 2 airway inflammation (and thereby aiding identification of a type 2 asthma phenotype). However, bronchial biopsy is an invasive procedure with significant short-term morbidity. It also requires a dedicated facility and considerable laboratory support to maximise the information from the material sampled, which limits its use in routine clinical practice [29, 32]. Sputum analysis, while well tolerated, must be performed in laboratories with relevant expertise, is relatively time-consuming and is not always successful (with reported success rates ranging from 74% to 94%), leading to bias in reporting [33–39]. F_{eNO} adds an additional dimension to traditional clinical testing, with advantages including the non-invasive nature of the test, the ease of repeat measurements and its relatively simple use in patients with severe airflow obstruction, where other techniques may be difficult to perform [40].

F_{eNO} has been shown to have comparable accuracy to peripheral blood eosinophilia in predicting sputum eosinophilia in adults with asthma, irrespective of factors such as severity, degree of atopy and smoking status [41]. In addition, F_{eNO} levels correlate well with the level of inflammation and decrease in response to ICS treatment [42]. However, whilst ICS treatment is a strong suppressor of F_{eNO} [43], its effect on PBEs is probably weak [44]. Conversely, treatment with oral corticosteroids (OCS) appears to have more influence on PBEs than on F_{eNO} [45].

Although F_{eNO} generally correlates with eosinophilia, this is not always the case, as F_{eNO} and eosinophilia result from inflammatory processes that involve different type 2 cytokine pathways; the relative production of the corresponding cytokines determines the level of each biomarker [42]. While cytokines IL-4 and IL-13 are involved in regulating IgE synthesis and increasing F_{eNO} levels, IL-5 is the main cytokine

involved in the development, recruitment and activation of eosinophils. This supports the concept that F_{eNO} should not be considered a surrogate marker for sputum eosinophils but rather a parallel marker of airway inflammation often, but not always, associated with eosinophilia [42, 46–48].

Measuring both F_{eNO} levels and blood eosinophil counts may provide more information than using either alone, as they are both valid, but distinct, biomarkers for type 2 inflammation [49–52]. It has been suggested that both F_{eNO} levels and blood eosinophil counts should be incorporated in future diagnostic algorithms [53]. There is also some evidence that simultaneously increased F_{eNO} levels and blood eosinophil counts are associated with a higher prevalence of uncontrolled asthma and moderate-to-severe bronchial hyper-responsiveness [50]. In a retrospective study of patients with severe asthma, the combined analysis of F_{eNO} levels and blood eosinophil counts identified patients with frequent severe exacerbations, which the authors concluded may help in formulating therapeutic strategies for comprehensive asthma control [52].

F_{eNO} and exacerbations

F_{eNO} is a predictive factor for asthma exacerbations, with increased levels of F_{eNO} being associated with a higher number of exacerbations [54–56]. Several systematic reviews of asthma management trials have shown that tailoring asthma medications based on F_{eNO} levels significantly reduces future exacerbation risk [57–60]. In a meta-analysis that compared the use of F_{eNO} to guide treatment with management based on clinical symptoms or asthma guidelines or both, the number of adults who had one or more asthma exacerbations was significantly lower in the F_{eNO}-guided group than in the control group (odds ratio (OR) 0.60) [59]. However, there was no statistically significant difference between the groups for exacerbations requiring hospitalisation (OR 0.14) or rescue OCS (OR 0.86).

In a similar comparative analysis in children, the number of children having one or more asthma exacerbations was significantly lower in the F_{eNO}-guided group than in the control group (OR 0.58) [58]. As in the adult meta-analysis, there was no statistically significant difference between the groups for exacerbations requiring hospitalisation (OR 0.75) [59]. Furthermore, F_{eNO} has been shown to be more strongly correlated with exacerbations than PBE counts (r=0.42, p=0.0008 *versus* r=0.34, p=0.0078) [56]. However, there was high prevalence of the use of OCS (56% of patients) in this study, which might have suppressed the PBE signal more than the F_{eNO} signal.

In a study using National Health and Nutrition Examination Survey (NHANES) data (2007–2008 and 2009–2010), F_{eNO} and blood eosinophil values provided independent information on the prevalence of current asthma, the occurrence of asthma events and the prevalence of wheeze [49].

