Review

Biomarkers in severe asthma: Identifying the treatable trait

Aanchal Gvalani[1], Amita Athavale[2], Disha Gupta[1]

[1]Medical Affairs, GlaxoSmithKline, Mumbai, Maharashtra, India, [2]Department of Pulmonary Medicine and EPRC, Seth GS Medical College and KEM Hospital, Mumbai, Maharashtra, India

ABSTRACT

Asthma is a chronic condition of bronchial hyper-reactivity associated with inflammation ranges from mild to severe form. It affects 1 – 18% of the population globally and it is estimated that > 300million people in the world have asthma. Of this 5 – 10% have severe asthma. while the proportion of patients suffering from severe are smaller, the morbidity and mortality are higher in this group. With the advances in our understanding of the pathophysiology of asthma there is a need to understand the role of various biomarkers. We live in an era of precision medicine and today there is a clear unmet need to understand targeted therapies. This review aims to raise awareness to the available biomarkers used in clinical practice in India and their role in predicting response to targeted therapies.

KEY WORDS: Biomarkers, severe asthma, t2 inflammation

Address for correspondence: Dr. Aanchal Gvalani, Medical Affairs, GlaxoSmithKline, Mumbai, Maharashtra, India.
E-mail: aanchal.a.gvalani@gsk.com

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INTRODUCTION

Epidemiology and burden

Asthma is a condition of bronchial hyper-reactivity associated with inflammation. It is a chronic inflammatory disease that ranges from mild to the severe form. Asthma affects 1–18% of the population globally and it is estimated that >300 million people in the world have asthma. Of this, 5–10% have severe asthma. Although the severe form affects a smaller proportion of patients, it is responsible for a larger component of the overall disease burden.[1,2]

DEFINITION AND PHENOTYPES

Severe asthma has been defined by the European Respiratory Society (ERS)/American Thoracic Society (ATS) as "the phenotype, which requires treatment with high-dose inhaled corticosteroids plus a second controller (and/or systemic corticosteroids) to prevent it from becoming 'uncontrolled' or which remains uncontrolled despite this therapy".[3]

Despite the guideline-based approach to the management of severe asthma, many continue to have uncontrolled symptoms making it pertinent to improve our understanding of the underlying pathophysiological mechanisms.[4]

The recognition of heterogeneity among patients with severe asthma and acknowledgment of the importance of a more precise classification of severe asthma led to the concept of phenotyping asthma.[5] In the field of severe asthma, these subtypes are now called phenotypes or endotypes based on observable characteristics or specific biological mechanisms.[6]

This phenotyping has clinical importance. The categorisation based on severity, progression, and

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responsiveness to therapy helped understand physicians, which patients remain refractory to standard therapy and which subset requires a newer therapeutic target.

Today, severe asthma is a heterogeneous syndrome with variable clinical presentations, underlying mechanisms, and outcomes with specific phenotypes identified based on biomarkers.[3-6]

Biomarkers that reflect the underlying pathophysiology of the disease (endotype) and can assist in further classifying asthma phenotypes and determining a patient's disease phenotype or endotype become crucial, particularly when selecting targeted immunomodulatory therapeutics.

PATHOPHYSIOLOGY

Severe asthma is a chronic inflammatory disease with a marked heterogeneity [see Figure 1, *adapted from Brusselle et al., 2014*] in etiology, pathophysiology, and clinical aspects, leading to the identification of different phenotypes (early onset atopic/allergic, eosinophilic, exercise-induced, obesity, and paucigranulocytic).[7] Subsequently, considering the molecular mechanisms underlying the pathophysiology of bronchial inflammation, the concept of endotypes has been studied to develop targeted therapy.[8]

For many years, from a pathogenic perspective, the focus of research has been on the role of T-cells in the initiation and perpetuation of inflammation. T helper 2 (Th2) cells have been identified as the cells involved in controlling immunoglobulin E (IgE) production due to the generation of interleukin (IL)-4 and IL-13 and influencing the functioning of eosinophils through the actions of IL-5.[9]

The presence of Th2 cells in the bronchoalveolar lavage from atopic asthmatics was clearly demonstrated; however, subsequent analyses demonstrated that at least two different endotypes could be proposed based on the degree of Th2 inflammation and called T2 "high" and T2 "low".[9,10]

Allergic (or atopic) asthma represents the most frequent endotype of asthma representing over 60% of the cases, whereas the non-atopic eosinophilic phenotype represents about 25–30% of them. Approximately 5–10% of patients suffering from asthma have severe refractory asthma. Based on existing data, 55% are eosinophilic forms, 20% are neutrophilic forms, 18% are paucigranulocytic, and 6% mixed form.[8-10]

