Phenotyping of Severe Asthma in the Era of Broad-Acting Anti-Asthma Biologics

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Severe asthma is associated with significant morbidity and mortality despite the maximal use of inhaled corticosteroids and additional controller medications, and has a high economic burden. Biologic therapies are recommended for the management of severe, uncontrolled asthma to help to prevent exacerbations and to improve symptoms and health-related quality of life. The effective management of severe asthma requires consideration of clinical heterogeneity that is driven by varying clinical and inflammatory phenotypes, which are reflective of distinct underlying disease mechanisms. Phenotyping patients using a combination of clinical characteristics such as the age of onset or comorbidities and biomarker profiles, including blood eosinophil counts and levels of fractional exhaled nitric oxide and serum total immunoglobulin E, is important for the differential diagnosis of asthma. In addition, phenotyping is beneficial for risk assessment, selection of treatment, and monitoring of the treatment response in patients with asthma. This review describes the clinical and inflammatory phenotypes of asthma, provides an overview of biomarkers routinely used in clinical practice and those that have recently been explored for phenotyping, and aims to assess the value of phenotyping in severe asthma management in the current era of biologics. **(J Allergy Clin Immunol Pract 2024;12:809-23)**

Key words: *Biomarker; Phenotyping; Eosinophil; Fractional exhaled nitric oxide; Biologic; Severe asthma*

Severe asthma is associated with greater morbidity and mortality than nonsevere disease, and has a significant economic burden.[1,2] An estimated 262 million people globally had asthma in 2019,[3] and it is generally estimated that 5% to 10% of

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Abbreviations used
AHR- Airway hyper-responsiveness
BEC- Blood eosinophil count
CI- Confidence interval
FeNO- Fractional exhaled nitric oxide
HRQoL- Health-related quality of life
ICS- Inhaled corticosteroid
ILC2- Type 2 innate lymphoid cell
ISAR- International Severe Asthma Registry
OCS- Oral corticosteroid
SARP- Severe Asthma Research Program
T2- Type 2
Th- T-helper
TSLP- Thymic stromal lymphopoietin
U-BIOPRED- Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes
UKSAR- UK Severe Asthma Registry

patients with asthma have severe disease that in some cases remains uncontrolled despite the maximal use of inhaled corticosteroids (ICS) and additional controller medications.[4] Adjunct treatments, such as biologic therapies, are recommended for these patients to help to prevent exacerbations, improve symptoms,[5,6] and reduce ICS and oral corticosteroid (OCS) use in those with corticosteroid-dependent asthma.

The effective management of severe asthma requires consideration of the marked heterogeneity in disease triggers and drivers, the severity of symptoms, the nature of inflammation, the response and adherence to treatment, and often the presence of multiple comorbidities.[7] This heterogeneity has driven research to define asthma phenotypes that ultimately reflect different underlying disease mechanisms, including pathogenetic processes and responses to environmental exposures.[8,9] These phenotypes are not fixed and can evolve over time in response to new environmental triggers, such as viral infections, cigarette smoking, and air pollution.[10]

Two broad classes of phenotypes are commonly used to help to differentiate and manage severe asthma: clinical phenotypes and inflammatory phenotypes (Figure 1).[11] Biomarkers are key to defining asthma phenotypes, although they should not be used in isolation from clinical characteristics including age, age of asthma onset, obesity, or the presence of allergic sensitization. Phenotyping patients is important for the differential diagnosis of asthma, risk assessment, treatment selection, and monitoring of the treatment response.[12] Accurate identification of the phenotype has been especially important for the choice of biologic to treat severe asthma and for predicting the response to treatment. Until recently, all of the approved biologics targeted specific immunologic pathways downstream in the inflammatory cascade (Table I).[13-35] A broad-acting biologic—tezepelumab—was approved for the treatment of severe asthma in 2021 by the US Food and Drug Administration and in 2022 by the European Medicines Association.[31,32] Unlike other approved biologics, its target, the epithelial cytokine thymic stromal lymphopoietin (TSLP), is upstream in the inflammatory cascade and is involved in both the initiation and persistence of airway inflammation in asthma.[36,37] Based on efficacy results from randomized controlled trials, the label indication is not restricted to a biomarker-defined phenotype (Table I).[31,32] However, phenotyping may still be required in patients receiving tezepelumab because the efficacy of tezepelumab in suppressing exacerbations and improving lung function has been shown to be greater with increasing type 2 (T2) biomarker levels in patients with severe, uncontrolled asthma.[34]

Using available published literature, this review aims to assess the value of phenotyping in severe asthma management in the current era of biologics by (1) summarizing clinical and inflammatory phenotypes in the context of asthma heterogeneity, (2) discussing the types of biomarkers that are routinely used, (3) providing evidence to support the use of 1 or more biomarkers in combination to distinguish between overlapping asthma phenotypes, and (4) discussing the role of phenotyping in the assessment and management of severe asthma.

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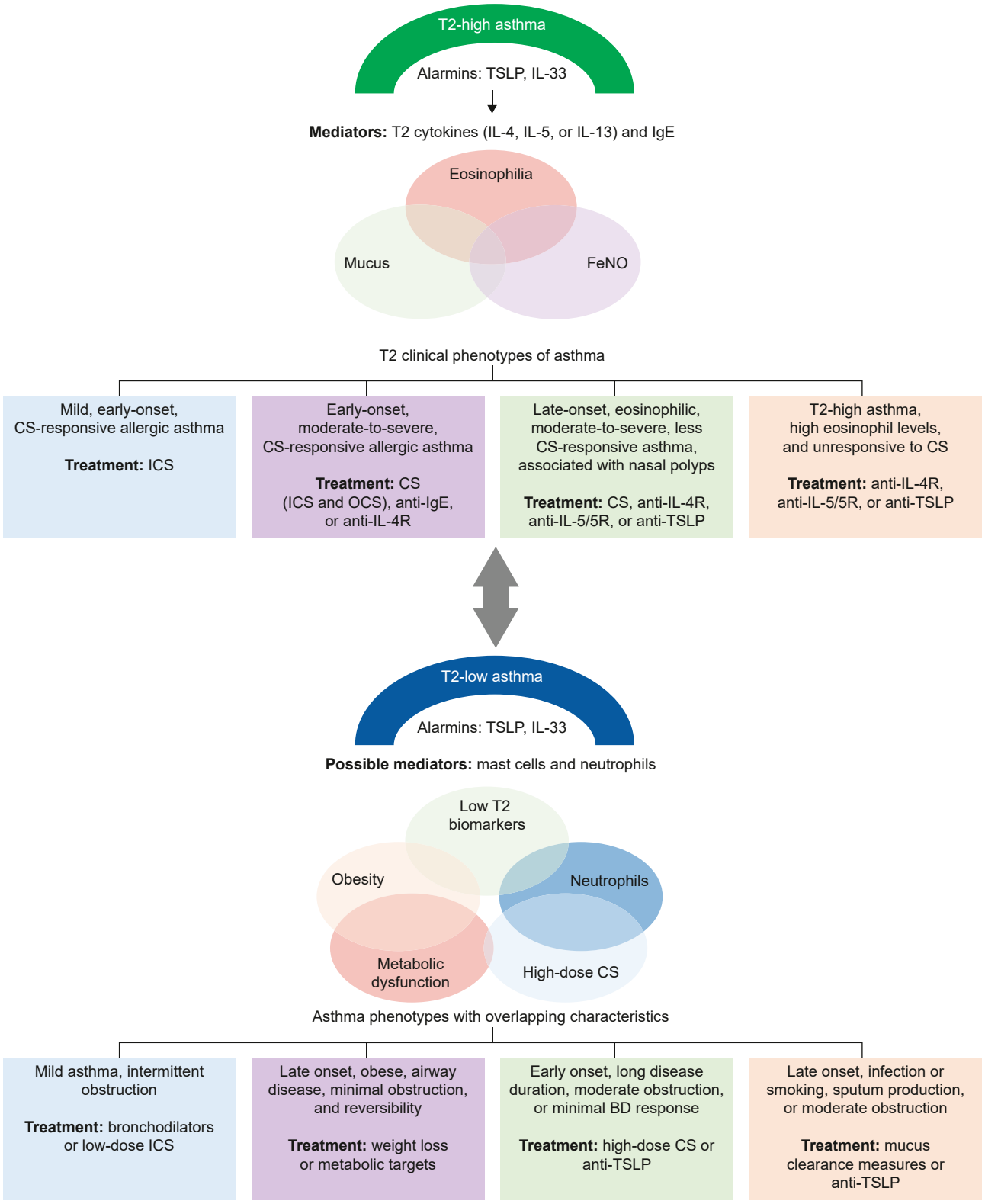

