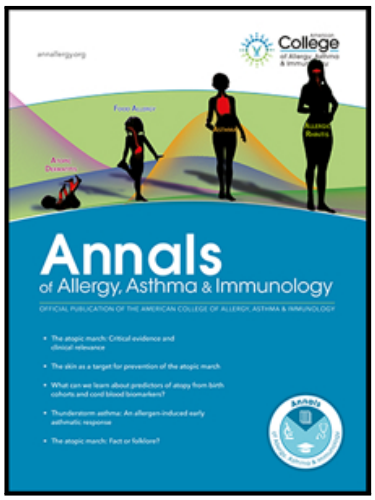
Journal Pre-proof



After the escalator: narrative review of biomarker-guided asthma care

Morgane Gronnier MD , Sanjay Ramakrishnan MDPhD , Richard W Beasley DSc , Ian D. Pavord FMedSci , Simon Couillard MDMSc

PII: S1081-1206(26)00231-0
DOI: https://doi.org/10.1016/j.anai.2026.05.027
Reference: ANAI 5344

To appear in: *Annals of Allergy, Asthma Immunology*

Received date: 27 February 2026
Revised date: 4 May 2026
Accepted date: 19 May 2026

Please cite this article as: Morgane Gronnier MD , Sanjay Ramakrishnan MDPhD , Richard W Beasley DSc , Ian D. Pavord FMedSci , Simon Couillard MDMSc , After the escalator: narrative review of biomarker-guided asthma care, *Annals of Allergy, Asthma Immunology* (2026), doi: https://doi.org/10.1016/j.anai.2026.05.027

KEY MESSAGES

HIGHLIGHTS

- Current symptom-focused stepwise escalation fails to achieve control in two-thirds of patients
- Blood eosinophils and FeNO predict exacerbations and corticosteroid/biologic treatment responses
- Biomarkers may help streamline diagnostic pathways and target therapeutic escalation, but require threshold validation
- Low biomarkers identify T2-low patients requiring alternative treatable trait management
- Further trials and studies investigating innovative ways of using biomarker-guided care – for example with 'fast and slow lane' prioritization schemes – are urgently needed.

TITLE: **After the escalator: narrative review of biomarker-guided asthma care**

Authors: Morgane Gronnier[1-2], MD ORCID 0009-0002-7138-4518

Sanjay Ramakrishnan[3,4], MD PhD ORCID 0000-0002-3003-7918

Richard W Beasley[5] DSc ORCID 0000-0003-0337-406X

Ian D. Pavord[6] FMedSci ORCID 0000-0002-4288-5973

Simon Couillard[1*], MD MSc; ORCID: 0000-0002-4057-6886

From: [1]Faculté de médecine et des sciences de la santé, Université de Sherbrooke, 3001 12[e] Avenue Nord, Sherbrooke (Québec), QC J1H 5N4, Canada [2]Centre hospitalier Universitaire d'Amiens, Amiens-Picardie, France [3]Institute for Respiratory Health and UWA Medical School, University of Western

Australia, Perth, Australia;[4] Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Perth, Australia [5]Medical Research Institute of New Zealand, Wellington, New Zealand ; [6]Respiratory Medicine Unit and Oxford Respiratory NIHR BRC, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom;

Key words: airways, asthma, biomarkers, biologics, eosinophils, exacerbations, FeNO, diagnosis, prognosis, management, type-2 inflammation, therapy

Correspondence to: *Dr Simon Couillard, Centre Hospitalier Universitaire de Sherbrooke, 3001, 12e Avenue Nord, pièce 2616, Sherbrooke (Québec), Canada, J1H 5N4 Phone: +1-819-822-6735, Fax: +1-819-822-6763, E-mail: s.couillard@usherbrooke.ca

Manuscript word count: 3991 /4000

AUTHOR DECLARATION OF CONFLICTS OF INTEREST:

- **MG** has no conflict to declare.
- **SR** has received salary support from the National Institute for Health and Social Care Research (NIHR) UK and the Charlie's Foundation for Research, unrelated to this work. SR also declares speaker fees from GSK, Sanofi, Chiesi, Boehringer Ingelheim and AstraZeneca, and conference travel support from GSK, Sanofi and AstraZeneca unrelated to this work. SR also declares GSK and Sanofi advisory board membership, unrelated to this work. SR is the deputy convenor of the COPD special interest group for the Thoracic Society Australia & New Zealand (TSANZ).
- **RB:** RB has received institutional research funding from AstraZeneca, Teva, HRC (NZ), Cure Kids (NZ) and Perpetual Guardian; personal fees from AstraZeneca, Avillion and Teva; is Chair of the Asthma Foundation of New Zealand adolescent and adult asthma guidelines, a reviewer for GINA and an ex-member of the GOLD Board.
- **IDP**: In the last 5 years, IDP has received speaker's honoraria for speaking at sponsored meetings from AstraZeneca, Circassia, AmgenNovartis, Chiesi, Sanofi/Regeneron, Menarini, and GSK, and payments for organising educational events from AstraZeneca, GSK, Sanofi/Regeneron, and Teva. He has received honoraria for attending advisory panels with Genentech, Sanofi/Regeneron, AstraZeneca, GSK, Novartis, Teva, Merck, Circassia, and Amgen. He has received sponsorship to attend international scientific meetings from, GSK, AstraZeneca, Sanofi and Regeneron.
- **SC** reports the following: he has received non-restricted research grants from the NIHR Oxford BRC, the Quebec Respiratory Health Research Network, the Association Pulmonaire du Québec, the Academy of Medical Sciences, AstraZeneca, bioMérieux, Circassia Niox Group, and Sanofi-

Genzyme-Regeneron; he is the holder of the Association Pulmonaire du Québec's Research Chair in Respiratory medicine and is a Clinical research scholar of the Fonds de recherche du Québec; he received speaker honoraria from AstraZeneca, GlaxoSmithKline, Sanofi-Regeneron, Circassia Niox Group and Valeo Pharma; he received consultancy fees for FirstThought, Apogee Therapeutics, Upstream Bio, AstraZeneca, GlaxoSmithKline, Sanofi-Regeneron, Access Biotechnology and Access Industries; he has received sponsorship to attend/speak at international scientific meetings by/for AstraZeneca and Sanofi-Regeneron. He is an advisory board member and detains stock options for Biometry Inc – a company which is developing a FeNO device (myBiometry); he is co-inventor for the patent filed as 'Method for alleviating dyspnea with neuromodulation'. He advised the Institut national d'excellence en santé et services sociaux (INESSS) for an update of the asthma general practice information booklet for general practitioners as well as therapeutic indications for Enerzair, and is a member of the asthma steering committee of the Canadian Thoracic Society.

Funding information: Supported by the Association Pulmonaire du Québec's Research Chair in respiratory medicine, the Fonds de Recherche du Québec, and the Fondation J.A. DeSève.

Author statement: MG and SC drafted the manuscript; MG drafted the figures; all authors contributed to manuscript writing, approved the final version, and agreed to submit the manuscript; SC is the guarantor of the manuscript.

Artificial intelligence statement: At time of manuscript writing, generative AI (ChatGPT) was used to proofread text which was originally written by authors.

Abstract

Asthma, the most common chronic respiratory disease, is characterized by variable symptoms, airflow limitation, and airway inflammation. Current management relies largely on a symptom-focused stepwise escalation approach which often leads to suboptimal outcomes. This review examines how type-2 (T2) inflammatory biomarkers – blood eosinophils and fractional exhaled nitric oxide (FeNO) – can complement symptom-based assessment to optimize care pathways. We synthesize evidence for biomarker-guided management across five critical decision points: diagnostic triage, inhaled

corticosteroid (ICS) initiation and dose escalation, acute attack phenotyping, and biologic selection. Across trials and observational cohorts, biomarker-high patients derived substantially greater benefit with ICS-based therapy, while biomarker-low patients had a worse benefit-harm profile. Each section is balanced by a review of data in disfavor of biomarker-based management. Indeed, tests for type-2 inflammation may be criticized in terms of accessibility or variability and require threshold validation. Nevertheless, the cumulated evidence suggests that future trials and studies of biomarker integration into diagnostic and treatment pathways may help streamline management for the most at-risk patients, from diagnosis to treatment intensification. The analogy of 'fast and slow lanes' for diagnostic and treatment algorithms is developed. The utility of alternative treatable traits including chronic airway infection, persistent airflow limitation, and breathing pattern disorders is also explored. The quality and counterpoints of the reviewed evidence emphasize that biomarkers should complement rather than replace comprehensive clinical assessment to optimize care for all phenotypes. Trials and studies of interventions tailored according to blood eosinophils, FeNO, and other key treatable traits are urgently needed across diagnostic and treatment algorithms for asthma.

INTRODUCTION

Asthma is a chronic respiratory disease affecting at least 300 million people worldwide.[1] It is the most common respiratory disease across the lifespan[2], one of the top 20 chronic conditions for global ranking of disability-adjusted life years in children[3], and a leading cause of missed school or workdays.[4–6] It is referred to as either a syndrome[7] or a disease defined by a variable intensity of respiratory symptoms, airflow limitation, and lower airway inflammation.[5,6]

Since the 1950s, inflammation has been recognized as a key driver of asthma risk, with anti-inflammatory therapy as a cornerstone of its management.[8] The Global Initiative for Asthma (GINA)[5] recommended a stepwise escalation approach of care[9,10] implemented since 1995[5,11] enabling standardized care across a broad range of clinical presentations and severity. However, several observations suggest opportunities for improvement with this approach.

Asthma stands out as one of the main chronic illnesses where escalation to high-intensity therapy relies solely on symptoms[12–15]-a strategy based on subjective criteria with notable physician-patient divergence.[16,17] Its impact on treatment outcomes remains unclear [18–20] with total asthma control in only one-third of patients while exposing the majority to high-dose inhaled corticosteroids (ICS) with potential systemic side effects[16,17,21] underscoring the need for alternative precision medicine-based approaches.

Building on the Oxford Asthma Attack Risk Scale (ORACLE) studies, this review examines evidence supporting accessible type-2 (T2) inflammatory biomarkers – specifically blood eosinophils and fraction of exhaled nitric oxide (FeNO) – from initial diagnosis through treatment escalation. We review the rationale for biomarker-guided escalation beyond ICS-formoterol anti-inflammatory reliever (AIR) therapy, including high-dose ICS, oral corticosteroid (OCS) bursts for severe attacks, and biologics balancing evidence with limitations to define optimal clinical use.

METHODS

This narrative review is based on a targeted synthesis of the literature, including studies selected for their clinical importance and methodological quality, alongside a review of current guidelines (GINA, British Thoracic Society (BTS), European Respiratory Society (ERS) and American Thoracic Society (ATS)).[5,22,23]

WHAT IS THE ORACLE FRAMEWORK?

Recently, as part of precision asthma management, the Lancet Commission on asthma[7] and the ORACLE framework proposed a biomarker-enhanced 'predict and prevent' approach specifically targeting asthma attacks, alongside other treatable traits.[24–29]

The ORACLE framework (Figure 1)[25] uses blood eosinophil count and FeNO to predict asthma attacks through prognostic stratification for ages 12 and older. Attributable risk estimates initially came from five clinical trial analyses across asthma severity evaluating independent associations between various factors, including these biomarkers, and attack risk.[30–34] A larger individual patient-level meta-analysis of 22 randomised controlled trial (RCT) control groups (ORACLE2)[27,35] robustly confirmed these findings, as did a Bayesian Network analysis of the International Severe Asthma Registry.[36,37] These studies showed that blood eosinophils and FeNO have comparable prognostic value and are additive to established risk factors such as prior-year attacks and GINA treatment step.[38,39] For example, based on the risks at the 75th and 25th percentiles of the sample distribution, a blood eosinophil count of 0.42 vs 0.14 $\times 10^9$/L or a FeNO of 42 vs 14 parts per billion (ppb) was associated with a 16% (95% CI 12 to 21%) or 11% (7 to 15%) increased rate of severe asthma attacks, respectively. Trial-level analyses showed T2 anti-inflammatory therapy reduces this excess risk,[24] with targeted biologics reducing exacerbation rates up to tenfold in patients with elevated T2 biomarkers. Combined elevation of both markers further improves prognostic accuracy and may thus serve to identify high-risk patients for personalized treatment.[32,35]

The ORACLE papers[20,24–26] confirmed three key findings regarding T2 biomarkers in asthma stratification.