Another group of cells that research shows to play a role in airway inflammation is the thymic stromal lymphopoietin (TSLP). Following the activation of airway epithelial cells, a wide range of cytokines including the TSLP is produced, which affect dendritic cells, natural killer cells, mast cells, and T cells, indicative of the broad role TSLP play in inflammatory processes. Recent studies have established that high levels of TSLP are associated with airway inflammatory disease in humans demonstrating that allergen-activated basophils also

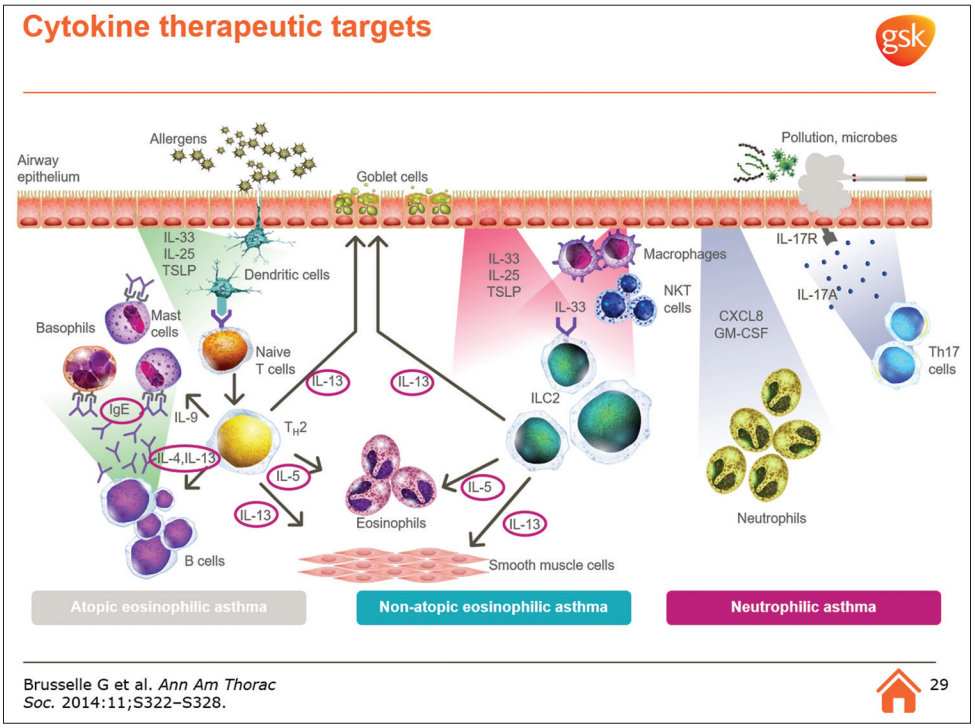

Figure 1: Severe asthma mechanism and cytokine targets

produce TSLP and thus also may be important in the initiation of T_H2 responses.[10,11]

It is important to underline that among all severe forms of asthma, particularly in young subjects, a specific IgE sensitisation is demonstrable in a significant proportion of patients. For patients with severe uncontrolled asthma, monoclonal antibodies (mAbs) against IgE or IL-5 are now available as add-on treatments to inhaled corticosteroid (ICS) plus long-acting β2-agonist (LABA) therapy. For Global Initiative for Asthma (GINA) Step 5 patients, a targeted therapy approach is recommended.[12,13]

BIOMARKERS

What is a biomarker?

As per the World Health Organization (WHO), a biomarker can be any substance, structure, or process that can be measured in the body and that can influence or predict the incidence of an outcome or disease. In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic, that is, objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[12]

In severe asthma, biomarkers can be applied to determine the patient's phenotype and help evaluate treatment response, thereby improving the precision therapeutic approach to severe asthma.

Biomarkers can be diagnostic or predictive in nature, and applying these biomarkers to identify phenotypes has been very purposeful. For example, early-onset allergic asthma is characterised by the presence of allergen-specific (IgE, and late-onset hypereosinophilic asthma is characterised by elevated peripheral blood and sputum eosinophils.[9,11-13]

Eosinophil and IgE levels can be used as diagnostic biomarkers for these phenotypes. However, blood eosinophils are commonly present in early-onset allergic asthmatics, and allergen-specific IgE may be present in late-onset, hyper-eosinophilic asthmatics. Therefore, these commonly used biomarkers may give clues as to the phenotype or endotype.[13,14]

In the following sections, we will discuss the use of commonly used biomarkers to identify phenotypes of severe asthma as there remains an unmet need to understand and discover clinically available biomarkers to target therapies for greater exacerbation control and improved quality of life.