FIGURE 1. Clinical and inflammatory phenotypes of asthma. Reproduced and adapted from Ray et al, 2020[11] with permission from the American Physiological Society. *BD*, Bronchodilator; *CS*, corticosteroid; *FeNO*, fractional exhaled nitric oxide; *ICS*, inhaled corticosteroid; *OCS*, oral corticosteroid; *T2*, type 2; *TSLP*, thymic stromal lymphopoietin.

TABLE I. Biologics approved for the treatment of severe asthma, and the biomarkers associated with their use

Biologic	Molecular target	FDA first approval date, current asthma indication	EMA first approval date, current asthma indication	Phenotyping biomarkers required by label or suggested by guidelines	Effects on asthma outcomes
Omalizumab	IgE	• June 2003[13] • Moderate-to-severe, persistent asthma in patients aged ≥6 years with a positive skin test result or *in vitro* reactivity to a perennial aeroallergen, and symptoms that are inadequately controlled with ICS[13]	• October 2005[14] • Age ≥12 years: add-on therapy to improve asthma control in patients with severe, persistent allergic asthma who have a positive skin test result or *in vitro* reactivity to a perennial aeroallergen, who have reduced lung function (FEV_1 <80%), who have frequent daytime symptoms or nighttime awakenings, and who have had multiple documented severe asthma exacerbations despite daily high-dose ICS plus a LABA[14] • Age 6 to <12 years: same indication but without the FEV_1 criterion[14]	• FDA and EMA dosing schedules: require baseline serum IgE to be ≥30 IU/mL[13,14] • ERS/ATS guidelines: suggest a BEC cutoff of ≥260 cells/μL and a FeNO cutoff of ≥19.5 ppb to identify patients with severe allergic asthma more likely to benefit from anti-IgE treatment[6]	• Reduces asthma exacerbations and OCS requirement; improves asthma control[15] • Reduces health care resource utilization in patients with allergic asthma[16]
Mepolizumab	IL-5	• April 2015[17] • Add-on maintenance treatment of patients with severe asthma aged ≥12 years and with an eosinophilic phenotype[17]	• December 2015[18] • Add-on treatment for severe refractory eosinophilic asthma in adults, adolescents, and children aged ≥6 years[18]	• GINA guidelines: BEC threshold according to local criteria, for example, ≥150 cells/μL or ≥300 cells/μL[5] • ERS/ATS guidelines: suggest a BEC cutoff of ≥150 cells/μL to guide anti-IL-5 initiation in adults with severe asthma and a history of asthma exacerbations*[6]	• Reduces asthma exacerbations and OCS requirement[19] • Improves asthma control in patients with eosinophilic asthma[19]
Benralizumab	IL-5Rα	• November 2017[20] • Add-on maintenance treatment of patients with severe asthma aged ≥12 years and with an eosinophilic phenotype	• January 2018[21] • Add-on maintenance treatment in adult patients with severe eosinophilic asthma inadequately controlled despite high-dose ICS plus a LABA	• GINA guidelines: BEC threshold according to local criteria, for example, ≥150 cells/μL or ≥300 cells/μL[5] • ERS/ATS guidelines: suggest a BEC cutoff of ≥150 cells/μL to guide anti-IL-5 initiation in adults with severe asthma and a history of asthma exacerbations*[6]	• Reduces asthma exacerbations and OCS requirement • Improves lung function in patients with severe, uncontrolled asthma and eosinophilic inflammation[22,23]
Reslizumab	IL-5	• March 2016[24] • Add-on maintenance treatment of patients with severe asthma aged ≥18 years and with an eosinophilic phenotype	• August 2016[25] • Add-on therapy in adult patients with severe eosinophilic asthma inadequately controlled despite high-dose ICS plus another medicinal product for maintenance treatment	• GINA guidelines: BEC threshold according to local criteria, for example, ≥150 cells/μL or ≥300 cells/μL[5] • ERS/ATS guidelines: suggest a BEC cutoff of ≥150 cells/μL to guide anti-IL-5 initiation in adults with severe asthma and a history of asthma exacerbations*[6]	• Reduces the risk of asthma exacerbations and OCS use, and improves lung function and HRQoL in patients with high BECs[26,27]

Dupilumab	IL-4Rα	• October 2018[28] • Add-on maintenance treatment of patients with moderate-to-severe eosinophilic or OCS-dependent asthma aged ≥6 years	• March 2019[29] • Adults and adolescents aged ≥12 years as add-on maintenance treatment for severe asthma with T2 inflammation characterized by raised BEC and/or raised FeNO, who have inadequately controlled asthma with high-dose ICS plus another medicinal product for maintenance treatment	• GINA guidelines: payers usually require T2 biomarkers above locally specified levels (eg, BEC ≥150 cells/μL and ≤1500 cells/μL; or FeNO ≥25 ppb) or a need for maintenance OCS[5]	• Reduces severe exacerbations and OCS requirement, and improves lung function and asthma control in patients with uncontrolled, moderate-to-severe asthma[30]
Tezepelumab	TSLP	• December 2021[31] • Add-on maintenance treatment of adult and pediatric patients aged ≥12 years with severe asthma	• September 2022[32] • Add-on maintenance treatment in adults and adolescents aged ≥12 years with severe asthma inadequately controlled despite high-dose ICS plus another medicinal product for maintenance treatment	• No requirement for phenotype or biomarkers[31,32] • Can be used in patients with no elevations in T2 biomarkers • Biomarkers can predict the magnitude of the response because the response is greater with higher baseline T2 biomarkers[33]	Reduces exacerbations and improves symptoms, asthma control, lung function, and HRQoL[34]

BEC, blood eosinophil count; *EMA*, European Medicines Agency; *ERS/ATS*, European Respiratory Society/American Thoracic Society; *FDA*, US Food and Drug Administration; *FeNO*, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; *GINA*, Global Initiative for Asthma; *HRQoL*, health-related quality of life; *ICS*, inhaled corticosteroid; *LABA*, long-acting β_2 agonist; *OCS*, oral corticosteroid; *T2*, type 2; *TSLP*, thymic stromal lymphopoietin.
*Conditional recommendation, low-quality evidence.