First, similar to the use of lipid markers and blood pressure in cardiovascular risk management[14], relative risks of exacerbation, and treatment benefit effects with blood eosinophils and FeNO utilization were

consistent across populations, though absolute risk and benefit were greater in higher-risk groups. The ORACLE framework targets modifiable attack risk via T2 biomarkers, disease severity, and prior attacks. In ORACLE2, prior attacks nearly doubled future risk, severe disease increased risk by ~50% versus moderate asthma, and combined high eosinophils and FeNO conferred comparable, treatment-modifiable risk.[26] This risk is evident across diverse patient profiles, including patients of both sexes and those with normal spirometry and no bronchodilator reversibility, provided biomarker levels and/or prior attack history indicate high risk.[40]

Second, biomarkers quantify risk reducible by anti-inflammatory therapy, distinguishing high-risk responders from low-risk non-responders (40–60% exacerbation reduction in biomarker-elevated populations).[24,31] Moreover, when ICS and OCS dose are titrated using biomarkers of T2 airway inflammation, greater reduction in severe exacerbations is achieved versus standard stepwise escalation.[41]

Third, blood eosinophils and FeNO have complementary prognostic (both predict asthma attacks[25], with FeNO more consistently predicting lung function decline[42–46]) and theragnostic value (both predict T2 targeting therapy response[24]) reflecting distinct T2 immune components and compartments. Blood eosinophils respond to interleukin (IL)-5 levels in circulation and the body's overall reservoir of these effector cells, while FeNO serves as an IL-13-driven epithelial marker signaling (including cytokines, chemokines, and alarmins) within the airways.[45,47] Different biomarker scenarios thus indicate distinct treatment opportunities with ICS and/or T2 targeting biologics (Figure 2).[48]

Caveats and next steps

Although ORACLE2, based on 22 high-quality RCTs, is a major advance in identifying high-risk, treatment-responsive patients, its generalizability is limited by selected control-arm populations with

adherence differing from real-world practice. Implementation also requires considering factors affecting T2 biomarkers, which are well characterized and easily integrated into interpretation. (Table 1).[48–51]

Furthermore, ORACLE focused on blood eosinophils, whereas sputum eosinophils are associated with an increased asthma attacks risk[34] and a predictor of a good response to ICS,[31] although in practice, they are more difficult to measure.

The ORACLE framework represents significant progress in asthma risk stratification, moving beyond symptom-based assessment alone.[20] Continued validation across diverse populations, especially among children, is required to determine the value of its implementation in key disease timepoints: diagnosis, stratification, and treatment decisions.

SUSPECTED ASTHMA: BIOMARKER-ENABLED TRIAGE VS PHYSIOLOGICAL CONFIRMATION

Current diagnostic challenges

Asthma is diagnosed by the recognition of specific symptoms, such as wheezing, dyspnea, cough, bronchorrhea, and chest discomfort, combined with objective evaluation of variable airflow limitation. Symptoms are variable in intensity and over time and may manifest in many other pulmonary and extrapulmonary conditions. These considerations pose a diagnostic challenge throughout the clinical journey.[52–56] Accordingly, over- and under-diagnosis is rampant (30-35% misdiagnosed, 20-70% undiagnosed).[53–56] The universal guideline “test before treating” is relevant, but developing a diagnostic framework emphasising both physiology and biology remains challenging.[57–60]

The two main methods recommended to diagnose asthma are post-bronchodilator reversibility on spirometry and, when non-diagnostic (>85% of cases[60]), airway hyperresponsiveness via bronchial provocation testing (BPT) measuring the methacholine dose causing a 20% fall in forced expiratory

volume in 1 second (FEV_1) (PD20)).[5,59] These tests reflect the physiological mechanisms and have been incorporated into guidelines to objectively diagnose asthma when clinical criteria alone are insufficient, from age 5 years onwards. [5]

Limitations of current physiological testing

However, several limitations challenge widespread implementation: bronchodilator reversibility has poor sensitivity and specificity for asthma [61], and BPT is time-consuming (1 hour of time for adults and 1.5 to 2 hours for children, often requiring parental support).[62] High demand and procedural complexity [59,62], have submerged physiology laboratories (especially since the COVID-19 pandemic). At the Centre Hospitalier Universitaire (CHU) of Sherbrooke, about 91% of asthma diagnostic tests are requested by non-pulmonologists[63] and in many centres, primary care requests are low priority and subject to long delays.

Biomarkers as diagnostic tools: supporting evidence

Given these limitations, T2 biomarkers have emerged as potential diagnostic tools. Beyond predicting asthma attacks[25,26,37] and lung function decline[42–46], they potentially offer rapid, non-invasive, technically simple tests[50] that may contribute to the diagnostic process. Several studies have compared the diagnostic performance of FeNO and blood eosinophils against bronchial provocation testing (Table 2), and these data are currently being synthesized in an ongoing meta-analysis.[64]

Currently, European[57] and United-Kingdom (UK)[23] guidelines have recognised biomarker elevation**,** FeNO >40-50 ppb (both), or blood eosinophils 'outside the normal range' (in UK), as effective, cost-efficient diagnostic alternative when spirometry is nondiagnostic (even before spirometry in UK), in patients with symptoms suggestive of asthma.[57,65] A FeNO level ≥40 ppb being 90% is specific for asthma diagnosis in suspected adults.[65–67] Biomarker-based diagnostic strategies could offer several benefits

compared to BPT after non diagnostic spirometry, including fewer delays, faster care, better well-being, improved cost-effectiveness, and lower carbon footprint – cutting wait times may reduce unnecessary inhaler emissions.[68–70]

Limitations and concerns related to T2 biomarker diagnosis

Despite these advantages, several limitations restrict integration of biomarkers in diagnostic pathways.

Variability limits reliability, as biomarker levels fluctuate between measurements and fewer than 20% of asthmatics have FeNO ≥50 ppb on a single assessment.[49,71] Multiple confounding factors influence biomarker values (Table 1): children produce less NO due to smaller mucosal surface [72], and in adults, obesity, hormonal changes, or comorbidities like chronic rhinitis or eczema can alter readings.[71,73,74]

These considerations, along with the limited evidence supporting the diagnosis of asthma based on biomarkers, particularly in children, explain why FeNO is not included in the latest European guidelines for children.[75]

Second, optimal thresholds remain uncertain. "Rule in" thresholds should target a positive likelihood ratio (LR+) ≥ 10[76], together with specificity ≥90% and positive predictive value (PPV) ≥80%. However, few studies meet these rigorous criteria. Furthermore, the heterogeneity of the studies (Table 2) – which report prevalence rates higher than those observed in general practice – suggests a higher pre-test probability of asthma, which could lead to an overestimation of the diagnostic performance of FeNO and limit the generalizability of these results to settings where prevalence is lower. Additional studies which incorporate the impact of pre-test probability such as observed in primary care are needed.

Finally, biomarker-based approaches risk missing T2-low asthma (representing 30-50% of cases)[77] or misdiagnosed non-asthmatic inflammatory conditions, highlighting the need for comprehensive clinical assessment.[78]

Biomarkers could serve as a potentially useful adjunct in the diagnostic workup when spirometry is insufficient to establish a diagnosis or as a triage tool to prioritize BPTs where T2 biomarkers are elevated to enhance diagnostic interpretation for healthcare providers.[79]

THERAPEUTIC DECISION-MAKING:

ICS initiation: universal therapy versus biomarker stratification

Current consensus supports AIR-alone as the preferred initial treatment for adolescents and adults with mild asthma rather than short-acting beta$_2$-agonists (SABA) alone.[5] Several randomized trials show that, as-needed low-dose ICS-formoterol or ICS-albuterol significantly reduce severe exacerbations in mild asthma compared with short-acting bronchodilators alone, with benefits comparable to daily ICS but lower total exposure.[80–83]

In the Novel START trial there was no evidence of effect modification with severe exacerbation risk reduction due to ICS-formoterol reliever based on a wide range of characteristics such as demographic factors, smoking status, clinical characteristics, and blood eosinophil level, or FeNO. Patients with elevated blood eosinophils ($\geq$0.30 $\times 10^9$/L) on bronchodilator monotherapy had the highest exacerbation rates, while budesonide-formoterol reliever significantly reduced risk (Figure 3A).[31] High-eosinophil patients benefited from maintenance ICS treatment, but not T2-low patients who had a higher risk of exacerbations. This suggests biomarkers could identify patients who benefit most from maintenance ICS therapy. Similarly, ICS-naïve patients with elevated FeNO ($\geq$25 ppb) show more robust lung function improvements with ICS initiation than biomarker-low counterparts.[24]

T2-low patients receiving budesonide-formoterol reliever alone showed benefit, potentially reflecting formoterol's bronchodilator effects, non-eosinophilic anti-inflammatory mechanisms, or epithelial ICS effects independent of T2 inflammation.[80] Furthermore, although the risk of exacerbation is higher in T2-

high asthma, severe exacerbations occur in all phenotypes, but at lower absolute rates in T2-low populations. Concerns about potentially inadequate management of T2-low patients remain an obstacle to discontinuing or postponing ICS treatment based solely on biomarkers.

In summary, these data show that although all phenotypes in mild asthma benefit from AIR therapy alone[5], the use of biomarkers allows the identification of high T2 profile patients who will benefit from more careful monitoring.[84]

ICS dose escalation: symptom-driven versus biomarker guided intensification

Traditionally, escalation beyond low-dose ICS has followed standardized stepwise approaches based on persistent symptoms and exacerbations.[5] Established in the 1995 GINA report and consolidated over 30 years, this strategy matches treatment to disease burden.[11]

Pharmacologically, 80% to 90% of the maximum benefit of ICS in reducing exacerbations and improving lung function can be achieved with doses within the low to medium dose range.[85,86]

The therapeutic index of ICS – balancing efficacy against potential adverse effects – varies substantially by dose (Figure 4), leading clinicians to consider as-needed or low-dose ICS the "standard dose".[85,87]

Clinical reality shows 60% of patients remain partially or poorly controlled despite ICS therapy.[88] A UK study showed 35-45% of asthmatics remained uncontrolled one year after initiating medium-to-high dose ICS.[18] Studies show increasing ICS after an exacerbation does not reduce future events.[89,90] Another study found 70% of patients maintained the same ICS dose over 6 years without exacerbations or rescue use,[91] suggesting potential overtreatment when biomarkers are not considered.

This creates a therapeutic dilemma. Escalating to medium-to-high dose ICS offers limited additional efficacy, while introducing dose-dependent systemic adverse effects. Yet some patients may benefit

significantly from intensified treatment that cannot be predicted by symptom assessment or exacerbation history alone.[80]

Two large population-based cohorts of asthma patients – involving over 160,000 patients in the UK[87] and over 500,000 in Northern Europe[92] highlighted additional risks associated with higher ICS doses: major adverse cardiovascular events (MACE), pneumonia, arrhythmia, and pulmonary embolism, alongside historically recognized risks of adrenal suppression, cataracts, fractures, and diabetes. [85,93–96] In large observational cohorts, medium ICS doses more than doubled the relative risks of these events, and high doses increased them up to fourfold.[87] Whether these risks outweigh the benefits of increasing from low to high-dose ICS as not been prospectively studied.These findings prompted GINA's recent shift to recommend limited-duration trials (3-6 months) of high-dose ICS followed by gradual tapering if unsuccessful,[97] reserving high-dose treatment for patients deriving clear benefit.

Type-2 biomarkers have emerged as potential tools to identify patients most likely to benefit from ICS dose escalation, avoiding unnecessary exposure in non-responders. The CAPTAIN trial provided evidence for this approach.[32] This six-arm RCT evaluated fluticasone furoate (FF)–vilanterol, with or without umeclidinium, across doses in 2,416 patients with moderate asthma, including baseline blood eosinophils and FeNO. After 12 months, fluticasone furoate (FF) 100 vs. FF200 showed substantial differences in annual moderate-to-severe exacerbations and trough FEV1 depending on T2 biomarker profiles. Notably, the higher FF dose led to a six-fold greater reduction in severe attacks in patients with dual T2-high inflammation (T2 ‘High/high’: blood eosinophil count ≥0.3×10⁹/L and FeNO ≥50 ppb) compared to dual ‘T2-low/low’ patients (blood eosinophil count <0.15×10⁹/L and FeNO <25 ppb) (Figure 3B).[98]

However, several limitations challenge biomarker-guided escalation: In CAPTAIN, T2-high patients showed large benefits (reduced exacerbations, improved FEV1), while symptom improvement in T2-low

patients appeared more due to long-acting antimuscarinic than ICS escalation, highlighting a clinically relevant discordance between inflammation control and patient-prioritized symptom relief.[32,98] ICS reduction guided by symptoms or biomarkers does not worsen lung function in severe [99] or mild-to-moderate asthma (BASALT),[16] yet patients often refuse dose reduction when symptomatic, indicating unmet therapeutic needs.[100] Moreover, T2-low patients with significant symptoms risk undertreatment in purely biomarker-driven strategies.[101]

In summary, while symptom assessment and patient preferences remain important, T2 biomarkers offer objective guidance to identify the subgroup of patients who derive substantial benefit from high-dose ICS escalation while protecting the majority from unnecessary exposure to systemic adverse effects.

Monitoring of adherence

Up to 80% of patients on high-dose ICS lack optimal adherence.[102] Assessing adherence is crucial but often overlooked, as nonadherence means patients are undertreated. Efforts should prioritize optimizing current use rather than prescribing more medication.