BIOMARKERS IN NON-T2 INFLAMMATION

Recruitment of inflammatory cells to the airways is one of the crucial features of asthma.[15] The proportion of eosinophils and neutrophils in induced sputum samples are used to determine the inflammatory phenotype; however, only a few studies so far evaluated the pathophysiological mechanisms of asthma that drive the disease in relation to the asthma phenotype.[16] The next section describes the biomarkers predominant in non-T2 inflammation.

SPUTUM NEUTROPHILS

Not all patients with severe asthma are characterised by eosinophilia.[17] Severe asthma can also be characterised by neutrophilic inflammation. The mechanism underlying neutrophilic inflammation and the functional role of these cells in disease progression remain unclear.[18,19]

The cut-off for sputum neutrophilia is less clear than for sputum eosinophilia because even in healthy individuals, neutrophils are the most abundant inflammatory cells in induced sputum.[20] Additionally, the use of corticosteroids may affect sputum cellular profiles by suppressing T2 inflammation. In patients with severe asthma receiving high doses of inhaled and oral corticosteroids, high numbers and percentages of neutrophils have been identified in bronchoalveolar lavage and biopsy specimens.[21]

To identify neutrophilic asthma phenotype sputum neutrophils are used instead of blood neutrophils.[21] Blood neutrophils are known to be poor predictors of sputum neutrophil count.[22] The neutrophilic asthma phenotype is usually defined by sputum neutrophils greater than 61– 76%, depending on the reference control population. In a study conducted by Moore *et al.*, the cut-off level for sputum neutrophilia (>40%) was defined based on the median level of sputum neutrophils in patients with asthma.[21-23] Defining a cut-off value for sputum neutrophilia seems difficult because age and medication use are important confounding factors.[21,22]

INTERLEUKINS: IL-17, IL-8

IL-17 receptor-targeted therapies are under evaluation today as they have an indirect effect on neutrophil recruitment. Airway neutrophilia is associated with a type 17 (Th17) immune response, which induces chemokine IL-8 in structural cells.[22,23] Several triggers have been shown to induce IL-17 and recruitment of neutrophils to the airways, including infection, air pollution, cigarette smoke, irritants, intensive exercise and cold air.[24]

Depending on the trigger, different biomarkers have been evaluated that correlate with the degree of exposure or the degree of inflammation. The presence of IL-17 promotes indirect recruitment of neutrophils, thereby inducing airway hyper-responsiveness and as such has become an attractive target for patients with neutrophilic asthma.[25,26]

In clinical trials, sputum IL-8 levels have been found to be increased in patients with sputum neutrophils by

64% compared with those with the non-neutrophilic phenotype. However, this correlation has not been tested for accuracy.[27,28]

BIOMARKERS IN T2 INFLAMMATION

It is now established that type-2 inflammatory pathways are involved in asthma, and many studies suggest that about 50% of SA patients present with type-2 inflammation, as measured by eosinophilia or high levels of fractional exhaled nitric oxide (FeNO).[29] C Miranda *et al*.[30] distinguished early-onset severe asthma patients, characterised by allergen sensitivity, allergic symptoms, eosinophilia and higher serum IgE levels, and late-onset severe asthma subjects, with lower lung function than early-onset ones, despite a shorter duration of illness and significantly more symptoms, if presenting with persistent eosinophils at the onset.[29,30]

Eosinophilic inflammation, which occurs in allergic and nonallergic patients with asthma, is driven by several inflammatory cells, not only those representing adaptive immunity such as the Th2 cells described above but also those representing innate immunity including the type 2 innate lymphoid cells (ILC2).[31] Both Th2 and ILC2 cells are primarily responsible to produce the most effector cytokines including IL-5 and IL-13.[32] This inflammatory pathway of asthma has been labeled as Th2 high asthma. The presence of eosinophils is important in determining asthma phenotypes and is a consequence of T2-related inflammation described above. Because IL-5 and IL-13 are produced not only by Th2 CD4 T cells but also by mast cells, eosinophils and basophils, the term T2 inflammation rather than Th2 inflammation reflects the diverse origin of these cytokines. Many proposed asthma biomarkers are linked to these T2 inflammatory pathways.[32,33]

EOSINOPHILS

Histologically, asthma is also characterised by the recruitment of eosinophils into the large airway wall and lumen along with mucus plugging and airway thickening. The number of these cells that are seen is now congruent with symptoms, severity, worsening of lung function and near-fatal events.[34]

Eosinophilic asthma is found in approximately 50% of adult patients recruited at tertiary centers. Early-onset eosinophilic asthma is usually associated with atopy, whereas atopy is less common in the subgroup of late-onset eosinophilic asthma patients. Increased type 2 cytokines and eosinophils in the airways have been associated with allergic asthma but also nonallergic triggers have been found to induce or exacerbate type 2-driven inflammation.[35,36]

Blood eosinophil count, either using the percent of blood eosinophils or the absolute blood eosinophil count, is one of the most used predictive biomarkers for the Th2 high asthma phenotype.[37,38] This test is easy to perform and readily available at most medical centers.