F_{eNO} and lung function

Higher levels of F_{eNO} have been shown to be associated with a decline in lung function [61–64]. In a prospective 5-year follow-up study of 200 adults with newly diagnosed asthma, high F_{eNO} levels (⩾57 ppb) were associated with a more rapid decline in lung function [61]. In a 3-year prospective study in Japanese adults with stable, controlled asthma [62], F_{eNO} levels >40.3 ppb were shown to have 43% sensitivity and 86% specificity for identifying patients with a rapid decline in forced expiratory volume in 1 s (FEV_1). In a study of Korean children with atopic or non-atopic asthma, higher F_{eNO} levels were associated with reduced lung function in children with atopic asthma [63]. High F_{eNO} levels (⩾20 ppb) were associated with worse lung function in children and adolescents aged 6–18 years with persistent asthma compared with those who had low F_{eNO} levels (<20 ppb) [64].

In a study of patients included in the NHANES (2007–2012), combined high F_{eNO} levels and blood eosinophil counts identified patients with a higher risk of reduced lung function and wheezing symptoms [51].

Clinical utility of F_{eNO} measurements

The role of F_{eNO} in asthma diagnosis

Current National Institute for Health and Clinical Excellence (NICE) guidelines in the UK recommend the use of F_{eNO} for the initial diagnosis of patients with suspected asthma [28]. NICE standards for a positive F_{eNO} test are >40 ppb in adults and >35 ppb in children (5–16 years) (table 1) [28]. However, the pre-test probability of asthma will impact on subsequent clinical decision-making with regard to the F_{eNO} measurement. A single positive test in isolation is insufficient to make a diagnosis of asthma, irrespective of the pre-test probability, and additional bronchial provocation testing can be beneficial to determine airway hyper-responsiveness [28].

The recently published Scottish consensus statement on the role of F_{eNO} in adult asthma suggests cut-off values for F_{eNO} of >40 ppb in adult patients who are ICS naïve to support asthma diagnosis and F_{eNO} >25 ppb for adult patients taking ICSs [65]. In the Global Initiative for Asthma (GINA) report [15],

TABLE 1 Fractional exhaled nitric oxide (F_{eNO}) cut-offs in different guidelines

Guidelines	F_{eNO} cut-offs	Justification
NICE [28]	**Adults**	
	Positive: >40 ppb	
	Children (5–16 years)	
	Positive: >35 ppb	
Scottish consensus statement [65]	**ICS-naïve patients**	
	>40 ppb	
	Patients taking ICS	
	>25 ppb	
GINA [15]	**Adults**	Associated with eosinophilic inflammation (in non-smokers)
	⩾20 ppb	
ATS/ERS [40]	**Adults**	
	High: >50 ppb	Eosinophilic inflammation and, in symptomatic patients, responsiveness to corticosteroids likely
	Intermediate: 25–50 ppb	Cautious interpretation required
	Low: <25 ppb	Eosinophilic inflammation and responsiveness to corticosteroids less likely
ATS/ERS [40]	**Children**	
	High: >35 ppb	Eosinophilic inflammation and, in symptomatic patients, responsiveness to corticosteroids likely
	Intermediate: 20–35 ppb	Cautious interpretation required
	Low: <20 ppb	Eosinophilic inflammation and responsiveness to corticosteroids less likely

ATS: American Thoracic Society; ERS: European Respiratory Society; GINA: Global Initiative for Asthma; ICS: inhaled corticosteroid; NICE: National Institute for Health and Care Excellence.

⩾20 ppb F_{eNO} in conjunction with other characteristics, such as blood eosinophils ⩾150 cells·μL^{-1} and/or sputum eosinophils ⩾2%, could indicate patients with type 2 immune response (table 1).