CLINICAL PHENOTYPES IN SEVERE ASTHMA

Well-established severe asthma clinical phenotypes are based on a combination of clinical characteristics that have been validated in clustering analyses of patients.[5,7,38-43] These include the timing of asthma onset (early vs late), atopy status (allergic vs nonallergic), lung function (persistence of airflow limitation), treatment response (OCS resistance and OCS-related comorbidities), and other comorbidities (eg, obesity, chronic rhinosinusitis with or without nasal polyps, gastroesophageal reflux disease, atopic dermatitis, urticaria, eosinophilic esophagitis, and aspirin-exacerbated respiratory disease).[44-46] Notably, some of the clinical comorbidities that coexist in some patients may also improve with the use of biologics for the treatment of severe asthma.[47]

A cluster analysis by Haldar et al[38] identified distinct clinical asthma phenotypes with varying responses to treatment regimens. Although a direct relationship between eosinophilic inflammation and symptoms has traditionally been used to guide treatment, the cluster analysis suggested that a symptom-led approach would be effective in guiding treatment for patients with early-onset, mild-to-moderate asthma only, because they have concordance between inflammation and symptoms. In contrast, patients with refractory asthma, those who are obese, and those who have a noneosinophilic phenotype have a disconnect between inflammation and symptoms, which predisposes them to failure of the conventional treatment regimens.[38] This disconnect supports the measurement of airway eosinophil counts in these patients and implies that failing to measure biomarkers may lead to erroneous prognostication and therapeutic outcomes. Similarly, the Severe Asthma Research Program (SARP) identified 5 phenotypic clusters of severe asthma, distinguished primarily by a combination of clinical phenotypes and biomarkers.[39,43]

INFLAMMATORY PHENOTYPES AND THE BIOLOGY OF SEVERE ASTHMA

Clinical asthma phenotypes are the products of underlying inflammatory processes, which may be categorized into 2 main overarching inflammatory phenotypes defined by the predominant immunological pathways driving the disease pathology: eosinophilic and noneosinophilic.[48] T2 immune responses in the airways are a type of airway inflammation mediated mainly by eosinophils, mast cells, basophils, T-helper (Th) 2 cells, T2 innate lymphoid cells (ILC2s), and IgE-producing B cells.[49] Upstream in the inflammatory cascade, the epithelial cytokines IL-33, IL-25, and TSLP (known as alarmins) regulate the maturation of CD4+ T cells to induce a Th2 adaptive immune response and to activate ILC2s.[49] The major downstream T2 cytokines in the airways are IL-5, IL-4, and IL-13, all of which play distinct roles in severe asthma pathology (Figure 2). Increased production of IL-5 induces hypereosinophilia, IL-4 mediates an isotype switch in B cells leading to the synthesis of IgE and subsequent elevations in levels of total and specific IgE, and IL-13 regulates the production of nitric oxide and smooth muscle contraction, with subsequent bronchial hyperresponsiveness.[37,49] Eosinophilic or T2-high phenotypes of severe asthma, which encompass allergic (adaptive) and nonallergic (innate or eosinophilic with ILC2 activation) asthma phenotypes, are characterized by elevated blood eosinophil counts (BECs) and fractional exhaled nitric oxide (FeNO) levels, and have been extensively reviewed.[11] Clinically, T2-high asthma is frequently

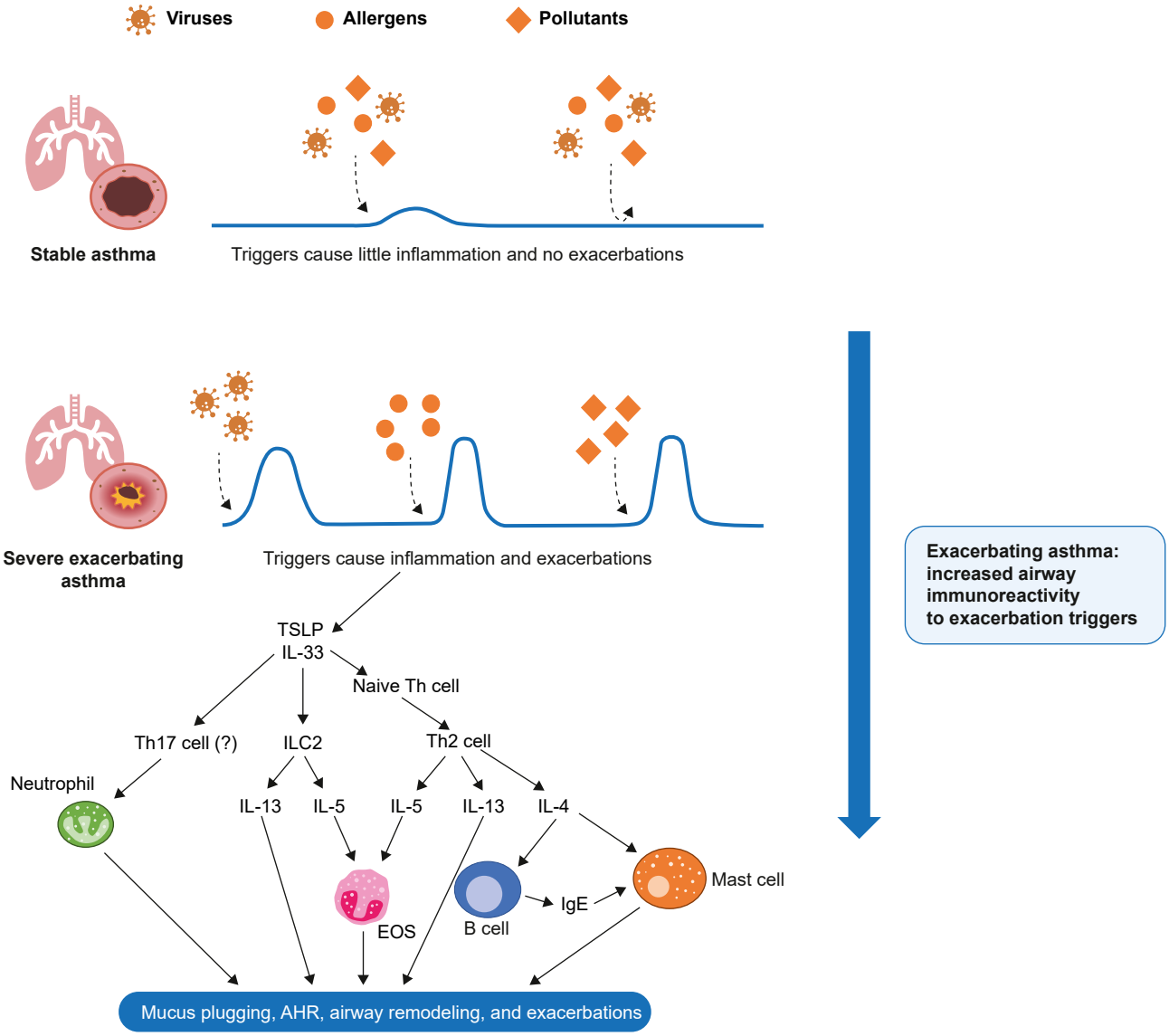

FIGURE 2. Increased airway immunoreactivity is a cardinal feature of exacerbating asthma. Severe exacerbating asthma is characterized by increased airway immunoreactivity to asthma triggers, which cause a flare in inflammation, asthma pathology, and symptoms. In contrast, patients with stable asthma do not have an aggravated response to asthma triggers. *AHR*, Airway hyper-responsiveness; *EOS*, eosinophil; *ILC2*, type 2 innate lymphoid cell; *Th*, T-helper; *TSLP*, thymic stromal lymphopoietin.