Different contexts require different adherence assessments, from pharmacy refill checks to chipped inhalers or FeNO suppression testing.[52,103,104] Confirming adequate pickup must be combined with checking adequate technique, as up to 30% of patients make at least one critical error[105], meaning little or no medication reaches the lungs.[106,107]

FeNO suppression testing literature highlights mechanistic and clinical utilities. Daily FeNO monitoring with chipped inhalers at very high-dose ICS may provide mechanistic insight, since normalised FeNO indicates corticosteroid sensitivity.[47] Conversely, FeNO nonsuppression implies corticosteroid resistance.[103] Clinically, this translates to the former group (FeNO suppressors) experiencing better response to ICS, remaining more steadily adherent to their therapy at follow-up.[104,108] The latter group

(FeNO nonsuppressors) represent only 30% of the difficult-to-treat asthma population, yet will likely need escalation to T2 targeting biologics.[52,109]

Blood eosinophils should be very low (<0.1×10^9/L) or undetectable under OCS or anti-IL-5 therapy,[110,111] and the opposite may suggest nonadherence. Interestingly, though ICS are seen as having little effect on blood eosinophils, data show higher ICS doses do partially suppress eosinophils.[52,110,112]

Practical constraints limit widespread implementation: FeNO monitoring access remains limited, particularly in primary care; confounding factors (Table 1) complicate interpretation; and optimal frequency, thresholds, and cost-effectiveness require validation.

In summary, including T2 biomarkers in asthma management may prompt timely and appropriately targeted adherence checks. For example, a FeNO ≥40 ppb or blood eosinophil count ≥0.1×10^9/L despite OCS or anti-IL-5/5R should prompt a review of adherence.

Asthma attacks

Severe asthma attacks lead to morbidity, healthcare utilisation and avoidable deaths.[5,7] Despite their significance, the standard of care in acute asthma has not changed in the last decades, as guidelines recommend a 'one-size-fits-all' treatment approach with OCS for exacerbations to reduce hospital admission and relapses.[7,113,114]

Several studies have documented the heterogeneity of attacks according to sputum, blood, exhaled air, and microbiomic profiles.[115–124] Importantly, inflammatory phenotypes are often dynamic and indistinguishable from the point of view of initial symptoms or lung function.[123,125]

The PRISMA (Phenotyping the Responses to Systemic Corticosteroids in the Management of Asthma Attacks) study [118,119] assessed severe asthma attacks before and after OCS [125,126]. Asthma attacks were

categorised using a three-level system: T2-low/low (blood eosinophils <0.15×10^9/L and FeNO <25 ppb), T2-high/high (eosinophils ≥0.3×10^9/L and FeNO ≥35+ ppb), and T2-mid (patients not in T2-low/low and high/high groups). Among 53 attacks, 30% were T2-low/low, 51% were T2-mid, and 19% were T2-high/high. T2-high patients were more often male, had higher rates of nasal polyps, previous T2-high episodes, better bronchodilator responsiveness, and lower FEV1/Forced Vital Capacity (FVC), while lower T2 patients exhibited more symptoms consistent with abnormal breathing patterns. Response to OCS varied by phenotype: T2-high patients improved FEV1 substantially (0.390 ± 0.512 L), whereas T2-low patients showed no benefit (0.017 ± 0.153 L). Sixty-two percent experienced adverse effects attributable to OCS. Baseline FeNO and eosinophils were the only significant predictors of OCS response, demonstrating that T2 biomarkers can identify which patients benefit from systemic corticosteroids during acute exacerbations, distinguish asthma attack types by underlying biology. Although these findings require confirmation in larger-scale placebo-controlled clinical trials, they suggest precision medicine in the acute setting could enable more targeted use of OCS to the patients most likely to benefit while avoiding unnecessary side effects.

Biomarker guided use of biologics in acute severe asthma was investigated in the ABRA trial, a phase 2 RCT testing benralizumab for acute asthma or chronic obstructive pulmonary disease (COPD) attacks in 158 adults with elevated eosinophils (≥0.3×10^9/L).[127] Patients were randomized to benralizumab plus five days of oral prednisolone, benralizumab plus placebo, or prednisolone plus placebo injection. Benralizumab, alone or with steroids, reduced treatment failure by 75% versus prednisolone alone, preventing one failure per four treated patients. Symptom scores over 28 days also improved significantly with benralizumab. These phase 2 results mark a promising reframing of the management of acute asthma crises, yet requires further regulatory trials to allow adoption in clinical practice.

Managing biologic therapies

The introduction of biologic therapies, such as anti-IgE monoclonal antibodies (omalizumab), anti-IL-5/IL-5R (mepolizumab, benralizumab, reslizumab), anti-IL-4/IL-13 (dupilumab) and anti-thymic stromal lymphopoeitin (TSLP) (tezepelumab), was a major advance in patient care.[128] Type-2 biomarkers blood eosinophils and FeNO have played a key role in this context.[109,129] Current guidelines emphasise the use of elevated biomarkers to confirm eligibility for biologic therapies and predict response, [5]as T2-high patients show better clinical response to biologics, with greater reduction of exacerbations, symptoms, and improved pulmonary function than patients with low biomarkers.[109]

However, guidelines provide limited guidance on selecting between specific agents in the absence of head-to-head trials and emerging data suggest biomarker profiles could help distinguish patients who respond to specific biologics, but prospective validation is needed.[109]

As FeNO is only suppressed by dupilumab or tezepelumab,[130,131] these agents may potentially be preferred in severe "FeNO-predominant" asthma. [109] Shrimanker et al. demonstrated in a post hoc analysis of the phase 3 QUEST and phase 2b DREAM studies that patients with FeNO >25 ppb but blood eosinophils $<0.15\times10^9$/L responded to dupilumab but not to mepolizumab. In contrast, patients with blood eosinophils $>0.5\times10^9$/L – consistent with an eosinophilic 'bomb'-driven i.e., large circulating pool of effector eosinophils, asthma profile–demonstrate very large and complete responses to anti-IL-5 biologics, independent of FeNO levels.[30] This suggests a hierarchy where marked eosinophilia predicts robust anti-IL-5 response regardless of epithelial inflammation markers, whereas isolated elevation of FeNO allows the identification of patients requiring treatment upstream of the inflammatory pathway (dupilumab/tezepelumab).

Important limitations temper reliance on biomarkers alone for biologic selection. Tezepelumab has demonstrated efficacy in patients with high eosinophils, high FeNO, and even T2-low phenotypes, [132,133]

suggesting some agents transcend biomarker boundaries. However, there is remaining uncertainty, as the efficacy of tezepelumab in type-2 low asthma has not been seen consistently.[131] Furthermore, eosinophilic patients are more likely to respond to any of the currently approved T2-targeting biologics. In the absence of head-to-head trials, the most appropriate algorithm to decide which biologic to prescribe remains prescriber-dependent.[134] Finally, individual variability means some biomarker-high patients may not respond while some biomarker-low patients may benefit, particularly with broader-acting agents.

After biologic initiation, biomarkers may guide ICS dose reduction to minimize long-term steroid exposure.

The SHAMAL trial showed a decrease in lung function in patients who responded well to benralizumab but reduced their ICS dose from high-dose maintenance ICS/formoterol to ICS-formoterol on demand, which coincided with an increase in FeNO levels.[135] Conversely, patients who tapered to low-to-moderate ICS/formoterol maintenance and reliever doses did not experience significant lung function deterioration.

FeNO-guided step-down can safely reduce corticosteroid burden, supporting at least moderate ICS dosing to prevent lung function decline while avoiding unnecessary exposure to high-dose ICS.[35,85,136]

Asthma attacks on mepolizumab are heterogeneous and may involve ongoing eosinophilic inflammation. The prospective MEX study of 45 first exacerbations used multiple assessments, including sputum analysis; half had ≥2% sputum eosinophils. High-eosinophil patients showed greater airflow obstruction, slightly elevated blood eosinophils, and higher FeNO. FeNO <20 ppb predicted non-eosinophilic, infection-related events, while higher FeNO indicated sputum eosinophilia.[122] Some patients improved when switching to benralizumab, suggesting complete eosinophil elimination may be needed.[137] Oral prednisolone appears to have broad anti-inflammatory effects in stable mepolizumab patients[138], potentially supporting its use in high FeNO exacerbations during mepolizumab treatment.[139]

Biomarkers could also indicate a voluntary or involuntary discontinuation of biologic. Patients with severe eosinophilic asthma who discontinued anti-IL-5 therapy experienced significant increases in blood eosinophils, symptoms, and exacerbation frequency within three to six months.[140] Tezepelumab shows similar patterns based on nine-month discontinuation data.[141] Therefore, verifying that patients experiencing exacerbations with increased biomarkers have continued their scheduled biological injections is important before concluding treatment failure.

Overall, biomarkers are increasingly central to targeted therapy: a better understanding of non-responding patients, monitoring of adherence, and achieving remission or, at the very least, the lowest dose of ICS required to control the disease. Looking forward, the earlier intervention with biomarkers may offer not only an opportunity to obtain clinical benefit in high risk patients before escalation to high dose ICS with its associated risks, but also potentially influence the natural history of the disease. Trials such as the ongoing HOTHOT study (NCT07309614) is evaluatingwhether early dupilumab initiation in high-risk patients (elevated eosinophils and FeNO) can achieve asthma remission, reduce exacerbation risk, and improve lung function.

Beyond type-2 biomarkers: implementation challenges and alternative treatable traits.

The widespread adoption of biomarker-based strategies faces several obstacles. Severe asthma primarily affects low- and middle-income countries, where even basic treatments remain inaccessible, let alone biomarker-based precision medicine.[142,143] Moreover, implementation in primary care is further constrained by reimbursement policies and the costs of specialized devices, even though long-term studies suggest potential economic benefits per patient. [144,145]

Despite the importance of the T2 inflammatory biomarkers in the ORACLE framework, other treatable traits must be assessed. Chronic airway infection predicts a good response to azithromycin [146–149] at far

lesser cost than a biologic. In the AMAZES trial, azithromycin prevented attacks and improved quality of life and pre-treatment sputum Haemophilus influenzae copy numbers were related to efficacy.[148]

Dyspnea may also reflect persistent airflow limitation, which occurs beyond severe disease, including developmental factors (lung growth disorders, early respiratory damage) or acquired lesions (smoking).[150–155] When present, FEV_1 and symptom scores will reliably improve with long-acting bronchodilators, with no consistent effect on the exacerbation rates.[32,153–155] Persistent airflow obstruction and chronic airway infections must be considered distinct treatable traits in asthma management. Recognizing and targeting these overlapping conditions (and low type-2 biomarkers in a patient who continues to experience exacerbations and/or symptoms may be helpful in this regard), along with associated comorbidities and modifiable environmental or lifestyle factors, enables a more personalized approach that may improve asthma control better than the traditional uniform stepwise treatment model.[21]

Finally, the recurrent airway attacks may represent 'pseudo-asthma' or a combination of asthma and other pathologies, such as dysfunctional breathing or inducible laryngeal obstruction. These situations may be best handled by multidisciplinary team evaluation and referral to pulmonary rehabilitation services (*e.g.* www.physiotherapyforbpd.org.uk).[52,87]

Conclusion

Type-2 inflammatory asthma is a distinct phenotype, identifiable by blood eosinophil counts and FeNO. These biomarkers are useful across initial diagnosis to established disease management. The T2 phenotype has greatest likelihood of confirmatory asthma diagnosis, represents higher risk of outcomes (attacks, systemic corticosteroid exposure, and lung function decline), and identifies patients who do particularly well following escalation of anti-inflammatory therapy (high-dose ICS or a biologic). Patient symptoms, longitudinal risk stratification, and monitoring for acute events remain essential pillars to determine which patients require re-assessment for potential treatment escalation. Together, these findings support revisiting purely symptom-based escalation, eventually enabling biomarker-guided 'fast and slow lanes' diagnostic and management schemes (Figure 5) that optimize therapy for those most likely to benefit while identifying alternative treatable traits, particularly when T2 inflammation is absent. Several limitations – including biomarker access, confounding factors, threshold uncertainty, and limited paediatric data – temper this transition and highlight the need for trials and studies to consolidate and expand the next era in precision asthma care.

REFERENCES

1. To T, Stanojevic S, Moores G, et al. Global asthma prevalence in adults: Findings from the cross-sectional world health survey. *BMC Public Health* 2012; 12: 204.

2. Ferrari AJ, Santomauro DF, Aali A, et al. Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases

and injuries in 204 countries and territories and 811 subnational locations, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. *The Lancet* 2024; 403: 2133–2161.

3. Asher I, Pearce N. Global burden of asthma among children. *International Journal of Tuberculosis and Lung Disease* 2014; 18: 1269–1278.

4. Zafari Z, Sadatsafavi M, Chen W, et al. The projected economic and health burden of sub-optimal asthma control in Canada. *Respir Med* 2018; 138: 7–12.

5. 2025 GINA Strategy Report - Global Initiative for Asthma - GINA. *https://ginasthma.org/2025-gina-strategy-report/*.

6. Porsbjerg C, Melén E, Lehtimäki L, et al. Asthma. *The Lancet* 2023; 401: 858–873.

7. Pavord ID, Beasley R, Agusti A, et al. After asthma: redefining airways diseases. *Lancet* 2018; 391: 350–400.

8. Morrow Brown H. Treatment of chronic asthma with prednisolone significance of eosinophils in the sputum. *The Lancet* 1958; 272: 1245–1247.