F_{eNO} measurement is also recommended by the ATS as part of the initial diagnosis of asthma and for monitoring of airway inflammation [40]. The ATS guidelines define high, intermediate and low F_{eNO} levels in adults as >50 ppb, 25–50 ppb and <25 ppb, respectively. In children, high, medium and low F_{eNO} levels are classified as >35 ppb, 20–35 ppb and <20 ppb, respectively (table 1) [40]. The ATS guidelines further advise against the use of reference values derived from a "normal" population when interpreting F_{eNO} levels, as the distribution of F_{eNO} in an unselected population is skewed such that the upper limits overlap with the range of values obtained in populations with asthma [40]. One immediate observation to be made from the various guideline cut-offs is the range of values adopted, which might reflect differences in the evidence base used to arrive at the chosen thresholds, but nevertheless appear arbitrary. The use of fixed cut-off levels is problematic, since (as discussed in the Limitations section) F_{eNO} can be influenced by a number of factors unrelated to the disease. The absence of evidence-based, patient-adjusted cut-offs has been cited as one of the remaining unresolved issues with F_{eNO} measurement [53]. A joint ERS–Global Lung Function Initiative task force is currently developing subject-specific F_{eNO} values [66], as have been successfully achieved previously for spirometry, lung volumes and diffusion capacity [67, 68].

F_{eNO} as a predictor of treatment response

An F_{eNO} level >50 ppb in adults is a strong indicator that the patient is likely to be responsive to ICS therapy [69]. In an observational, single-centre study conducted at an outpatient asthma and allergy specialty clinic in the USA, treatment decisions were first based on the results of symptoms, clinical examination and spirometry, then any treatment changes based on F_{eNO} measurements were documented [70]. Without F_{eNO} measurement, the physician's assessment of airway inflammation was incorrect in 50% of patients, and F_{eNO} measurement substantially altered the treatment decisions in 36% of patients. In another real-world study involving 337 specialist asthma practices in the USA that investigated the impact of F_{eNO} measurement on asthma management, F_{eNO} measurement enabled doctors to assess underlying airway inflammation, which led to a significant revision of the treatment plans compared with clinical assessment alone [71]. The clinical assessment agreed with F_{eNO} measurement in only 56% of cases. After F_{eNO} measurement, doctors altered the treatment plan in 31% of cases and changed ICS prescriptions in 90% of cases [71].

In a randomised controlled study conducted primarily in the UK, a significant interaction was observed between F_{eNO} levels at baseline and treatment groups (ICS *versus* placebo), indicating the magnitude of treatment response depends on the F_{eNO} level at baseline [30]. For every 10-ppb increase in baseline F_{eNO},

the change in the Asthma Control Questionnaire (ACQ)-7 mean score increased by 0.071 (p=0.044) more in the patients using ICS than placebo. Baseline F_{eNO} also had a strong association with improvement in cough severity in this study, with higher F_{eNO} values associated with greater odds of a clinical response, defined as an improvement of 20 mm or more on the visual analogue scale for cough symptoms [30]. A UK observational study assessing the ability of F_{eNO} to diagnose asthma and predict response to ICS therapy concluded the true utility of the F_{eNO} test to be in detecting the presence of underlying type 2 inflammation, identifying patients in whom ICS response is highly unlikely, thus guiding the appropriate use of ICSs in asthma treatment [72].

The use of F_{eNO} to guide asthma management in pregnant women appears to be as effective, if not more so, than in other adults [73]. In a double-blind, randomised trial of inflammatory marker-based management of asthma in pregnancy, a treatment algorithm based on F_{eNO} level and ACQ score led to a significant reduction in asthma exacerbations and less use of β_2 agonists compared with a clinical algorithm. Although the study was not specifically powered to assess perinatal outcomes, F_{eNO}-guided management resulted in a normalisation of babies' birthweights and reduced rates of neonatal admissions and preterm deliveries (both of which are increased in asthmatic pregnancies) [73]. Although further studies are needed, there is some evidence that F_{eNO} has the potential to be a useful and cost-effective tool for titration of ICS dose and in guiding management of asthma therapies [59, 74–77].