associated with allergic rhinitis, chronic rhinosinusitis with and without nasal polyps, and other ear, nose, and throat comorbidities.[50]

The International Severe Asthma Registry (ISAR) identified 5 asthma phenotypes based on relative BECs and levels of FeNO and serum total IgE.[7] Although these phenotypes and the variations in biomarker levels between them suggest distinct underlying inflammatory pathways, overlapping clinical characteristics suggest that clinical phenotypes may not always match inflammatory phenotypes.[10] This further supports the need for the measurement of biomarkers to guide treatment.

Noneosinophilic or T2-low asthma is relatively understudied.[10] It is characterized by low levels of T2 biomarkers, neutrophilic or paucigranulocytic inflammation, and a nonallergic/noneosinophilic response.[51-53] Noneosinophilic or T2-low asthma has been linked to the activation of Th1 and/or Th17 cells.[10,36] TSLP is thought to be an upstream player in noneosinophilic or T2-low inflammation, activating dendritic cells to induce the polarization of naive T cells toward a Th17 phenotype,[54] leading to the release of IL-17.[36] Although there has been considerable interest in anti-IL-17 antibodies for the treatment of moderate-to-severe asthma, findings from clinical studies of brodalumab, a biologic targeting the IL-17 receptor, have not shown any improvement in asthma symptoms or lung function.[55] It is also important to note that noneosinophilic asthma is less frequently observed in real-world clinical practice than previously reported.[56] However, identifying a noneosinophilic or T2-low asthma phenotype in patients is challenging because of the lack of reliable, definitive biomarkers. In the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) study (ClinicalTrials.gov identifier:

TABLE II. Factors contributing to nonresponsiveness to corticosteroid treatment in patients with T2-low asthma

Potential causative factor	Examples
Genetic factors	• Defective GRα expression, binding and nuclear translocation[61] • Increased GRβ expression in airway epithelial cells[62] • Defective histone acetylation[62]
Respiratory infections	• *Chlamydia pneumoniae*, *Haemophilus influenzae*, influenza A virus, respiratory syncytial virus[61,62]
Exogenous factors	• Cigarette smoking[62] • A high-fat diet or obesity[62]
Intrinsic factors	• Absence of or low eosinophilic airway inflammation as a target for ICS treatment in noneosinophilic asthma[63] • Corticosteroid resistance measured by nonsuppression of FeNO, reported in patients who were older and had a low baseline BEC[64]

BEC, Blood eosinophil count; *FeNO*, fractional exhaled nitric oxide; *GR*, glucocorticoid receptor; *ICS*, inhaled corticosteroid; *T2*, type 2.

NCT01976767), which evaluated molecular phenotypes of asthma by analyzing sputum cell transcriptomics, the non-T2 phenotype was inferred from the absence of raised T2 biomarkers and, thus, the exclusion of T2-high asthma.[10,37,57] However, biomarker measurements must be repeated several times before the exclusion of a T2-high phenotype.[5] Moreover, by the time a patient undergoes asthma phenotyping, it can be difficult to ascertain their T2 status if they are already receiving ICS per treatment guidelines[5] or if they are receiving maintenance OCS, because both of these therapies reduce T2 biomarker levels.[58,59] A recent report from Gautam et al[60] proposed a modular signaling network, in which patients may have variable degrees of T2 and non-T2 inflammation, with some patients having low T2 biomarker levels despite high alarmin levels.

Clinically, noneosinophilic or T2-low asthma has been associated with obesity, smoking, very late onset (age >50 or >65 years, depending on the study), a lower risk of asthma attacks, and nonresponsiveness to corticosteroid treatment (Table II).[11,53,61-66] Although treatment guidelines for asthma recommend a stepwise increase in the ICS dose to control symptoms and reduce exacerbations without specifying T2-high or T2-low asthma,[5] 95% of patients with severe asthma and low levels of T2 biomarkers (blood eosinophils, FeNO, and periostin, another biomarker indicative of high IL-13 levels) at baseline demonstrated an increase in levels of these biomarkers during careful down-titration of corticosteroid (ICS and OCS) therapy.[67] In a study from the UK Severe Asthma Registry (UKSAR), 9.4% of the cohort fulfilled the definition of T2-low asthma (BEC <150 cells/μL; FeNO level <25 ppb) at registration.[68] Compared with patients who had T2-high asthma, those who had T2-low asthma were more likely to be women and have a higher prevalence of depression and anxiety, a higher rate of current smoking, and more maintenance OCS use. Of note, although BECs at registration were used to define patients' T2 status, analysis of historical BECs highlighted a high median (minimum, maximum) value of 350 cells/μL (130, 600) in the T2-low group, suggesting that most patients had an underlying eosinophilic phenotype.[68] Separately, in the ISAR cohort, the population with severe asthma was described as predominantly women, overweight, and in the age group of 55 to 79 years.[69] Analysis of biomarker distribution in the ISAR cohort found that 31% of patients had low BECs (<150 cells/μL) and 43% had low FeNO levels (<25 ppb); however, the underlying phenotypes were not assessed.[69]

The UK Refractory Asthma Stratification Programme biomarker study (ClinicalTrials.gov identifier: NCT02717689) assessed the proportion of patients with severe asthma who were able to reduce their corticosteroid (OCS and ICS) dose at the end of the study (week 48) in 2 patient groups.[67] One group underwent biomarker-directed corticosteroid reduction with protocolized weaning of OCS and ICS, and a control group continued to receive standard of care therapy based on asthma symptoms, lung function, and recent exacerbation history. Overall, biomarker-directed corticosteroid dose adjustment did not result in a greater proportion of patients reducing their corticosteroid dose compared with the control group. However, a *post hoc* analysis of this study in patients with uncontrolled asthma at baseline (defined as an Asthma Control Questionnaire-7 score ≥1.5) showed that a greater proportion of patients who underwent biomarker-directed corticosteroid reduction were receiving a lower dose of corticosteroid (OCS and ICS) treatment at the end of the study compared with the control group, (26.4% vs 5.7% of patients at week 48; adjusted odds ratio, 5.78 [95% confidence interval (CI): 1.27-26.2]; $P = .023$), with no difference in the exacerbation rate or worsening of asthma control, consistent with corticosteroid overtreatment in the T2-biomarker low group. Overall, 23.0% of the 209 patients who received biomarker-directed care had T2-low asthma at study entry. However, only 11 of these 209 patients (5.0%) were able to either down-titrate their ICS dose to the lowest dose and remain T2 biomarker-low, or remain serially biomarker-low without a reduction in corticosteroid treatment. This is consistent with the UKSAR data, suggesting that the T2-low phenotype is rare in severe asthma and, when seen clinically, is reflective of T2 biomarker suppression with corticosteroid treatment. Taken together, if a patient with T2 biomarker-low asthma receiving high-dose ICS or OCS treatment remains symptomatic (frequent) or exacerbation prone (less frequent), then consideration of nonasthma alternative mechanisms (eg, inducible laryngeal obstruction, dysfunctional breathing, and obesity) along with corticosteroid reduction is an essential step in the management of asthma. Therefore, the assessment of biomarker levels is an integral part of clinical phenotyping and is critical for guiding treatment in severe asthma. The recommended steps for phenotyping patients who are receiving corticosteroids and the significance of biomarkers are described in Figure 3.[67]