9. Melhorn J, Howell I, Pavord ID. Should we apply a treatable traits approach to asthma care? *Annals of Allergy, Asthma and Immunology* 2022; 128: 390–397.

10. Bush A, Pavord ID. Stop the Asthma Treatment Elevator, We Need to Get Off! *Am J Respir Crit Care Med* 2025; 211: 698–699.

11. *Global initiative for asthma global strategy for asthma management and prevention nhlbi/who workshop report*, https://ginasthma.org/wp-content/uploads/2019/01/1995-GINA.pdf (January 1995, accessed 15 July 2025).

12. Goupil R, Tsuyuki RT, Santesso N, et al. Hypertension Canada guideline for the diagnosis and treatment of hypertension in adults in primary care. *CMAJ Canadian Medical Association Journal* 2025; 197: E549–E564.

13. Morin SN, Feldman S, Funnell L, et al. Clinical practice guideline for management of osteoporosis and fracture prevention in Canada: 2023 update. *CMAJ Canadian Medical Association Journal* 2023; 195: E1333–E1348.

14. Conroy RM, Pyörälä K, Fitzgerald AP, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: The SCORE project. *Eur Heart J* 2003; 24: 987–1003.

15. Diabetes Canada | Clinical Practice Guidelines, https://guidelines.diabetes.ca/cpg (accessed 22 July 2025).

16. Calhoun WJ, Ameredes BT, King TS, et al. Comparison of physician-, biomarker-, and symptom-based strategies for adjustment of inhaled corticosteroid therapy in adults with asthma: the BASALT randomized controlled trial. *JAMA* 2012; 308: 987–997.

17. Fuhlbrigge A, Marvel J, Electricwala B, et al. Physician–Patient Concordance in the Assessment of Asthma Control. *Journal of Allergy and Clinical Immunology: In Practice* 2021; 9: 3080-3088.e1.

18. Buhl R, Heaney LG, Loefroth E, et al. One-year follow up of asthmatic patients newly initiated on treatment with medium- or high-dose inhaled corticosteroid-long-acting β2-agonist in UK primary care settings. *Respir Med* 2020; 162: 105859.

19. Tran TN, Kerkhof M, Le TT, et al. Association of Stepping Up to High-Dose Inhaled Corticosteroids and Risk of Future Asthma Exacerbations: Data From the United States. *J Allergy Clin Immunol Pract* 2026; 14: 122-135.e10.

20. Couillard S, Meulmeester FL, Castro M, et al. Evaluating symptom burden to characterise, predict, and prevent asthma attacks: a patient-level analysis of randomised trials and translational prospective studies. *Lancet Respir Med*. (In press)

21. Beasley R, Braithwaite I, Semprini A, et al. Optimal Asthma Control: Time for a New Target. *American Journal of Respiratory and Critical Care Medicine* 2020; 201: 1480–1487.

22. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; 43: 343–373.

23. British Thoracic Society. *Asthma: diagnosis, monitoring and chronic asthma management (BTS, NICE, SIGN)*, https://www.nice.org.uk/guidance/ng245/resources/asthma-diagnosis-monitoring-and-chronic-asthma-management-bts-nice-sign-pdf-66143958279109 (27 November 2024).

24. Couillard S, Do WIH, Beasley R, et al. Predicting the benefits of type-2 targeted anti-inflammatory treatment with the prototype Oxford Asthma Attack Risk Scale (ORACLE). *ERJ Open Res* 2021; 8: 00570.

25. Couillard S, Laugerud A, Jabeen M, et al. Derivation of a prototype asthma attack risk scale centred on blood eosinophils and exhaled nitric oxide. *Thorax* 2022; 77: 199–202.

26. Meulmeester FL, Celis-Preciado CA, Ramakrishnan S, et al. Multivariable prognostic relations of asthma attack risk factors in the ORACLE patient-level meta-analysis. *European Respiratory Journal* 2024; 64: OA3761.

27. Couillard S, Steyerberg E, Beasley R, et al. Blood eosinophils, fractional exhaled nitric oxide and the risk of asthma attacks in randomised controlled trials: protocol for a systemic review and control arm patient-level meta-analysis for clinical prediction modelling. *BMJ Open* 2022; 12: e058215.

28. Couillard S, Petousi N, Smigiel KS, et al. Toward a Predict and Prevent Approach in Obstructive Airway Diseases. *J Allergy Clin Immunol Pract* 2023; 11: 704–712.

29. Riemann S, Meulmeester FL, Mailhot-Larouche S, et al. Sex does not modify prediction of asthma attacks by clinical risk factors and Type-2 biomarkers. *Chest*; 0. Epub ahead of print 31 January 2026. DOI: 10.1016/J.CHEST.2025.12.049.

30. Shrimanker R, Keene O, Hynes G, et al. Prognostic and predictive value of blood eosinophil count, fractional exhaled nitric oxide, and their combination in severe asthma: A post hoc analysis. *Am J Respir Crit Care Med* 2019; 200: 1308–1312.

31. Pavord ID, Holliday M, Reddel HK, et al. Predictive value of blood eosinophils and exhaled nitric oxide in adults with mild asthma: a prespecified subgroup analysis of an open-label, parallel-group, randomised controlled trial. *Lancet Respir Med* 2020; 8: 671–680.

32. Lee LA, Bailes Z, Barnes N, et al. Efficacy and safety of once-daily single-inhaler triple therapy (FF/UMEC/VI) versus FF/VI in patients with inadequately controlled asthma (CAPTAIN): a double-blind, randomised, phase 3A trial. *Lancet Respir Med* 2021; 9: 69–84.

33. Busse WW, Wenzel SE, Casale TB, et al. Baseline FeNO as a prognostic biomarker for subsequent severe asthma exacerbations in patients with uncontrolled, moderate-to-severe asthma receiving placebo in the LIBERTY ASTHMA QUEST study: a post-hoc analysis. *Lancet Respir Med* 2021; 9: 1165–1173.

34. Kraft M, Brusselle G, FitzGerald JM, et al. Patient characteristics, biomarkers and exacerbation risk in severe, uncontrolled asthma. *European Respiratory Journal* 2021; 58: 2100413.

35. Meulmeester FL, Mailhot-Larouche S, Celis-Preciado C, et al. Inflammatory and clinical risk factors for asthma attacks (ORACLE2): a patient-level meta-analysis of control groups of 22 randomised trials. *Lancet Respir Med* 2025; 13: 505–516.

36. Couillard S., Ramakrishnan S. In the Land of Asthma, Four Risk Factors to Rule Them All. *Chest* 2025; 168: 277–279.

37. Yadav CP, Chakraborty A, Price DB, et al. Prediction pathway for severe asthma exacerbations: a Bayesian Network analysis. *Chest* 2025; 168: 301–316.

38. Bloom CI, Palmer T, Feary J, et al. Exacerbation patterns in adults with Asthma in England A population-based study. *Am J Respir Crit Care Med* 2019; 199: 446–453.

39. Suruki RY, Daugherty JB, Boudiaf N, et al. The frequency of asthma exacerbations and healthcare utilization in patients with asthma from the UK and USA. *BMC Pulm Med* 2017; 17: 1–11.

40. Couillard S, Mailhot-Larouche S, Meulmeester F, et al. Normal FEV1 without reversibility in asthma trials – a placebo patient-level meta-analysis. *Am J Respir Crit Care Med*.

41. Mailhot-Larouche S, Celis-Preciado C, Heaney LG, et al. Identifying super-responders: A review of the road to asthma remission. *Annals of Allergy, Asthma and Immunology* 2025; 134: 31–45.

42. Hancox RJ, Pavord ID, Sears MR. Associations between blood eosinophils and decline in lung function among adults with and without asthma. *European Respiratory Journal* 2018; 51: 1702536.

43. Coumou H, Bel EH, Zwinderman AH, et al. Predictors of accelerated decline in lung function in adult-onset asthma. *Eur Respir J* 2018; 51: 1701785.

44. Pavord ID, De Prado Gómez L, Brusselle G, et al. Biomarkers Associated with Lung Function Decline and Dupilumab Response in Patients with Asthma. *Am J Respir Crit Care Med* 2024; 209: 1031–1033.

45. Park HY, Chang Y, Kang D, et al. Blood eosinophil counts and the development of obstructive lung disease: The Kangbuk Samsung Health Study. *European Respiratory Journal* 2021; 58: 2003823.

46. Çolak Y, Afzal S, Marott JL, et al. Type-2 inflammation and lung function decline in chronic airway disease in the general population. *Thorax* 2024; 79: 349–358.

47. Couillard S, Shrimanker R, Chaudhuri R, et al. Fractional Exhaled Nitric Oxide Nonsuppression Identifies Corticosteroid-Resistant Type 2 Signaling in Severe Asthma. *Am J Respir Crit Care Med* 2021; 204: 731–734.

48. Couillard Castonguay S. Fractional exhaled nitric oxide non-suppression in asthma - ORA - Oxford University Research Archive, https://ora.ox.ac.uk/objects/uuid:3312c52e-e5a0-4a5d-ac6d-15df48d44f5c (2021, accessed 15 July 2025).

49. Alving K, Malinovschi A. Basic aspects of exhaled nitric oxide. *Exhaled Biomarkers* 2025; 1–31.

50. Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011; 184: 602–615.

51. Hartl S, Breyer MK, Burghuber OC, et al. Blood eosinophil count in the general population: Typical values and potential confounders. *European Respiratory Journal* 2020; 55: 1901874.

52. Couillard S, Jackson DJ, Wechsler ME, et al. Workup of Severe Asthma. *Chest* 2021; 160: 2019–2029.

53. Aaron SD, Vandemheen KL, FitzGerald JM, et al. Reevaluation of Diagnosis in Adults With Physician-Diagnosed Asthma. *JAMA* 2017; 317: 269–279.

54. Aaron SD, Boulet LP, Reddel HK, et al. Underdiagnosis and overdiagnosis of asthma. *Am J Respir Crit Care Med* 2018; 198: 1012–1020.

55. Yang C l., Simons E, Foty R g., et al. Misdiagnosis of asthma in schoolchildren. *Pediatr Pulmonol* 2017; 52: 293–302.

56. Looijmans-Van Den Akker I, Van Luijn KRS, Verheij TJM. Overdiagnosis of asthma in children in primary care: A retrospective analysis. *British Journal of General Practice* 2016; 66: e152–e157.

57. Louis R, Satia I, Ojanguren I, et al. European Respiratory Society Guidelines for the Diagnosis of Asthma in Adults. *European Respiratory Journal* 2022; 60: 2101585.

58. 2024 GINA Main Report - Global Initiative for Asthma - GINA, https://ginasthma.org/2024-report/ (accessed 14 July 2025).

59. Yang CL, Hicks EA, Mitchell P, et al. Canadian Thoracic Society 2021 Guideline update: Diagnosis and management of asthma in preschoolers, children and adults. *Canadian Journal of Respiratory, Critical Care, and Sleep Medicine* 2021; 5: 348–361.

60. Selvanathan J, Aaron SD, Sykes JR, et al. Performance Characteristics of Spirometry With Negative Bronchodilator Response and Methacholine Challenge Testing and Implications for Asthma Diagnosis. *Chest* 2020; 158: 479–490.

61. Beasley R, Hughes R, Agusti A, et al. Prevalence, Diagnostic Utility and Associated Characteristics of Bronchodilator Responsiveness. *Am J Respir Crit Care Med* 2024; 209: 390–401.

62. Coates AL, Wanger J, Cockcroft DW, et al. ERS technical standard on bronchial challenge testing: General considerations and performance of methacholine challenge tests. *European Respiratory Journal* 2017; 49: 1601526.

63. Université de Sherbrooke. Internal data collated .

64. Roy M-A, Gronnier M, Manisha Ramphul A, et al. Diagnostic accuracy of fractional exhaled nitric oxide (FeNO) with or without blood eosinophils in childhood, adolescent, and adult asthma: protocol for a systematic review and meta-analysis. *medRxiv* 2024; 2024.07.14.24310394.

65. Recommendations | Asthma: diagnosis, monitoring and chronic asthma management (BTS, NICE, SIGN) | Guidance | NICE.

66. Li J-H, Han R, Wang Y-B, et al. Diagnostic possibility of the combination of exhaled nitric oxide and blood eosinophil count for eosinophilic asthma. *BMC Pulm Med* 2021; 21: 259.

67. Celis-Preciado CA, Lachapelle P, Couillard S. Exhaled nitric oxide (FeNO): Bridging a knowledge gap in asthma diagnosis and treatment. *Clinical and Experimental Allergy* 2023; 53: 791–793.

68. Schneider A, Brunn B, Hapfelmeier A, et al. Diagnostic accuracy of FeNO in asthma and predictive value for inhaled corticosteroid responsiveness: A prospective, multicentre study. *EClinicalMedicine* 2022; 50: 101533.

69. Couillard S, Sykes DL, Abdo M, et al. Viewpoint: a white paper for a greener design, conduct and reporting of clinical trials in respiratory medicine. *European Respiratory Journal* 2025; 65: 2401832.