F_{eNO} and adherence to therapy

F_{eNO} has been used to monitor adherence to ICS therapy, as persistently high F_{eNO} levels can be an indication of non-adherence [26, 40, 43]. In a study of patients with "difficult asthma", defined as patients who remained symptomatic despite treatment at GINA steps 4 and 5, an F_{eNO} suppression test differentiated patients who were adherent or non-adherent to ICS treatment. After 7 days of directly observed ICS (DOICS) treatment, non-adherent patients experienced a significantly greater reduction from baseline in F_{eNO} levels compared with adherent patients (52.4% *versus* 20.4%; p<0.003) (figure. 2) [43]. A rapid fall in F_{eNO} after DOICS treatment can therefore identify patients who are presumed to have refractory disease but are actually not receiving optimal ICS treatment [43]. In a recent study in severe asthma centres in the UK, an F_{eNO} suppression test delivered using remote monitoring technology was shown to be a simple and effective method to identify which patients were adherent to, and those who derived benefit from, ICS/long-acting β_2-adrenergic receptor agonist (LABA) treatment [26].

F_{eNO} as a biomarker in severe asthma

Severe asthma is a heterogeneous disease and can be divided into several phenotypes according to inflammatory, clinical and functional characteristics [78]. These phenotypes may have prognostic value and therapeutic implications. The pathophysiology of severe asthma is poorly understood and it is therefore difficult to treat. However, from our current understanding of type 2 inflammation and the importance of its components to the pathophysiology of asthma, several key factors have been identified, including IgE, eosinophils and the IL-4/IL-13 pathway.

To help select appropriate biologics for severe asthma, a limited number of biomarkers are currently available, including IgE, PBEs and F_{eNO}, each of which reflects the characteristics of the underlying

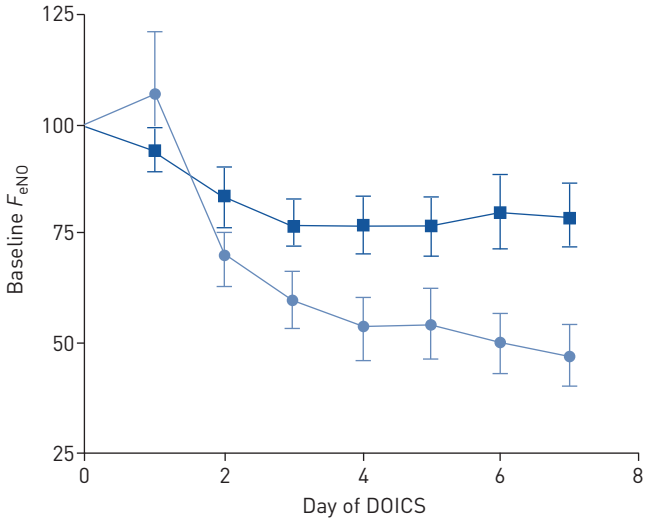

FIGURE 2 Fractional exhaled nitric oxide (F_{eNO}) levels in adherent and non-adherent patients on inhaled corticosteroids (ICS) therapy after directly observed ICS (DOICS) treatment. Non-adherent (n=9; circles) and adherent patients (n=13; squares). Reproduced from [43] with permission from the publisher.

inflammatory profile and specifically the presence of type 2 inflammation [5, 79, 80]. Periostin has also been validated as a marker of type 2 inflammation although with limited clinical use as its levels are influenced by bone metabolism [79].

High F_{eNO} levels in severe asthma have been shown to identify patients with greatest airflow limitation and reversibility, highest sputum eosinophil counts and most emergency department visits and intensive care unit admissions, suggesting that grouping patients with severe asthma by F_{eNO} identifies the most aggressive asthma phenotype [81].

Biomarker-guided management options

A number of monoclonal antibody (mAb)-directed biologics are now available, directed against inflammatory targets, including omalizumab (anti-IgE), mepolizumab (anti-IL-5), reslizumab (anti-IL-5), benralizumab (anti-IL-5 receptor α) and dupilumab (anti-IL-4 receptor α) (table 2) [82–91].