INFLAMMATORY BIOMARKERS IN SEVERE ASTHMA

Biomarkers are important not only for phenotyping asthma but also for diagnosis, evaluation of disease risk and severity, prognosis (risk of exacerbations), treatment selection, and

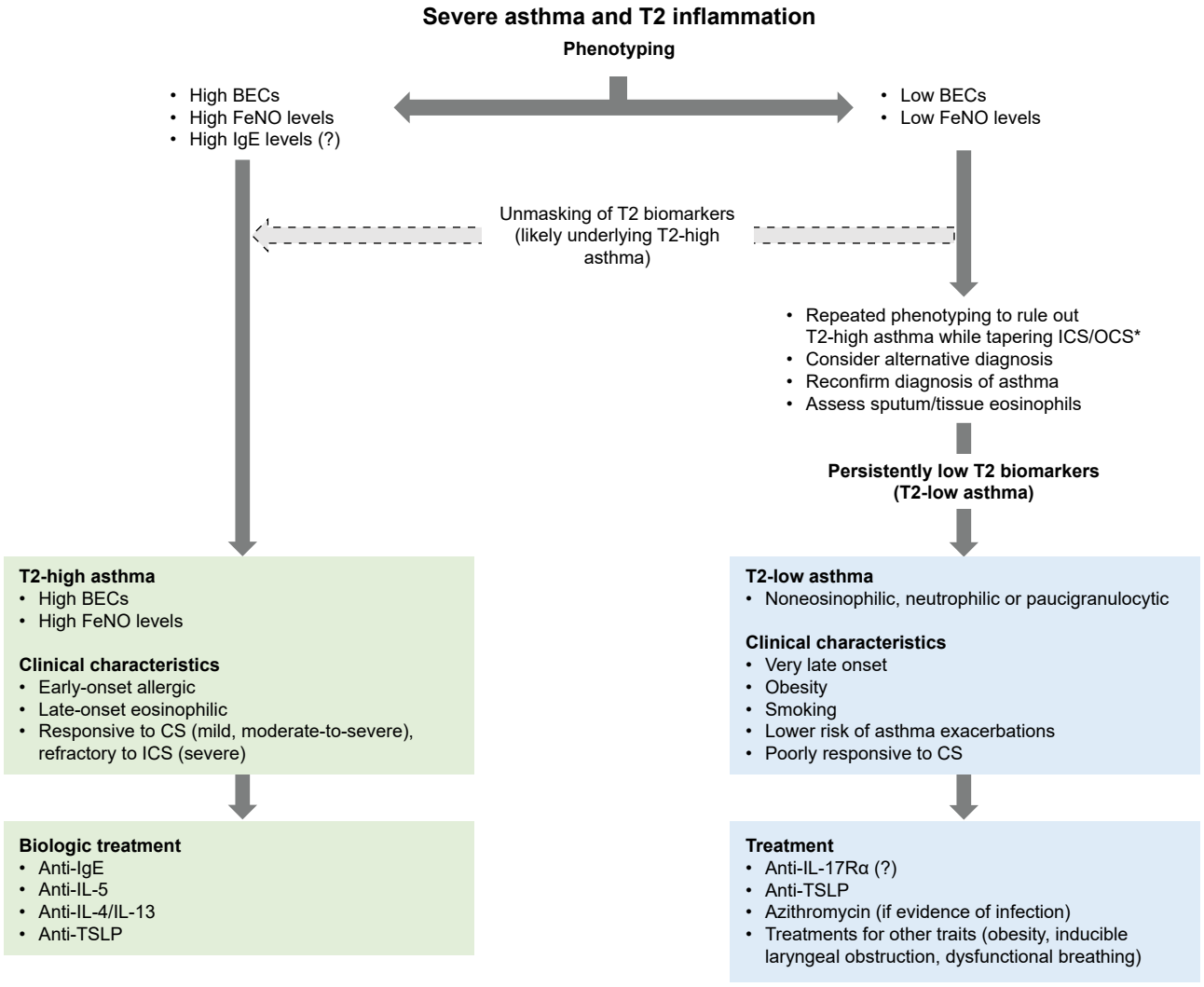

FIGURE 3. Phenotyping and therapeutic approaches for the management of patients with T2-high and T2-low asthma. *Heaney et al.[67] *BEC*, Blood eosinophil count; *CS*, corticosteroid; *FeNO*, fractional exhaled nitric oxide; *ICS*, inhaled corticosteroid; *OCS*, oral corticosteroid; *T2*, type 2; *TSLP*, thymic stromal lymphopoietin.

monitoring the response to treatment.[70] Blood eosinophils, FeNO, and serum total IgE are the most widely used biomarkers for the diagnosis of both T2-high and T2-low asthma in clinical practice. Patients with high levels of T2 inflammatory biomarkers are classified as having T2-high asthma. However, it is notable that 30% to 50% of patients have variable T2 status over time according to established cutoffs.[71]

Eosinophils play a key role in long-term airway inflammation,[72] contributing to immune response modulation, airway hyper-responsiveness (AHR), airway remodeling, and especially asthma exacerbations.[49] Eosinophilic asthma accounts for approximately 57% to 70% of patients with severe asthma.[7,73] Elevated peripheral BECs, alone or in combination with high FeNO levels, are associated with high levels of airway inflammation, poor asthma control, and an increased risk of severe exacerbations.[49,66,74] Assessment of sputum cell counts is important when BECs are within the normal range because sputum eosinophils can be elevated in 25% of patients with asthma in the absence of increased BECs. Higher eosinophil counts are also associated with poorer asthma control and a higher exacerbation risk.[75] BECs of at least 150 cells/μL are indicative of T2 inflammation[5] and are typically associated with at least 1 or 2 exacerbations requiring treatment with OCS.[76]

In patients with T2 inflammation, increased production of both IL-4 and IL-13 leads to the overexpression of inducible nitric oxide synthase in airway epithelial cells via the signal transducer and activator of transcription 6 pathway. This results in the overproduction of nitric oxide by the airway epithelium.[77,78] The American Thoracic Society 2021 guidelines state that high FeNO levels (≥20 ppb in children and ≥25 ppb in adults) are recommended to determine the T2 status of a patient with severe asthma and, therefore, to predict the response to biologic treatment.[79] FeNO levels have been shown to predict and positively correlate with the number of asthma exacerbations in randomized controlled trials[66,80-83] and can be measured via a simple, noninvasive, point-of-care test.[84] The ease of measurement of FeNO levels and their tendency to decrease after

appropriate ICS treatment are the basis for their use in monitoring ICS adherence (via the FeNO suppression test).[64,85]