70. Pouliot L, Désy L, Lemieux SÈ, et al. The carbon footprint of diagnostic delays in asthma. *ERJ Open Res* 2025; 11: 00577–02024.

71. Alving K, Teague WG. Further Fine-Tuning of the Interpretation of FeNO Measurement in Asthma. *Journal of Allergy and Clinical Immunology: In Practice* 2024; 12: 118–119.

72. Pedroletti C, Högman M, Meriläinen P, et al. Nitric oxide airway diffusing capacity and mucosal concentration in asthmatic schoolchildren. *Pediatr Res* 2003; 54: 496–501.

73. Harris EA, Hunter ME, Seelye ER, et al. Prediction of the physiological dead space in resting normal subjects. *Clin Sci* 1973; 45: 375–386.

74. Jagadish I, Shah D, Priessnitz J, et al. Sex-based hormonal influences on asthma biomarkers. *Journal of Allergy and Clinical Immunology*; 0. Epub ahead of print January 2026. DOI: 10.1016/J.JACI.2025.12.1010.

75. Gaillard EA, Kuehni CE, Turner S, et al. European Respiratory Society clinical practice guidelines for the diagnosis of asthma in children aged 5–16 years. *European Respiratory Journal* 2021; 58: 2004173.

76. Deeks JJ, Altman DG. Statistics Notes: Diagnostic Tests 4: Likelihood Ratios. *BMJ: British Medical Journal* 2004; 329: 168–169.

77. Kyriakopoulos C, Gogali A, Bartziokas K, et al. Identification and treatment of T2-low asthma in the era of biologics. *ERJ Open Res* 2021; 7: 00309–02020.

78. Maniscalco M, Fuschillo S, Mormile I, et al. Exhaled Nitric Oxide as Biomarker of Type 2 Diseases. *Cells* 2023; 12: 2518.

79. Gronnier M, Désy L, Pouliot L, et al. Integrating blood eosinophils and exhaled nitric oxide (FeNO) in asthma diagnostic pathways for adults and children: the PROPULSION SANTÉ observational study with translational sub-studies (DIVE, DIVE2)—protocols. *BMJ Open Respir Res* 2025; 12: 3750.

80. Beasley R, Holliday M, Reddel HK, et al. Controlled Trial of Budesonide-Formoterol as Needed for Mild Asthma. *N Engl J Med* 2019; 380: 2020–2030.

81. O'Byrne PM, FitzGerald JM, Bateman ED, et al. Inhaled Combined Budesonide–Formoterol as Needed in Mild Asthma. *New England Journal of Medicine* 2018; 378: 1865–1876.

82. Bateman ED, Reddel HK, O'Byrne PM, et al. As-Needed Budesonide–Formoterol versus Maintenance Budesonide in Mild Asthma. *New England Journal of Medicine* 2018; 378: 1877–1887.

83. LaForce C, Albers F, Danilewicz A, et al. As-Needed Albuterol–Budesonide in Mild Asthma. *New England Journal of Medicine* 2025; 393: 113–124.

84. Pauwels RA, Loefdahl CG, Postma DS, et al. Effect of inhaled formoterol and budesonid on exacerbations of asthma. *Pneumologie* 1998; 52: 140–141.

85. Beasley R, Harper J, Bird G, et al. Inhaled corticosteroid therapy in adult asthma time for a new therapeutic dose terminology. *Am J Respir Crit Care Med* 2019; 199: 1471–1477.

86. Noble JH, Warhurst S, Cullen R, et al. The Dose-Response of Inhaled Corticosteroids in Combination Inhaled Corticosteroid/Long Acting Beta2-Agonist Maintenance Therapy for Asthma: A Systematic Review and Meta-Analysis. *Chest* 2025; 168: 1304–1316.

87. Bloom I, Yang F, Hubbard R, et al. Association of Dose of Inhaled Corticosteroids and Frequency of Adverse Events. *Am J Respir Crit Care Med* 2025; 211: 54–63.

88. Bateman ED, Boushey HA, Bousquet J, et al. Can guideline-defined asthma control be achieved? The gaining optimal asthma control study. *Am J Respir Crit Care Med* 2004; 170: 836–844.

89. Tran TN, Kerkhof M, Le TT, et al. Association of Stepping Up to High-Dose Inhaled Corticosteroids and Risk of Future Asthma Exacerbations: Data From the United States. *J Allergy Clin Immunol Pract* 2026; 14: 122-135.e10.

90. Pavord ID, Tran TN, Jones RC, et al. Effect of Stepping Up to High-Dose Inhaled Corticosteroids in Patients With Asthma: UK Database Study. *Journal of Allergy and Clinical Immunology: In Practice* 2023; 11: 532–543.

91. Bloom CI, de Preux L, Sheikh A, et al. Health and cost impact of stepping down asthma medication for UK patients, 2001–2017: A population-based observational study. *PLoS Med* 2020; 17: e1003145.

92. von Bülow A, Hansen S, Sandin P, et al. Use of High-Dose Inhaled Corticosteroids and Risk of Corticosteroid-Related Adverse Events in Asthma Findings From the NORDSTAR Cohort. *Journal of Allergy and Clinical Immunology: In Practice* 2025; 13: 1609-1619.e5.

93. Suissa S, Kezouh A, Ernst P. Inhaled corticosteroids and the risks of diabetes onset and progression. *American Journal of Medicine* 2010; 123: 1001–1006.

94. Weatherall M, Clay J, James K, et al. Dose-response relationship of inhaled corticosteroids and cataracts: A systematic review and meta-analysis. *Respirology* 2009; 14: 983–990.

95. Masoli M, Weatherall M, Holt S, et al. Inhaled fluticasone propionate and adrenal effects in adult asthma: Systematic review and meta-analysis. *European Respiratory Journal* 2006; 28: 960–967.

96. Weatherall M, James K, Clay J, et al. Dose-response relationship for risk of non-vertebral fracture with inhaled corticosteroids. *Clinical and Experimental Allergy* 2008; 38: 1451–1458.

97. Global initiative for COPD (GOLD). Global Strategy for Prevention, Diagnosis and Management of COPD. *https://goldcopd.org/2021-gold-reports/* .

98. Couillard S, Pavord ID. Fluticasone furoate: CAPTAIN of fluticasones in type 2 inflammatory asthma. *Respirology* 2022; 27: 184–186.

99. Heaney LG, Busby J, Hanratty CE, et al. Composite type-2 biomarker strategy versus a symptom–risk-based algorithm to adjust corticosteroid dose in patients with severe asthma: a multicentre, single-blind, parallel group, randomised controlled trial. *Lancet Respir Med* 2021; 9: 57–68.

100. Heaney LG, Djukanovic R, Woodcock A, et al. Research in progress: Medical Research Council United Kingdom Refractory Asthma Stratification Programme (RASP-UK). *Thorax* 2015; 71: 187.

101. Thomas D, Hamada Y, Gibson P, et al. Diagnosis and treatment options for T2-low asthma. *J Allergy Clin Immunol Pract* 2025; 13: 1527–1539.

102. Hekking PPW, Wener RR, Amelink M, et al. The prevalence of severe refractory asthma. *Journal of Allergy and Clinical Immunology* 2015; 135: 896–902.

103. Couillard S, Shrimanker R, Lemaire-Paquette S, et al. Longitudinal changes in sputum and blood inflammatory mediators during FeNO suppression testing. *Thorax* 2022; 77: 933–938.

104. Heaney LG, Busby J, Bradding P, et al. Remotely Monitored Therapy and Nitric Oxide Suppression Identifies Nonadherence in Severe Asthma. *Am J Respir Crit Care Med* 2019; 199: 454–464.

105. Sanchis J, Gich I, Pedersen S. Systematic Review of Errors in Inhaler Use: Has Patient Technique Improved Over Time? *Chest* 2016; 150: 394–406.

106. Vanderman AJ, Moss JM, Bailey JC, et al. Inhaler misuse in an older adult population. *Consultant Pharmacist* 2015; 30: 92–100.

107. Al-Jahdali H, Ahmed A, AL-Harbi A, et al. Improper inhaler technique is associated with poor asthma control and frequent emergency department visits. *Allergy, Asthma and Clinical Immunology* 2013; 9: 8.

108. Butler CA, McMichael AJ, Honeyford K, et al. Utility of fractional exhaled nitric oxide suppression as a prediction tool for progression to biologic therapy. *ERJ Open Res* 2021; 7: 00273–02021.

109. Couillard S, Jackson DJ, Pavord ID, et al. Choosing the Right Biologic for the Right Patient With Severe Asthma. *Chest* 2025; 167: 330–342.

110. Moran AM, Ramakrishnan S, Borg CA, et al. Blood Eosinophil Depletion with Mepolizumab, Benralizumab, and Prednisolone in Eosinophilic Asthma. *Am J Respir Crit Care Med* 2020; 202: 1314–1316.

111. Thorn GW, Renold AE, Wilson DL, et al. Clinical Studies on the Activity of Orally Administered Cortisone. *New England Journal of Medicine* 1951; 245: 549–555.

112. Evans PM, O'Connor BJ, Fuller RW, et al. Effect of inhaled corticosteroids on peripheral blood eosinophil counts and density profiles in asthma. *Journal of Allergy and Clinical Immunology* 1993; 91: 643–650.

113. Rowe BH, Spooner CH, Ducharme FM, et al. Corticosteroids for preventing relapse following acute exacerbations of asthma. *Cochrane Database Syst Rev* 2007; CD000195.

114. Ramakrishnan S, Couillard S. Antibiotics for asthma attacks: Masking uncertainty. *European Respiratory Journal* 2021; 58: 2100183.

115. Baigelman W, Chodosh S, Pizzuto D, et al. Sputum and blood eosinophils during corticosteroid treatment of acute exacerbations of asthma. *Am J Med* 1983; 75: 929–936.

116. Alfaro C, Sharma OP, Navarro L, et al. Inverse correlation of expiratory lung flows and sputum eosinophils in status asthmaticus. *Ann Allergy* 1989; 63: 251–254.

117. Fahya, b J V., Kimb KW, Liub J, et al. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. *J Allergy Clin Immunol* 1995; 95: 843–852.

118. Ordoñez CL, Shaughnessy TE, Matthay MA, et al. Increased Neutrophil Numbers and IL-8 Levels in Airway Secretions in Acute Severe Asthma Clinical and Biologic Significance. *Am J Respir Crit Care Med* 2000; 161: 1185–1190.

119. Ghebre MA, Pang PH, Diver S, et al. Biological exacerbation clusters demonstrate asthma and chronic obstructive pulmonary disease overlap with distinct mediator and microbiome profiles. *Journal of Allergy and Clinical Immunology* 2018; 141: 2027-2036.e12.

120. Diver S, Haldar K, McDowell PJ, et al. Relationship between inflammatory status and microbial composition in severe asthma and during exacerbation. *Allergy: European Journal of Allergy and Clinical Immunology* 2022; 77: 3362–3376.

121. Pizzichini MMM, Pizzichini E, Clelland L, et al. Sputum in severe exacerbations of asthma: Kinetics of inflammatory indices after prednisone treatment. *Am J Respir Crit Care Med* 1997; 155: 1501–1508.

122. McDowell PJ, Diver S, Yang F, et al. The inflammatory profile of exacerbations in patients with severe refractory eosinophilic asthma receiving mepolizumab (the MEX study): a prospective observational study. *Lancet Respir Med* 2021; 9: 1174–1184.

123. McDowell PJ, Busby J, Hanratty CE, et al. Exacerbation Profile and Risk Factors in a Type-2-Low Enriched Severe Asthma Cohort A Clinical Trial to Assess Asthma Exacerbation Phenotypes. *Am J Respir Crit Care Med* 2022; 206: 545–553.

124. Maneechotesuwan K, Prapruetkit N, Chankham J, et al. Paradoxical eosinophilic and cytokine responses to oral corticosteroid treatment in patients with asthma exacerbations. *Journal of Allergy and Clinical Immunology: Global* 2024; 3: 100238.

125. Celis-Preciado C, Leclerc S, Duval M, et al. Phenotyping the Responses to Systemic Corticosteroids in the Management of Asthma Attacks (PRISMA). *European Respiratory Journal* 2025; 65: 2402391.

126. Celis-Preciado CA, Leclerc S, Duval M, et al. Phenotyping the Responses to Systemic Corticosteroids in the Management of Asthma Attacks (PRISMA): protocol for an observational and translational pilot study. *BMJ Open Respir Res* 2023; 10: e001932.

127. Ramakrishnan S, Russell REK, Mahmood HR, et al. Treating eosinophilic exacerbations of asthma and COPD with benralizumab (ABRA): a double-blind, double-dummy, active placebo-controlled randomised trial. *Lancet Respir Med* 2025; 13: 59–68.

128. Brusselle GG, Koppelman GH. Biologic Therapies for Severe Asthma. *New England Journal of Medicine* 2022; 386: 157–171.

129. Couillard S, Pavord ID, Heaney LG, et al. Sub-stratification of type-2 high airway disease for therapeutic decision-making: A 'bomb' (blood eosinophils) meets 'magnet' (FeNO) framework. *Respirology* 2022; 27: 573–577.