Omalizumab, an anti-IgE mAb, was the first biological therapy to be approved as an add-on therapy for adults and children aged ⩾6 years with severe persistent allergic asthma which is uncontrolled despite the

TABLE 2 Type 2-directed therapies based on monoclonal antibodies: key clinical trials in asthma

Target	Drug	Patient characteristics and biomarkers	Main response
Free IgE	Omalizumab [82, 83]	Severe asthma on ICS/LABA; atopic status, serum IgE 30–1500 IU·mL^{-1} (EU Label)	Reduced asthma exacerbations Improved mean AQLQ scores
IL-4Rα	Dupilumab [84]	Moderate-to-severe-uncontrolled asthma; FEV_1 reversibility; persistent symptoms (ACQ-5 ⩾1.5); exacerbation in past year	Decrease in asthma exacerbations Improvement in FEV_1 and % change in FEV_1 Reductions in mean ACQ-5 and AQLQ scores
IL-5	Mepolizumab [85, 86]	Severe asthma on ICS and LABA±OCS; blood eosinophils ⩾150 cells·μL^{-1} at screening or ⩾300 cells·μL^{-1} in past year	Reduced exacerbation rates Decrease in maintenance OCS Improvement in FEV_1 Reductions in ACQ-5 and SGRQ scores
IL-5	Reslizumab [87]	Inadequately controlled moderate-to-severe eosinophilic asthma (⩾400 cells·μL^{-1} during screening; ACQ-7 ⩾1.5)	Decrease in asthma exacerbations Improvement in FEV_1 Reductions in mean ACQ-7 and AQLQ scores
IL-5Rα	Benralizumab [88, 89]	Severe asthma uncontrolled by medium/high-dose ICS+LABA for ⩾1 year; ⩾2 exacerbations in previous year (ACQ-6 ⩾1.5). Baseline stratification: eosinophils <300 and ⩾300 cells·μL^{-1}	Decrease in asthma exacerbations Improvement in FEV_1 Reduction in maintenance OCS Reductions in mean ACQ-6 and AQLQ scores
IL-13	Lebrikizumab [90]	Not well controlled on ICS/LABA; blood eosinophils; serum periostin	Did not consistently significantly reduce asthma exacerbations in patients with high type 2 biomarker levels Reductions in mean ACQ-5 and AQLQ scores
IL-13	Tralokinumab [91]	Severe uncontrolled asthma despite controller therapies (ACQ-6 ⩾1.5)	No significant reduction in exacerbation rate Reductions in mean ACQ-6 and AQLQ scores

ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; F_{eNO}: fractional exhaled nitric oxide; FEV_1: forced expiratory volume in 1 s; ICS: inhaled corticosteroid; IgE: immunoglobulin E; IL: interleukin; LABA: long-acting β_2-adrenergic receptor agonist; OCS: oral corticosteroids; SGRQ: St. George's Respiratory Questionnaire.

use of ICS/LABA. Type 2 biomarkers associated with omalizumab efficacy have been investigated in several studies [92, 93].

In an analysis of biomarkers in A Study of Omalizumab (Xolair) in Subjects With Moderate to Severe Persistent Asthma (EXTRA study), which included patients with uncontrolled severe persistent allergic asthma, high levels of F_{eNO} (⩾19.5 ppb), blood eosinophils (⩾260 cells·μL^{-1}) and serum periostin (⩾50 ng·mL^{-1}) were associated with a greater treatment effect of omalizumab on exacerbation frequency, although several other serum biomarkers (specific-to-total IgE ratios, serum tryptase, eosinophil cationic protein or soluble CD23) were unable to predict outcomes with omalizumab [93].

Recently, in the prospective, real-world, PRospective Observational Study to evaluate Predictors of clinical Effectiveness in Response to Omalizumab (PROSPERO) study in patients with moderate-to-severe allergic asthma, 87% of patients had a positive treatment response to omalizumab (measured by several parameters), irrespective of baseline biomarker levels of blood eosinophils or F_{eNO} [92]. Therefore, the utility of blood eosinophil and F_{eNO} levels as predictors of treatment outcomes with omalizumab remains uncertain.

Mepolizumab [94–96] and reslizumab [97, 98] are mAbs that target IL-5, and benralizumab [99, 100] is a mAb that targets the IL-5 receptor. They are approved as add-on therapy for inadequately controlled severe refractory eosinophilic asthma in adults (all three agents) and in children aged ⩾6 years (mepolizumab). Blood IgE counts and blood and sputum eosinophil counts, have been used as biomarkers to identify patients for whom treatment is likely to result in clinically significant reductions in exacerbations [5, 47, 101].