Elevated serum total IgE levels, driven by IL-4-induced immunoglobulin class switching in B cells, are associated with allergic asthma and potentially reduced lung function.[49,86] However, both individuals with atopic and those with nonatopic asthma can have high serum total IgE levels,[87] reflective of T2 inflammation (T2-high asthma), whereas low or normal values do not exclude the presence of allergic asthma.[87,88] In a recent prospective study testing a panel of 43 perennial aeroallergens in 176 patients with severe asthma in Belgium, 68% of patients tested positive for at least 1 perennial aeroallergen, 43% for at least 4 perennial aeroallergens, and 13% for more than 10 perennial aeroallergens.[89] A higher number of prospective positive tests was associated with increased serum total IgE levels, reaching an 18-fold increase in patients with more than 10 positive tests versus those with nonpositive tests.

Although serum total IgE levels may be useful to prompt consideration of alternative or overlapping diagnoses (eg, allergic bronchopulmonary aspergillosis), they have not demonstrated prognostic value (to estimate the risk of asthma exacerbations) or theragnostic value (to estimate the likelihood of a response to treatment) when identifying a suitable biologic therapy.[34,81,90] The main clinical application for measuring serum total IgE levels is to estimate the optimal dosage of the anti-IgE antibody omalizumab for add-on treatment in moderate-to-severe allergic asthma.[49,91] However, serum total IgE levels are of limited value as a marker of the response to omalizumab treatment and for informing changes in dosage because levels increase after drug administration owing to the formation of omalizumab:IgE complexes.[13] In contrast, allergen-specific IgE levels are more closely associated with the severity of asthma, measured by the number of specific allergen sensitizations from skin tests, as demonstrated among inner-city children with asthma.[92] High allergen-specific IgE levels in mucosal tissues are not known to be reflective of serum total IgE levels and, therefore, may provide insight into the role of localized IgE in the pathogenesis or characteristics of allergic asthma.[93]

Additional biomarkers of interest in asthma phenotyping

In addition to the biomarkers routinely used in asthma phenotyping, other biomarkers have acquired more interest. Periostin, a matricellular protein secreted by bronchial epithelial cells and lung fibroblasts in response to IL-4 and IL-13, has been explored as a potential T2 biomarker of asthma. Serum periostin is predictive of sputum and blood eosinophilia, and, therefore, eosinophilic airway inflammation in patients with severe asthma.[94] However, owing to a lack of standardized measurement techniques and validated predicted values, serum periostin is not widely used as a biomarker for asthma phenotyping.[95] Other potential T2 biomarkers include dipeptidyl peptidase-4 (expressed in lung epithelial cells, endothelial cells, and submucosal glands), urinary bromotyrosine, monocyte chemoattractant protein-4, and eotaxin-2.[96] Further clinical studies are required to ascertain their validity as biomarkers of asthma.[96]

Diagnosing non-T2 asthma phenotypes using biomarkers is challenging. In a retrospective analysis of the SARP-3 cohort study, systemic IL-6 levels, but not BECs, together with metabolic dysfunction (including high body mass index) and increased prevalence of diabetes and hypertension, were shown to be significantly associated with exacerbation-prone asthma. However, both elevated BECs and IL-6 levels were predictive of exacerbations in the study population.[97] Increased IL-6 levels correlated with impaired lung function in patients with allergic asthma[98] and were associated with high-dose ICS use in patients with adult-onset asthma.[99] These findings suggest that systemic IL-6 can be considered as a potential biomarker for non-T2 asthma, although it is not asthma specific and may be elevated in other diseases.[100]

Complementary aspects of biomarkers

In severe asthma, BECs and FeNO levels assess different components and compartments of T2 immunity, providing complementary information.[76] The complexity of the inflammatory processes in severe asthma suggests that clinically accessible biomarkers should be used in combination, providing a biomarker "profile" to distinguish overlapping phenotypes.[49,76] Sputum induction remains the gold standard for phenotyping severe asthma and exacerbations but is technically demanding and not widely available. In the ISAR cohort, 59% of patients were positive for either 2 or 3 biomarkers (BECs $\geq$300 cells/μL, FeNO levels $\geq$25 ppb, and/or serum total IgE $\geq$75 kU/L); FeNO and blood eosinophil positivity were more likely to occur together than with IgE positivity.[7] Although biomarkers were not included in the cluster analysis in the SARP cohort owing to their availability in only a subset of patients, BECs and FeNO levels were found to be similar in all clusters identified. In contrast, serum total IgE levels were higher in clusters with atopic asthma than in those with nonatopic asthma.[39] Elevated BECs and FeNO levels are associated with an increase in airflow limitation and mucus plugging, and an increased risk of severe asthma exacerbations, mediated by an increase in airway T2 cytokines, chemokines, and alarmins.[81,101,102]

ROLE OF PHENOTYPING IN THE ASSESSMENT AND MANAGEMENT OF SEVERE ASTHMA

It has long been recognized that not all symptoms in patients with a reported diagnosis of severe asthma are due to airway dysfunction.[103,104] Patients should be assessed using a systematic, multidisciplinary evaluation to determine the contribution of all comorbid conditions, including extrapulmonary factors such as obesity, anxiety or depression, and chronic rhinosinusitis with nasal polyps. In a pivotal study exploring different phenotypes in severe asthma, a cluster analysis identified a population of patients who had severe symptoms, were predominantly women and obese, and had low T2 biomarker levels.[38] More recently, in a similar cohort of patients with severe asthma, women had a consistently higher symptom burden than men, but when adjusted for obesity and anxiety or depression, there was no difference between sexes.[105] This suggests that careful clinical consideration involving a multidisciplinary diagnostic team is particularly necessary for symptomatic patients with a T2-low phenotype identified by the absence of elevated T2 biomarkers.[5] The T2-low phenotype is often a patient with T2 asthma whose treatment has been escalated following symptom-directed guidelines. In fact, a patient with T2-low asthma who is very symptomatic and receiving high-dose asthma treatment is often indicative of comorbid disease. This symptom-high/T2 biomarker-low group is often very adherent to treatment. Thus, before further treatment escalation, it is imperative that there is a systematic assessment to identify comorbidities

contributing to the symptom burden to (1) limit adverse events from inappropriate escalation of anti-inflammatory asthma treatment and (2) prevent an inappropriate label of "asthma treatment failure."[106]

The treatable traits paradigm suggests a targeted, personalized medicine approach to address the different components contributing to a patient's symptom burden and impaired health-related quality of life (HRQoL).[107] This approach was used in a randomized controlled trial in patients with severe asthma.[108]

Phenotyping asthma is complex, can be time-consuming, and may be complicated by ongoing corticosteroid treatment.[109] Furthermore, T2 biomarkers on their own are not sufficient to diagnose asthma accurately.[110,111] For example, FeNO levels are often elevated in patients with allergic rhinitis but decreased in current smokers with chronic obstructive pulmonary disease or asthma, or in patients with asthma treated with high ICS doses.[112,113] Targeting treatment to the patient-specific biology using biomarker-based phenotyping and careful systematic assessment of associated comorbid conditions are the principles of optimizing disease control.[109]

Unsurprisingly, patients with high BECs (≥150 cells/μL) and FeNO levels (≥25 ppb) and severe airway disease respond 3-fold to 5-fold more to treatment with biologics that target epithelial or T2 cytokines than patients with no evidence of T2 inflammation.[33,114,115] Therefore, phenotyping is important to assess the risk of exacerbations and/or lung function decline in patients with severe asthma, as well as to select biologic treatment optimally.