130. Castro M, Corren J, Pavord ID, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. *New England Journal of Medicine* 2018; 378: 2486–2496.

131. Menzies-Gow A, Corren J, Bourdin A, et al. Tezepelumab in Adults and Adolescents with Severe, Uncontrolled Asthma. *New England Journal of Medicine* 2021; 384: 1800–1809.

132. Carr T, Bernstein J, Cook B, et al. Efficacité du tézépélumab chez les patients asthmatiques sévères non contrôlés avec des comorbidités respiratoires : résultats de l'étude de Phase 3 NAVIGATOR. *Rev Fr Allergol* 2023; 63: 103596.

133. Corren J, Menzies-Gow A, Chupp G, et al. Efficacy of Tezepelumab in Severe, Uncontrolled Asthma: Pooled Analysis of the PATHWAY and NAVIGATOR Clinical Trials. *https://doi.org/101164/rccm202210-2005OC* 2023; 208: 13–24.

134. Côté A, Beaulé R, Boulay MÈ, et al. Poor Agreement Among Asthma Specialists on the Choice and Timing of Initiation of a Biologic Treatment for Severe Asthma Patients. *Journal of Allergy and Clinical Immunology: In Practice* 2025; 13: 1358-1366.e9.

135. Jackson DJ, Heaney LG, Humbert M, et al. Reduction of daily maintenance inhaled corticosteroids in patients with severe eosinophilic asthma treated with benralizumab (SHAMAL): a randomised, multicentre, open-label, phase 4 study. *The Lancet* 2024; 403: 271–281.

136. Price D, Ryan D, Burden A, et al. Using fractional exhaled nitric oxide (FeNO) to diagnose steroid-responsive disease and guide asthma management in routine care. *Clin Transl Allergy* 2013; 3: 1–10.

137. Jackson DJ, Burhan H, Menzies-Gow A, et al. Benralizumab Effectiveness in Severe Asthma Is Independent of Previous Biologic Use. *Journal of Allergy and Clinical Immunology: In Practice* 2022; 10: 1534-1544.e4.

138. Howell I, Yang F, Brown V, et al. Airway proteomics reveals broad residual anti-inflammatory effects of prednisolone in mepolizumab-treated asthma. *Journal of Allergy and Clinical Immunology* 2024; 154: 1146–1158.

139. Howell I, Mahdi M, Mahmood HR, et al. Fractional exhaled nitric oxide and the response to prednisolone for asthma attacks in patients treated with anti-IL5/5Rα therapy: a prospective observational study. *European Respiratory Journal* 2025; 66: 2501229.

140. Moore WC, Kornmann O, Humbert M, et al. Stopping versus continuing long-term mepolizumab treatment in severe eosinophilic asthma (COMET study). *European Respiratory Journal* 2022; 59: 2100396.

141. Menzies-Gow A, Wechsler ME, Brightling CE, et al. Long-term safety and efficacy of tezepelumab in people with severe, uncontrolled asthma (DESTINATION): a randomised, placebo-controlled extension study. *Lancet Respir Med* 2023; 11: 425–438.

142. Jayasooriya SM, Devereux G, Soriano JB, et al. Asthma: epidemiology, risk factors, and opportunities for prevention and treatment. *Lancet Respir Med* 2025; 13: 725–738.

143. García-Marcos L, Chiang CY, Asher MI, et al. Asthma management and control in children, adolescents, and adults in 25 countries: a Global Asthma Network Phase I cross-sectional study. *Lancet Glob Health* 2023; 11: e218–e228.

144. Sabatelli L, Seppälä U, Sastre J, et al. Cost-effectiveness and Budget Impact of Routine Use of Fractional Exhaled Nitric Oxide Monitoring for the Management of Adult Asthma Patients in Spain. *J Investig Allergol Clin Immunol* 2017; 27: 89–97.

145. Price D, Berg J, Lindgren P. An economic evaluation of NIOX MINO airway inflammation monitor in the United Kingdom. *Allergy* 2009; 64: 431–438.

146. Taylor SL, Ivey KL, Gibson PG, et al. Airway abundance of Haemophilus influenzae predicts response to azithromycin in adults with persistent uncontrolled asthma. *European Respiratory Journal* 2020; 56: 2000194.

147. Taylor SL, Leong LEX, Mobegi FM, et al. Long-Term Azithromycin Reduces Haemophilus influenzae and Increases Antibiotic Resistance in Severe Asthma. *Am J Respir Crit Care Med* 2019; 200: 309–317.

148. Gibson PG, Yang IA, Upham JW, et al. Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind, placebo-controlled trial. *The Lancet* 2017; 390: 659–668.

149. Thomas D, McDonald VM, Stevens S, et al. Effect of Azithromycin on Asthma Remission in Adults With Persistent Uncontrolled Asthma: A Secondary Analysis of a Randomized, Double-Anonymized, Placebo-Controlled Trial. *Chest* 2024; 166: 262–270.

150. Lange P, Celli B, Agustí A, et al. Lung-Function Trajectories Leading to Chronic Obstructive Pulmonary Disease. *New England Journal of Medicine* 2015; 373: 111–122.

151. Fletcher C, Peto R. The natural history of chronic airflow obstruction. *Br Med J* 1977; 1: 1645–1648.

152. Dharmage SC, Bui DS, Walters EH, et al. Lifetime spirometry patterns of obstruction and restriction, and their risk factors and outcomes: a prospective cohort study. *Lancet Respir Med* 2023; 11: 273–282.

153. Boulet LP, Abbott C, Brusselle G, et al. Baseline Characteristics and ICS/LAMA/LABA Response in Asthma: Analyses From the CAPTAIN Study. *Journal of Allergy and Clinical Immunology: In Practice* 2024; 12: 1244-1253.e8.

154. Kerstjens HAM, Engel M, Dahl R, et al. Tiotropium in Asthma Poorly Controlled with Standard Combination Therapy. *New England Journal of Medicine* 2012; 367: 1198–1207.

155. Sobieraj DM, Weeda ER, Nguyen E, et al. Association of inhaled corticosteroids and long-acting β-agonists as controller and quick relief therapy with exacerbations and symptom control in persistent asthma a systematic review and meta-analysis. *JAMA - Journal of the American Medical Association* 2018; 319: 1485–1496.

156. Bai H, Shi C, Yu S, et al. A comparative study on the value of lower airway exhaled nitric oxide combined with small airway parameters for diagnosing cough-variant asthma. *Ther Adv Respir Dis* 2023; 17: 1753466623118125.

157. Kasap Basioli N;, Tolic E;, Luis G-M, et al. Value of Fractional Exhaled Nitric Oxide in Diagnosing Mild Asthma Responsive to Inhaled Corticosteroids. *Journal of Clinical Medicine 2023, Vol 12, Page 3330* 2023; 12: 3330.

158. Hou L, Hao H, Huang G, et al. The value of small airway function parameters and fractional exhaled nitric oxide for predicting positive methacholine challenge test in asthmatics of different ages with FEV1 ≥ 80% predicted. *Clin Transl Allergy* 2021; 11: e12007.

159. Chen LC, Zeng GS, Wu LL, et al. Diagnostic value of FeNO and MMEF for predicting cough variant asthma in chronic cough patients with or without allergic rhinitis. *Journal of Asthma* 2021; 58: 326–333.

160. Fard MB, Samet M, Nodoushan MS, et al. Determination of a Cut-off Point for Exhaled Nitric Oxide in the Diagnosis of Asthma in an Iranian Population. *Tanaffos* 2021; 20: 150–155.

161. Urbankowski T, Przybyłowski T. Blood eosinophils, FeNO and small airways dysfunction in predicting airway hyperresponsiveness in patients with asthma-like symptoms. *Journal of Asthma* 2022; 59: 1376–1386.

162. Nekoee H, Graulich E, Schleich F, et al. Are type-2 biomarkers of any help in asthma diagnosis? *ERJ Open Res*; 6. Epub ahead of print 2020. DOI: 10.1183/23120541.00169-2020.

163. Liu J, Xu R, Zhan C, et al. Clinical utility of ultrahigh fractional exhaled nitric oxide in predicting bronchial hyperresponsiveness in patients with suspected asthma. *Postgrad Med J* 2019; 95: 541–546.

164. He L, Wei M, Luo J, et al. Re-evaluation of the diagnostic value of fractional exhaled nitric oxide & its impact in patients with asthma. *Indian Journal of Medical Research* 2018; 148: 441–448.

165. Feng-jia C, Xin-yan H, Geng-peng L, et al. Validity of fractional exhaled nitric oxide and small airway function indices in diagnosis of cough-variant asthma. *Journal of Asthma* 2018; 55: 750–755.

166. Porpodis K, Domvri K, Kontakiotis T, et al. Comparison of diagnostic validity of mannitol and methacholine challenges and relationship to clinical status and airway inflammation in steroid-naïve asthmatic patients. *Journal of Asthma* 2017; 54: 520–529.

167. Nickels AS, Lim KG. Evaluation of exhaled nitric oxide's ability to predict methacholine challenge in adults with nonobstructive spirometry. *Annals of Allergy, Asthma and Immunology* 2016; 117: 365-369.e1.

168. Wang Y, Li L, Han R, et al. Diagnostic value and influencing factors of fractional exhaled nitric oxide in suspected asthma patients. *Int J Clin Exp Pathol* 2015; 8: 5570.

169. Buslau A, Voss S, Herrmann E, et al. Can we predict allergen-induced asthma in patients with allergic rhinitis? *Clinical and Experimental Allergy* 2014; 44: 1494–1502.

170. Cirillo I, Ricciardolo FLM, Medusei G, et al. Exhaled Nitric Oxide May Predict Bronchial Hyperreactivity in Patients with Allergic Rhinitis. *Int Arch Allergy Immunol* 2013; 160: 322–328.

171. Katsoulis K, Ganavias L, Michailopoulos P, et al. Exhaled Nitric Oxide as Screening Tool in Subjects with Suspected Asthma without Reversibility. *Int Arch Allergy Immunol* 2013; 162: 58–64.

172. Voutilainen M, Malmberg LP, Vasankari T, et al. Exhaled nitric oxide indicates poorly athlete's asthma. *Clinical Respiratory Journal* 2013; 7: 347–353.

173. Schleich FN, Asandei R, Manise M, et al. Is FE NO50 useful diagnostic tool in suspected asthma? *Int J Clin Pract* 2012; 66: 158–165.

174. Matsunaga K, Hirano T, Akamatsu K, et al. Exhaled Nitric Oxide Cutoff Values for Asthma Diagnosis According to Rhinitis and Smoking Status in Japanese Subjects. *Allergology International*; 60, www.jsaweb.jp! (2011, accessed 16 July 2025).

175. Leon De La Barra S, Smith AD, Cowan JO, et al. Predicted versus absolute values in the application of exhaled nitric oxide measurements. *Respir Med* 2011; 105: 1629–1634.

176. Pedrosa M, Cancelliere N, Barranco P, et al. Usefulness Of Exhaled Nitric Oxide In Asthma Diagnosis. *Journal of Allergy and Clinical Immunology* 2010; 125: AB7.

177. Kowal K, Bodzenta-Lukaszyk A, Zukowski S. Exhaled nitric oxide in evaluation of young adults with chronic cough. *Journal of Asthma* 2009; 46: 692–698.

178. Schneider A, Tilemann L, Schermer T, et al. 'Diagnosing Asthma in General Practice with Portable Exhaled Nitric Oxide Measurement - Results of a Prospective Diagnostic Study: FENO ≤ 16 ppb better than FENO ≤ 12 ppb to rule out mild and moderate to severe asthma. *Respir Res* 2009; 10: 1–3.

179. David M, Chhajed PN, Tamm M, et al. Diagnostic Tests for Asthma in Firefighters. *Chest* 2007; 131: 1760–1767.

180. Fortuna AM, Feixas T, González M, et al. Diagnostic utility of inflammatory biomarkers in asthma: Exhaled nitric oxide and induced sputum eosinophil count. *Respir Med* 2007; 101: 2416–2421.

181. Heffler E, Guida G, Marsico P, et al. Exhaled nitric oxide as a diagnostic test for asthma in rhinitic patients with asthmatic symptoms. *Respir Med* 2006; 100: 1981–1987.

182. Smith AD, Cowan JO, Filsell S, et al. Diagnosing Asthma: Comparisons between Exhaled Nitric Oxide Measurements and Conventional Tests. *Am J Respir Crit Care Med* 2004; 169: 473–478.

183. Dupont LJ, Demedts MG, Verleden GM. Prospective Evaluation of the Validity of Exhaled Nitric Oxide for the Diagnosis of Asthma. *Chest* 2003; 123: 751–756.

184. Chatkin JM, Ansarin K, Silkoff PE, et al. Exhaled nitric oxide as a noninvasive assessment of chronic cough. *Am J Respir Crit Care Med* 1999; 159: 1810–1813.

185. Reddel HK, Bacharier LB, Bateman ED, et al. Global Initiative for Asthma Strategy 2021 Executive Summary and Rationale for Key Changes. *Am J Respir Crit Care Med* 2022; 205: 17–35.