Mepolizumab trials employed blood eosinophil cut-offs of ⩾150 cells/μL at baseline or ⩾300 cells/μL in the 12 months prior to allow inclusion of patients likely to achieve significant clinical benefit [101]. The absence of a pharmacodynamic response in F_{eNO} levels documented in trials with mepolizumab (in contrast to its depleting effect on blood eosinophils) suggests that F_{eNO} is not responsive to modulation through the IL-5 pathway and is potentially more impacted by other aspects of type 2 inflammation (*e.g.* IL-13) [101–103].

However, in a *post hoc* analysis [104] of the mepolizumab phase 2b Dose Ranging Efficacy and Safety with Mepolizumab in Severe Asthma (DREAM) study [102], patients with high baseline blood eosinophil levels experienced a greater reduction in exacerbations on mepolizumab treatment if they also had high baseline F_{eNO} levels (61%) than if they had low F_{eNO} levels (33%). Negligible reductions were observed in patients with low baseline blood eosinophil levels, irrespective of baseline F_{eNO} levels [104].

Lebrikizumab [90] and tralokinumab [91] are investigational anti-IL-13 mAbs that have completed 52-week, phase 3 trials in patients with uncontrolled asthma. Lebrikizumab did not consistently show significant reductions in asthma exacerbations in patients with high type 2 biomarker levels (periostin ⩾50 ng·mL^{-1} or blood eosinophils ⩾300 cells·μL^{-1}) [90]. Similarly, tralokinumab did not significantly reduce the annualised exacerbation rate compared with placebo in the overall study populations [91]. However, these studies did confirm that F_{eNO} was reduced by anti-IL-13 therapy [105], and the clinical efficacy observed was greater in those patients who had high levels of F_{eNO}, although the magnitude of benefit did not meet primary outcomes.

Dupilumab targets the shared receptor component for IL-4 and IL-13. It is approved in the USA as an add-on maintenance treatment in patients with moderate-to-severe asthma in patients aged ⩾12 years with an eosinophilic phenotype or with OCS-dependent asthma. It is approved in the European Union as an add-on maintenance treatment in patients aged ⩾12 years with type 2 severe asthma characterised by increased blood eosinophil and/or raised F_{eNO} levels who are inadequately controlled with high-dose ICS plus another medicinal product for maintenance treatment.

In clinical trials, dupilumab significantly reduced F_{eNO}, plus several additional biomarkers of type 2 inflammation (such as IgE). A transient increase in blood eosinophil levels was observed, which decreased close to baseline levels by the end of the treatment period [78, 97]. Raised baseline eosinophils (>150 cells·μL^{-1}) or F_{eNO} (>25 ppb) were both predictive of greater response to dupilumab, in terms of exacerbation reduction and improved FEV_1, suggesting both biomarkers may be potentially useful for informing treatment decisions and for monitoring biological response in patients with uncontrolled moderate-to-severe asthma [84, 106].

Cost-effectiveness of F_{eNO} measurement

Cost is often cited as a barrier to the use of F_{eNO}. However, F_{eNO} testing has been shown to be a cost-effective procedure [70, 107–111]. F_{eNO} measurement is considered by the NICE in the UK to be cost effective as an option to help diagnose asthma in adults and children, for asthma management in adults