PHENOTYPING TO GUIDE BIOLOGIC TREATMENT SELECTION FOR SEVERE ASTHMA

The first step in phenotyping for selection of a biologic treatment is to identify whether the patient has an exacerbation-prone phenotype,[97] followed by the presence of T2 inflammation. Several potential causes of a suboptimal response to biologic therapies have been described, including incorrect identification of the specific T2 pathways, comorbidities that reduce the room for improvement, insufficient treatment dose, autoimmune phenomena, infections, changes in the initial inflammatory phenotype, and adverse events caused by asthma medications.[116]

Most of the approved biologic therapies for severe asthma require or recommend phenotyping according to inflammatory biomarkers, as directed either by the product label or by treatment guidelines (Table I). Tezepelumab is approved for use in severe asthma without phenotypic restriction, based on the evidence obtained from its clinical trial program.

In the phase 3 NAVIGATOR trial (ClinicalTrials.gov identifier: NCT03347279), tezepelumab significantly reduced the annualized rate of asthma exacerbations compared with placebo, regardless of whether patients had elevated BECs or FeNO levels at baseline, and improved lung function, disease control, and HRQoL in patients with severe asthma. However, the relative and absolute reductions in exacerbation rates were markedly greater in patients with elevated T2 biomarkers than those with low T2 biomarker levels,[33] implying that biomarkers remain important to predict the maximal therapeutic response with tezepelumab. Tezepelumab has been shown to reduce exacerbations in patients with severe, uncontrolled asthma and low BECs (<300 cells/μL and <150 cells/μL) and FeNO levels (<25 ppb), as well as in patients with the clinically relevant "triple T2-low" phenotype (BECs <150 cells/μL, FeNO levels <25 ppb, and absence of perennial allergy).[34] Tezepelumab has also demonstrated broad anti-inflammatory effects by reducing serum levels of IL-5, IL-13, total IgE, and FeNO in the PATHWAY (ClinicalTrials.gov identifier: NCT02054130) and NAVIGATOR studies,[33,114] reducing circulating and airway eosinophils in the CASCADE study (ClinicalTrials.gov identifier: NCT03688074),[117] and attenuating AHR to methacholine and mannitol via T2-independent mechanisms (likely involving mast cell and airway smooth muscle effects).[117-119] However, in patients with a T2-low phenotype, it is also important to ensure asthma as the cause of symptoms and to address other identified treatable traits for optimal treatment.

In the phase 3 SOURCE study (ClinicalTrials.gov identifier: NCT03406078), tezepelumab did not demonstrate a significant OCS-sparing effect versus placebo in the overall population, although an improvement with tezepelumab versus placebo was observed in patients with baseline BECs of at least 150 cells/μL.[120] The ongoing open-label WAYFINDER study (ClinicalTrials.gov identifier: NCT05274815) will further evaluate the OCS-sparing effect of tezepelumab and will provide additional insights into whether anti-TSLP may have different targets and mechanisms of action in severe asthma compared with systemic corticosteroids.

Besides tezepelumab, other biologics that target epithelial alarmins are in development for severe asthma including anti-IL-33 antibodies (eg, anti-ST2 astegolimab, itepekimab, and tozorakimab).[121-123] Early clinical data from trials of anti-IL-33 antibodies in patients with asthma and chronic obstructive pulmonary disease suggest that this therapy could benefit patients with either T2 or non-T2 asthma.[122] Preclinical data on an anti-IL-25 antibody suggest that blocking IL-25 can suppress the T2 cytokine response to rhinovirus infection in bronchial epithelial cells obtained from patients with eosinophilic asthma.[124] Stem cell factor and its receptor, c-kit, are involved in the development of mast cells. The presence of mast cells in airways is associated with poor symptom control in patients with severe asthma. Early trials of imatinib, a tyrosine kinase inhibitor that blocks c-kit, showed that imatinib reduced AHR, mast cell counts, and tryptase levels in patients with severe asthma, suggesting mast cells as a potential therapeutic target for the treatment of asthma.[125]

CLINICAL VALUE OF ONGOING PHENOTYPE-DIRECTED CARE OF PATIENTS WITH SEVERE ASTHMA

Despite the availability of a next-generation therapeutic that is not restricted for use in specific asthma phenotypes, routine phenotyping of patients with severe asthma continues to be important for monitoring the response to treatment and to guide treatment decisions (eg, when to switch).[5] It is also important to note that a phenotype can change over time.

More recently, biomarkers have been used to predict clinical remission or a super-response in patients with asthma.[126,127] Currently, there is no standard definition for clinical remission in asthma. Proposed definitions from asthma societies and consensus groups generally include at least 12 months with no significant symptoms, no exacerbations, stable and optimized lung function, and no use of systemic corticosteroids.[128-130]

Beyond clinical remission, a complete remission definition would eventually also include that inflammatory biomarker levels are maintained at low levels. Therefore, stable biomarker levels over time in the absence of symptoms, exacerbations, and corticosteroid use can be used for monitoring the response to treatment and to predict clinical remission. Elevated levels of sputum T2 biomarkers (eg, IL-5, eosinophil peroxidase, and eotaxin-1) and low levels of neutrophils were demonstrated to predict remission after anti-IL-5 therapy in a small cohort of patients with severe eosinophilic asthma.[127] The largest real-world evaluation of clinical remission in patients with severe asthma treated with biologic therapies showed that patients who were composite T2-biomarker high (BEC $\geq$150 cells/μL and FeNO level $\geq$20 ppb) were 7.44-fold more likely to achieve clinical remission than those who were composite T2-biomarker low (95% CI: 1.73-31.95, $P = .007$).[131] Furthermore, real-world observational data from the Australian Mepolizumab Registry identified 24% (n = 61/252) of the cohort as super-responders (top 25% of responders, according to Australian Pharmaceutical Benefits Scheme—defined Asthma Control Questionnaire-5 responses) who achieved well-controlled symptoms and improved HRQoL and lung function after 6 months of treatment. In this study, super-responders had a higher T2 disease burden and fewer comorbidities at baseline than the bottom 25% of responders.[132] These findings suggest that appropriate biomarker phenotyping and targeting with the relevant biologic therapy at an early stage of disease may be beneficial in patients with severe asthma.