186. Patel R, Naqvi SA, Griffiths C, et al. Systemic adverse effects from inhaled corticosteroid use in asthma: a systematic review. *BMJ Open Respir Res* 2020; 7: 756.

FIGURE LEGENDS

FIGURE 1. The ORACLE framework. (A) Annual asthma attack rate based on the blood eosinophil count and FeNO. These spline curves show the estimated annual severe asthma attack rates based on biomarkers of type-2 inflammation, after adjusting for relative confounders (enrolled trial, GINA treatment step, FEV1%, ACQ-5, asthma attack in past 12 months), based on n=6513 patients enrolled across 22 clinical trials' control arms. Figure reproduced from reference [35]**. (B) The Prototype OxfoRd Asthma attaCk risk scaLE (ORACLE).** Numbers in each cell are predicted annual asthma attack rates requiring treatment with systemic corticosteroids if treatment is not changed. . *Risk factors are defined by GINA guidelines[185]: poor symptom control (ACQ score ≥1.5), low lung function (FEV1 <80% predicted), adherence issues, reliever over-use (>200-dose salbutamol cannister/month), intubation or intensive care unit admission for asthma previously, comorbidities (one of: chronic rhinosinusitis, obesity, psychiatric disease), environmental exposures (one of: smoking, allergen, pollution). Figure reproduced from reference [25.] FeNO, fractional exhaled nitric oxide; GINA, Global Initiative for Asthma

Figure 1

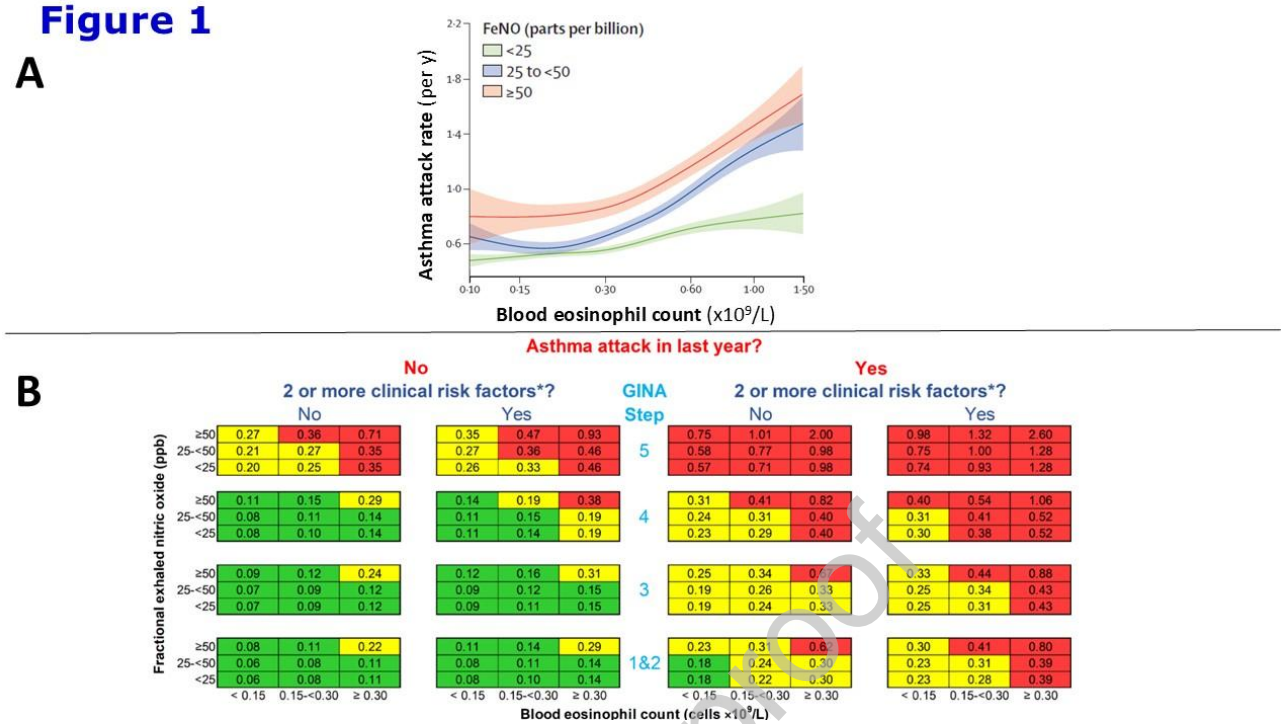


FIGURE 2

A two-biomarker, two-compartment framework for interpreting and using blood eosinophils and FeNO. FeNO is associated with increased levels of T2 cytokines, chemokines, alarmin, and MUC5AC in sputum in addition to predicting airway hyperresponsiveness, thus identifying unresolved airway inflammation. Blood eosinophils (not correlated with T2 markers in sputum), are correlated with serum IL-5. This suggests there are 2 distinct but additive compartments in T2 inflammation, the interleukin (IL)-4/IL-13 axis (the magnet) corresponding to FeNO driving chemotaxis of inflammatory cells (including eosinophils) to the airway, and the IL-5 dependent pool of effector eosinophils (bombs). Elevation in FeNO results in a greater risk of lung function decline, whereas elevations in either blood eosinophils and FeNO result in a higher risk of asthma attacks. Elevation in both biomarkers results in a further multiplicative increase in risk. Each represents a distinct treatment target: ICS and/or T2 targeting biologics. Considering the individualization of inflammatory compartments, biomarkers could be taken

into account when choosing biological treatment, alongside other clinical characteristics (nasal polyposis, etc.). eos, eosinophils; FeNO, fractional exhaled nitric oxide; IL, interleukin.

Figure 2

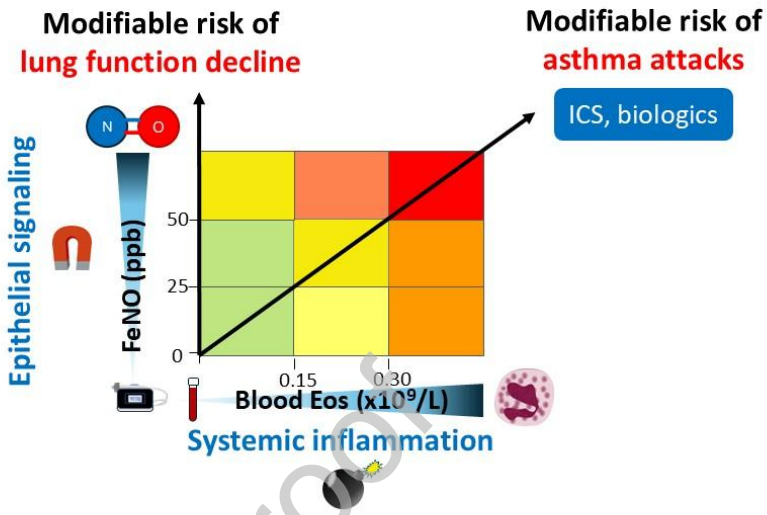


FIGURE 3. Severe exacerbations by T2 biomarkers and treatment strategy. Combined histogram derived from the NOVEL START[80] (top) and CAPTAIN [98,153] (bottom) trials, showing the proportion of patients with at least one severe asthma exacerbation, stratified by T2 biomarker profiles and treatment regimen. In NOVEL START, there was a greater incidence of exacerbation with SABA alone at higher eosinophil counts; the risk was offset by as-needed budesonide–formoterol (200/6 mcg per actuation) or maintenance budesonide (400 mcg/day: low-dose) plus as-needed salbutamol, highlighting the predictive value of blood eosinophils for ICS response. In CAPTAIN, T2-high/high profiles (eosinophils ≥0.30 × 10⁹/L and FeNO ≥50 ppb) had fewer exacerbations with high-dose ICS, whereas among T2-low/low patients (eosinophils < 0.15 × 10⁹/L and FeNO ≤25 ppb), escalation to higher ICS doses resulted in minimal additional reduction in the proportion of patients experiencing exacerbations. These findings support a biomarker-driven approach to optimize therapeutic decisions. FF, fluticasone furoate

Figure 3

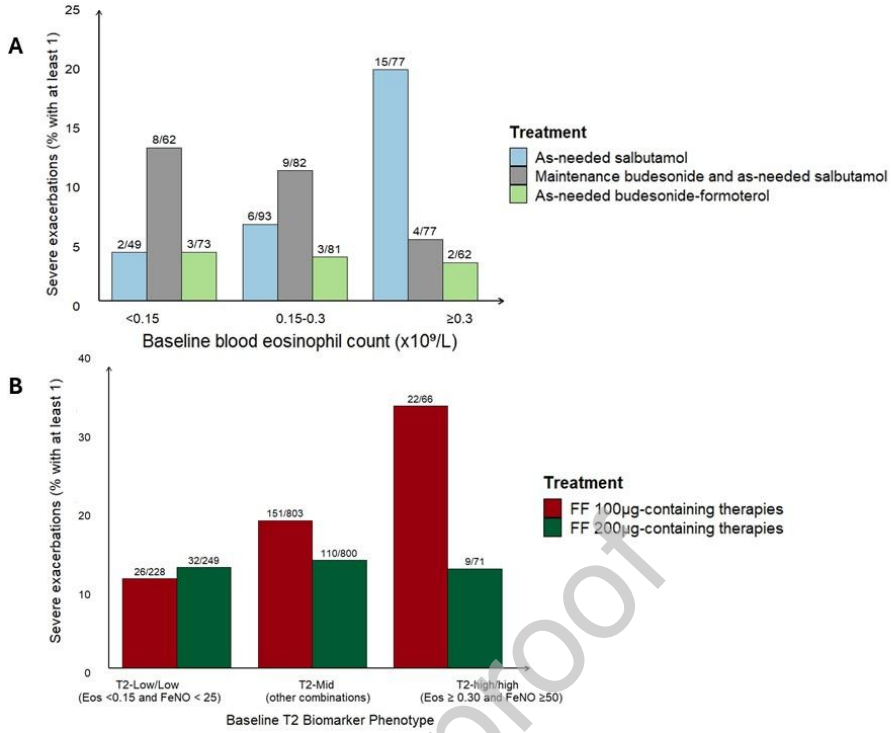


FIGURE 4. Benefit-to-adverse effects ratio of inhaled corticosteroids by T2 inflammatory phenotype and dose. Model illustrating the theoretical balance between clinical benefit and systemic adverse effects of ICS across escalating doses, stratified by T2 inflammatory phenotypes. In patient T2-high/high (eosinophils ≥0.30 × 10⁹/L and FeNO ≥50 ppb) the benefit-to-risk ratio increases steeply with dose, reflecting substantial expected therapeutic gain in this group. In T2-low phenotypes (eosinophils < 0.15 × 10⁹/L and FeNO < 25 ppb), dose escalation yields minimal therapeutic gain while disproportionately increasing the risk of systemic adverse effects, thereby shifting the balance toward harm. [21,31,92,186] In T2-mid phenotypes, the ratio increases moderately, but with greater uncertainty regarding net benefit.

ICS, inhaled corticosteroid; T2, type-2 inflammation.

Figure 4

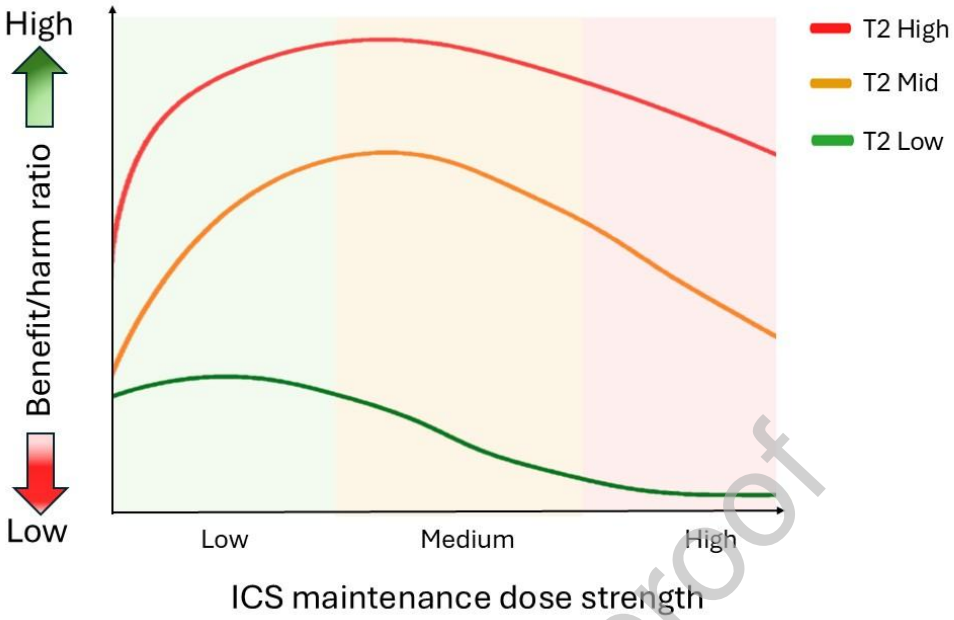


FIGURE 5. After the Escalator: slow and fast lanes for asthma diagnosis and management.A proposed biomarker-guided clinical framework based on the evidence supporting clinical utilities of raised type-2 biomarkers in suspected asthma and in established asthma. Incorporating these biomarkers into routine practice enables more personalized and biologically informed care pathways. In particular, patients with T2-high asthma may benefit from timely, targeted therapies, while those with T2-low asthma can be spared unnecessary escalation of anti-inflammatory treatment.AIR, anti-inflammatory reliever; dx, diagnostic; eos, eosinophils; FeNO, fractional exhaled nitric oxide; ICS, inhaled corticosteroids.