and to support symptomatic asthma management in people using ICSs [110]. In a UK cost-effectiveness study, diagnosis of asthma using F_{eNO} was found to cost GBP 43 less per patient than standard diagnostic methods and the use of F_{eNO} measurement for asthma management rather than lung function testing resulted in an annual cost-saving of GBP 341 and 0.06 quality-adjusted life-years (QALYs) gained for patients with mild-to-severe asthma, and an annual cost-saving of GBP 554 and 0.004 QALYs gained for patients with moderate-to-severe asthma [111]. In line with the NICE guidelines, the recently published Scottish consensus statement on the role of F_{eNO} in adult asthma also concluded that F_{eNO} can be a cost-effective tool in the diagnosis and management of asthma [70]. In a retrospective study in the USA using data from a Medicare database, F_{eNO} monitoring in patients with a history of exacerbations was associated with a substantial reduction in asthma-related emergency department claims and inpatient admissions [108]. Inpatient or emergency department charges per beneficiary per day were USD 6.46 with F_{eNO} monitoring compared with USD 16.21 before the use of F_{eNO} [108]. In a US decision-tree analysis comparing standard of care alone and in conjunction with F_{eNO} monitoring, the addition of F_{eNO} decreased annual expenditure from USD 2637 to USD 2228 per patient and increased expected per-patient annual QALYs from 0.767 to 0.844 *versus* standard of care alone [109]. In a US observational, single-centre study conducted at an outpatient specialty asthma and allergy clinic, use of F_{eNO} in addition to standard of care was estimated to save USD 629 per patient per year [108]. These cost savings in diagnosis, management and treatment optimisation are reflective of the benefits described in the above discussion.

Current limitations

Although F_{eNO} levels are higher in patients with asthma characterised by type 2 inflammation, they can also be elevated in other related conditions, such as eosinophilic bronchitis, allergic rhinitis, atopy and atopic dermatitis [112, 113]. F_{eNO} is also elevated in upper respiratory tract infections and in pulmonary infections of lung transplant patients and sometimes in patients with chronic obstructive pulmonary disease (COPD) [114, 115]. However, the exact role of F_{eNO} in COPD and more specifically for monitoring asthma–COPD overlap (ACO) in patients on ICS therapy is still unclear and needs to be defined. Moreover, the literature defining the role of F_{eNO} and the practical cut-off value in patients with ACO and established COPD is minimal [115].

Currently, F_{eNO} levels are being used to monitor type 2 asthma [38, 58, 59], and the latest GINA guidelines recommend cut-offs for both blood eosinophils and F_{eNO} to help define the type 2 asthma population [15]. However, the GINA guidelines do not recommend the use of F_{eNO} to guide treatment in the general asthma population [15].

F_{eNO} levels can also be affected (positively and negatively) by many other factors [40, 112, 116]. Smoking leads to a decrease in F_{eNO} (although values are still higher in smokers with asthma than in those without) [117]. Studies have also demonstrated an association with height and sex (the latter, however, might be attributable to differences in height). F_{eNO} may also be associated with age: children have lower levels, which increase significantly as they grow up [118], and elderly patients demonstrate elevated levels [117].

Variability of access to F_{eNO} testing can limit its availability. In the UK, for example, testing is ubiquitous in tertiary or specialist centres; however, globally, F_{eNO} measurements are not widely used, with some countries not supporting reimbursement of testing. Therefore, there is a wider need for increased education on the importance of F_{eNO} measurement in asthma management.

Conclusion

Advances in technology and standardisation have simplified the measurement of F_{eNO}, permitting its use as a biomarker in the assessment of inflammatory airway diseases, such as type 2 asthma. Measurements can be performed in a variety of settings and are easily repeatable. F_{eNO} monitoring in routine clinical practice could play a key role in helping doctors to improve the accuracy of diagnoses in patients who have non-specific respiratory symptoms and in identifying those patients more likely to respond to ICS. In addition, there is substantial evidence supporting the use of F_{eNO} for ongoing monitoring. F_{eNO} measurement can help to identify patients who have poor asthma control, those at greater risk of exacerbations and those at risk of progressive loss of lung function. Ongoing patient assessment using F_{eNO} can be beneficial in guiding corticosteroid dosing and monitoring patient adherence to corticosteroid therapy. F_{eNO} levels can also be used to help identify patients with asthma who are likely to benefit from personalised treatments with biological therapies targeting type 2 inflammation. In conclusion, biomarker-based stratification of airway disease towards precision medicine is a reality now but needs to evolve further with wider adoption. F_{eNO} has significant potential as part of such a biomarker-based approach to the management of airway disease in primary and secondary care, and the optimisation of

F_{eNO} testing methods in a variety of clinical settings as a non-invasive, readily available and affordable technology will be important in advancing effective asthma control.

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