Despite the conceptual value of measuring biomarkers after the initiation of biologic therapy, they are not routinely used to monitor treatment responses in clinical practice. The BEC offers value as a biomarker after commencement of treatment with all of the currently approved biologics, although with conflicting data for omalizumab.[133-136] Anti-IL-5 agents significantly decrease or completely diminish BECs;[17,20,24] thus, BEC as a biomarker does not predict a response or lack of response to anti-IL-5 therapy once initiated. However, a failure to lower BECs indicates that anti-IL-5 therapy is not working. FeNO levels remain elevated in patients receiving anti-IL-5 treatment. It is unclear whether persistence of high FeNO levels is associated with a different outcome to anti-IL-5 therapy.

Exacerbations in T2-low asthma are complex and poorly understood. Findings from the MEX study (ClinicalTrials.gov identifier: NCT03324230) challenge the practice of switching biologic therapies because of treatment failure without first profiling the inflammatory phenotype of ongoing asthma exacerbations.[137] The MEX study showed that, despite treatment with mepolizumab, approximately half of the asthma exacerbations were still eosinophilic. The remaining half (ie, non-eosinophilic exacerbations) were linked to an increase in symptoms and a decrease in lung function at the time of the exacerbation, which were similar to the effects observed for eosinophilic exacerbations.[137] Airway infection may be an important contributor to asthma symptoms in this T2 biomarker-low population, and a trend toward increased infections in patients with T2-low exacerbations has also been seen.[138] Understanding the mechanisms of and possible treatment options for T2-low, severe asthma remains an unmet clinical need (Figure 3). New approaches to phenotyping described below may be helpful in the identification, treatment, and management of T2-low asthma.

NEW APPROACHES TO PHENOTYPING FOR THE DIAGNOSIS OF SEVERE ASTHMA AND THE MANAGEMENT OF PATIENTS

Phenotyping early

Patients who are not responding well to low-dose ICS and a long-acting β_2-agonist should be phenotyped early based on BECs, FeNO levels, and lung function to assess treatment adherence and to support appropriate treatment decisions.[67] Elevated T2 biomarker levels have been associated with a decline in lung function, both in healthy individuals and in patients with asthma.[139-141] As such, early phenotyping using easily accessible biomarkers (blood eosinophils and FeNO) allows the early initiation of appropriate targeted treatment for patients with T2-high asthma, who typically have more severe asthma than those with T2-low asthma.[142] Early treatment can prevent airway remodeling by reducing inflammation and exacerbations, thus preventing lung function decline.[117] Furthermore, targeting the right patients early can prevent extrapulmonary consequences (dubbed "people remodeling"),[143] which can occur owing to recurrent, acute asthma attacks, airway remodeling, or frequent use of OCS and associated adverse events, all of which eventually lead to impaired HRQoL.[143]

Phenotyping based on BECs and FeNO levels using the prototype Oxford Asthma Attack Risk Scale (ORACLE) can provide a quantifiable measure of the risk of future exacerbations and can help to predict the reduction in that risk provided by T2 anti-inflammatory therapy.[115] This allows a biomarker-stratified risk assessment based on the additive prognostic information provided by BECs, which reflect the circulating levels of IL-5 and the pool of available effector cells, and FeNO levels, which reflect airway T2 activity and the recruitment of effector cells to the airways.[66] In contrast, a symptom-based risk assessment does not accurately reflect airway inflammation or predict exacerbations in T2-high asthma. Furthermore, because biomarkers are easily modified by T2-directed therapy, biomarker-based risk assessment based on early phenotyping in patients with severe asthma can be used to guide and monitor the response to treatment.[66] An expert consensus has proposed considering asthma remission, rather than response, as the treatment goal.[116,129]

Standardizing phenotype definitions

There is heterogeneity across research as to how asthma phenotypes are defined. Standardizing the definitions would help to facilitate comparisons between research studies, improve translation of phenotyping research into clinical practice, and help to identify patients who respond well to biologics. There is also a need to agree on standardized definitions for assessing phenotypes of exacerbations.

Phenotyping by proteomic signature

Proteomic technologies, although complex and expensive, are evolving to identify associations between genes, proteins, and diseases.[144] Analysis of the complete set of proteins found in the sputum of patients with asthma has allowed the identification of proteins with changing levels in response to the patient's condition.[144] As this technology continues to evolve, it may be useful in determining the phenotype in patient groups that are otherwise difficult to phenotype using routine methods (eg, patients with T2-low asthma). Therefore, phenotyping by proteomic signature should be considered in clinical practice with other methods, if feasible.

U-BIOPRED was the first project to apply multiple "omics methodologies" to stratify asthma and to provide information on disease mechanisms and phenotypes.[145,146] The project found that clustering using sputum proteomics and transcriptomics data yielded 4 stable and reproducible clusters that associate with different pathobiological pathways.[145] Schofield et al[147] identified 10 clusters based on similarities in proteomic features of the lining fluid of the bronchial epithelium, representing discrete molecular subphenotypes of asthma. In the longer term, proteomics may help to refine treatment options for defined groups of patients.[148,149]

Measuring AHR

AHR is a key feature of severe asthma and is a driver of poor asthma control. AHR is associated with a decline in lung function, an increased risk of developing asthma, and an increased risk of exacerbations in patients with asthma, and should be considered as a phenotypic trait in asthma management.[150,151] Used in the setting of a patient with exacerbations despite receiving high-dose ICS and a second controller, AHR to mannitol is a pathophysiological marker associated with an altered mast cell phenotype (with increased TSLP and carboxypeptidase A3) and has been linked to eosinophilic inflammation.[152] In addition, AHR improves with anti-TSLP treatment and thus may predict response.[117,118] The dependence of AHR on eosinophilic airway inflammation has been debated. The phase 2 CASCADE study suggested that there was no significant correlation between the reduction in AHR to mannitol with tezepelumab and baseline BEC.[117] However, in another single-arm, uncontrolled study, airway eosinophil depletion with benralizumab resulted in a clinically meaningful reduction in AHR in patients with severe, uncontrolled asthma. Although the latter study lacked a placebo control and thus could not definitively establish a treatment effect, the results suggest that eosinophilic airway inflammation can contribute to AHR.[153]

Phenotyping by breath analysis and imaging

The measurement of volatile organic compounds in exhaled breath using gas chromatography offers a noninvasive approach not only to distinguishing patients with and without asthma, but also to identifying those with atopy and allergic inflammation[154] or inflammatory phenotypes.[155] Imaging techniques including chest radiography, computed tomography, magnetic resonance imaging, optical coherence tomography, and positron emission tomography have traditionally been used to assess lung structure and function and to diagnose complications of severe asthma and associated conditions, including the identification of mucus plugs. However, studies using a combination of these imaging techniques have been able to identify unique patient groups and asthma phenotypes, suggesting that imaging results may also serve as potential biomarkers to monitor the response to therapy.[156,157] Further research is needed before breath analysis or imaging results can be used as biomarkers in routine clinical practice.

CONCLUSIONS

The findings summarized in this review provide insights into the importance of combining clinical and inflammatory phenotyping to make an accurate diagnosis of severe asthma, to provide accurate prognostic information, and to guide disease management. Such an approach aims to provide the opportunity to personalize treatment based on a patient's severe asthma phenotype, ensuring that they undergo optimal disease management and are not overtreated or undertreated. In the future, improved standardization of phenotype definitions could further aid the translation of research into clinical practice.

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