Figure 5

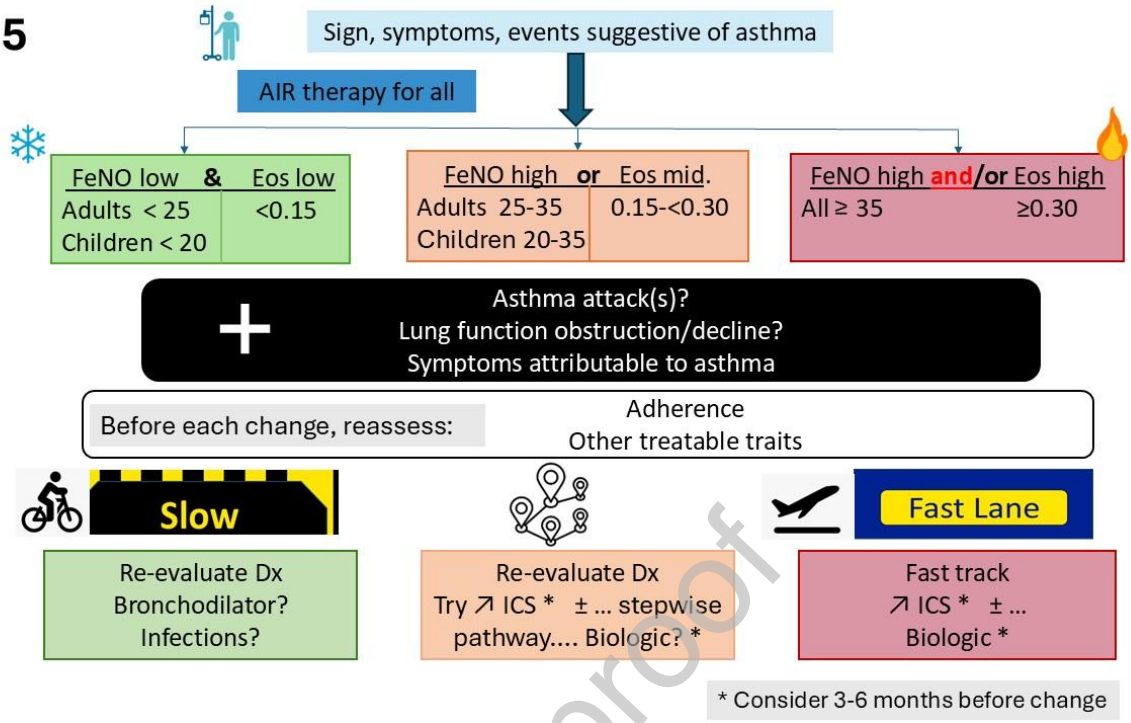


TABLE 1 – Selected factors* influencing type-2 biomarkers.

Factors influencing exhaled nitric oxide (FeNO) results

Decreasing values	Variable effect	Increasing values
◦ Inhaled > oral corticosteroids	◦ Age (pre-puberty > adolescence > adults)	◦ Atopy
◦ Anti-TSLP	◦ Diurnal variation (morning > evening)	◦ Nasal polyposis
◦ Anti-IL-4/-13		◦ Rhinitis
◦ Current smoking		◦ Recent respiratory viral infection
◦ Forced spirometry prior to the measurement		◦ Recent oral nitrate intake

Factors influencing blood eosinophil counts

Decreasing values	Variable effect	Increasing values	
◦ Oral > inhaled corticosteroids	◦ Age (pre-puberty > adolescence > adults)	◦ Anti-IL4R	◦ Atopic dermatitis
◦ Anti-IL-5/5R	◦ Diurnal variation (morning > evening)	◦ Atopy	◦ EGPA
◦ Anti-TSLP		◦ Nasal polyposis	◦ ABPA
		◦ Rhinitis	◦ HES
		◦ Recent respiratory viral infection	◦ Helminth infection

*List is non-exhaustive. ABPA, allergic bronchopulmonary aspergillosis; EGPA, eosinophilic granulomatosis with polyangiitis; HES, hypereosinophilic syndrome; IL, interleukin; TSLP, thymic stromal lymphopoietin. Adapted from [164–167]

TABLE 2 IDENTIFIED STUDIES SUPPORTING RECOMMENDATIONS ON BIOMARKER-BASED DIAGNOSIS OF ASTHMA IN NON-CONCLUSIVE SPIROMETRY.

Study Author (Year); Country, N	Clinical features	Setting	Referral Source	Study Design	Diagnostic Criteria (Gold standard) [1]	FeNO cut off (ppb)	FeNO cutoff Performance (%)	Blood Eos cut off ($\times 10^9$/L)	Blood Eos cutoff performance
Bai (2023)[156]; China, 307	CVA symptoms	Asthma clinic (outpatient)	No referral source specified	Prospective	BPT (Histamine)	27	Se : 78.9 Sp : 79.3	NA	NA
Karamarkovic (2023)[157]; Croatia, 282	Asthma symptoms	Asthma clinic (outpatient)	Primary care	Retrospective	BPT (Methacholine)	25	Se : 75.4 Sp : 47.9	NA	NA
Li (2019)[66]; China, 1542	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Retrospective	BPT (Methacholine)	32	Se : 90.7 Sp : 100	NA	NA
Hou (2021)[158]; China, 534	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Cross-sectional	BPT (Methacholine)	43	Se : 61.2 Sp : 84.1	NA	NA
Kellerer (2021)[68]; Germany, 393	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Retrospective	BPT (Metacholine)	46	Se : 23.4 Sp : 92.1	NA	NA

Chen (2021)[159]; China, 328	CVA symptoms	Asthma clinic (outpatient)	No referral source specified	Retrospective	BDT or BPT (Metacholine)	24.5	Se : 69.6 Sp : 72.9	NA	NA
Borhani Fard (2021)[160]; Iran, 87	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Cross-sectional	BPT (Methacholine)	39.5	Se : 48.6 Sp : 94.0	NA	NA
Urbankowski (2021)[161]; Poland, 42	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Cross-sectional	BPT (Metacholine)	24	Se : 70 Sp : 77.3	0.15	81/75

Study Author (Year); Country, N	**Clinical features**	**Setting**	**Referral Source**	**Study Design**	**Diagnostic Criteria (Gold standard)[1]**	**FeNO cutoff (ppb)**	**FeNO cutoff Performance (%)**	**Blood Eos cutoff ($\times 10^9$/L)**	BLOOD EOS CUTOFF PERFORMANCE
Nekooee[162] (2020); Belgium, 702	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Retrospective	BDT or BPT (Metacholine)	36	Se : 30 Sp : 85	NA	NA
Liu (2019)[163]; China, 259	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Retrospective	BPT(Methacholine)	43.5	Se : 75.6 Sp : 86.6	NA	NA
He (2018)[164]; China, 239	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Retrospective	BPT (Methacholine)	23.5	Se : 77.8 Sp : 67.9	NA	NA
Feng-jia (2017)[165]; China, 405	CVA symptoms	Asthma clinic (outpatient)	No referral source specified	Retrospective	BPT (Histamine)	25	Se : 81.3 Sp : 85	NA	NA
Porpodis (2017)[166]; Greece, 31	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Cross-sectional	BPT (Metacholine/ Mannitol)	20	Se : 50 Sp : 91.7	NA	NA
Nickels (2016)[167]; USA, 774	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Retrospective	BPT (Methacholine)	50	Se : 12 Sp : 89	NA	NA

Wang (2015)[168]; China, 408	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Retrospective	BPT (Methacholine)	64	Se : 52 Sp : 94.3	NA	NA
Buslau (2014)[169]; Germany, 73	AR	Asthma clinic (outpatient)	Volunteers (public ads/ university students)	Cross-sectional	BPT (Metacholine)	18	Se : 74.4 Sp : 61.1	0.12	69.2/52.8
Cirillo (2013)[170]; Italy, 211	AR	Asthma clinic (outpatient)	Mandatory health check-up (military personnel)	Cross-sectional	BPT (Methacholine)	37	Se : 79.1 Sp : 90.5	NA	NA

Study Author (Year); Country, N	Clinical features	Setting	Referral Source	Study Design	Diagnostic Criteria (Gold standard)[1]	FeNO cutoff	FeNO cutoff Performance (%)	Blood Eos cut off	Blood Eos cutoff performance
Katsoulis (2013)[171]; Greece, 112	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Prospective	BPT (Methacholine)	32	Se : 47 Sp : 85	NA	NA
Voutilainen (2013)[172]; Finaland, 87	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Prospective	BPT (Methacholine/ Histamine)	30	35/84	NA	NA
Schleich (2012)[173]; Belgium, 174	Asthma symptoms	Asthma clinic (outpatient)	Specialist respiratory physician	Prospective	BPT (Methacholine)	34	35/95	NA	NA
Matsunaga (2011)[174]; Japan, 366	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Cross-sectional	BPT	22	Se : 90.8 Sp : 83.9	NA	NA
Leon de la Barra (2011)[175]; New Zealand, 52	Asthma symptoms	Primary care – GP	Primary care	Retrospective	BDT or BPT (Methacholine)	50	Se : 76.5 Sp : 94.1	NA	NA
Pedrosa (2010)[176]; Spain, 115	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Prospective	BPT (Metacholine)	40	Se : 74.3 Sp : 72.5	NA	NA
Kowal (2009)[177]; Poland, 540	Asthma symptoms	Asthma clinic (outpatient)	Primary care	Retrospective	BPT (Methacholine)	40	Se : 88.3 Sp : 82.6	NA	NA

Schneider (2009)[178]; Germany, 126	Asthma symptoms	Primary care – GP	Primary care	Prospective	BDT or WBP or BPT (Metacholine)	46	Se : 32 Sp : 93	NA	NA
Miedinger (2007)[179]; Switzerland, 106	Occupational Asthma	Work-based screening Firefighter	No referral source specified	Cross-sectional	BPT (Methacholine/ Mannitol)	47	Se : 42 Sp : 96	NA	NA
Study Author (Year); Country, N	**Clinical features**	**Setting**	**Referral Source**	**Study Design**	**Diagnostic Criteria (Gold standard)[1]**	**FeNO cut off**	**FeNO cutoff Performance (%)**	**Blood Eos cut off**	**Blood Eos cutoff performance**
Fortuna (2007)[180]; Spain, 50	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Prospective	BPT (Metacholine)	20	Se : 74 Sp : 64	NA	NA
Arora (2006); USA, 172	Asthma symptoms	Asthma clinic (outpatient)	Referred by no Allergy physician	Prospective	BPT (Histamine)	46	Se : 17 Sp : 100	NA	NA
Heffler (2006)[181]; Italy, 48	AR and asthma symptoms	Allergy clinic	No referral source specified	Prospective	BDT or BPT (Metacholine)	36	Se : 78 Sp : 60	NA	NA
Smith (2004)[182]; New Zealand, 44	Asthma symptoms	Asthma clinic (outpatient)	Primary care	Prospective	BDT or BPT (hypertonic saline)	20	Se : 88 Sp : 79	NA	NA
Dupont (2003)[183]; Belgium, 240	Asthma symptoms	Asthma clinic (outpatient)	No referral source specified	Prospective	BDT or BPT (Histamine)	16	Se : 69.4 Sp : 90.0	NA	NA

Chatkin (1999)[1] [84]; Canada, 38	CVA symptoms	Asthma clinic (outpatient)	Referred by specialist	Cross-sectional	BDT or BPT (Metacholine)	30	Se : 75 Sp : 87	NA	NA

[1]The primary gold standard diagnosis consists of a direct methacholine provocation test with PC20 ≤8 mg/ml or PD20 ≤200 µg, or other direct and indirect provocation tests in accordance with European (preferred) and/or American guidelines (complementary) [25]. Direct methacholine tests with higher thresholds (PC20 ≤16 mg/ml, PD20 ≤400 µg) will also be considered in sensitivity analyses [26]. Spirometry with a bronchodilator response (BDR) is considered adequate for confirming asthma if it shows a variation of ≥12% and, for adults, an increase in FEV_1 of ≥200 ml from baseline in pre/post-BD testing ("standard criteria" for secondary analysis). Alternatively, a variation of >10% compared to predicted values for FEV_1 or FVC in pre/post-BD spirometry is acceptable ("alternative criteria" for secondary analysis).[64]

AR, allergic rhinitis; BDT, bronchodilator test; Blood Eos, blood eosinophil count; BPT, bronchial provocation test; CVA, cough variant asthma; FeNO, fractional exhaled nitric oxide; GP, general practitioner; NA, non-atopic; Se, sensitivity; Sp, specificity; WBP, whole body plethysmography.