Along with sputum eosinophilia and elevated FeNO, a mepolizumab trial showed decreased exacerbations with treatment using blood eosinophilia ≥150 at inclusion.[16,39]

Subsequent mepolizumab trials with encouraging results used blood eosinophilia as a sole biomarker for the inclusion of severe eosinophilic asthma patients in the trials. Two trials evaluating reslizumab required a blood eosinophil count of >400 cells/μL for inclusion, and both showed improved forced expiratory volume in 1 s (FEV1) and asthma-related quality of life with treatment.[39,40]

Trials for benralizumab, an anti-IL-5 receptor monoclonal antibody, stratified patients by eosinophil counts of ≥300 cells/μL and demonstrated that patients with eosinophilia responded to the medication. In an evaluation of benralizumab in asthmatics on chronic oral glucocorticoids, patients with blood eosinophil counts of at least 150 cells/μL were included and showed reductions in exacerbation rates and oral steroid dose. Another trial with benralizumab, which enrolled patients with poorly controlled asthma without specifically selecting patients based on blood eosinophilia, only showed a treatment effect in patients with a blood eosinophil count >400 cells/μL. Patients with elevated blood eosinophils also responded to dupilumab, an IL-4 receptor antibody, which blocks the downstream activation of the IL-4 and IL-13 pathways.[41,42]

Clinical trials, thus, have used eosinophil levels ranging from 150 to 400 as inclusion criteria. However, blood eosinophil levels may be elevated due to co-existing conditions, especially in countries with a high prevalence of parasitic infestations, thus limiting its use as a predictive biomarker.[43,44]

Both blood eosinophils and FeNO have been found to be better than the total serum IgE in identifying sputum eosinophilia in adult asthma patients, irrespective of their clinical profile. Blood eosinophil levels have also been shown to be a better predictor of exacerbation and response to treatment than IgE levels.[45]

In another study, blood eosinophils also showed the highest accuracy to predict sputum eosinophilia when compared with FeNO and serum periostin.[45,46]

It is currently hypothesised that the use of blood eosinophils as biomarkers could help personalise asthma management in patients with severe allergic asthma. Although still in study and a source of debate, the persistent eosinophilic phenotype in adults might be a real candidate for specific therapies, thus potentially interfering with the natural history of SA with high exacerbation rates.

INTERLEUKIN 5

Improved understanding of the contribution of eosinophils to various chronic inflammatory conditions, most notably allergic asthma, has encouraged the development of monoclonal antibodies specifically targeting mediators and surface receptors involved in eosinophil expansion and activation.[46] The pivotal role of IL-5 in eosinophil biology, its high specificity for this leukocyte subset, and its involvement in the majority of eosinophilic conditions make it an important target for the treatment of eosinophil-mediated disorders. Among the factors contributing to eosinophil maturation, IL-5 is the most specific.[47] Interleukin-5 acts on eosinophils at multiple functional levels and time points during their lifespan. Besides stimulating proliferation, differentiation and maturation of IL-5Rα-expressing eosinophil-committed progenitors in the marrow, IL-5 contributes to eosinophil egress from the marrow toward the intravascular compartment. When produced in tissues, this cytokine also synergises with chemotactic factors such as eotaxin-1 (CCL11) to attract eosinophils (homing) and primes these cells for activation in response to various mediators. Finally, IL-5 prolongs eosinophil survival in concert with other anti-apoptotic factors. Thus, increased IL-5 production induces (hyper) eosinophilia, both by stimulating eosinophil production and reducing peripheral apoptosis.[46,47] Two types of antibodies have been developed to target eosinophils: antibodies against IL-5 (mepolizumab and reslizumab), and an antibody against the IL-5-receptor-alpha-chain (IL-5Rα) (benralizumab). Both types of antibodies prevent IL-5 from engaging its receptor, and in addition, anti-IL-5Rα antibodies induce target cell lysis. They have been shown to reduce circulating eosinophil counts rapidly in humans with various disorders.[48,49]

FENO (FRACTION OF EXHALED NITRIC OXIDE)

FeNO is produced by NO synthases, some of which can be induced by cytokines, and these elevated NO levels can be detected in diseases with prominent IL-4 and IL-13 expression such as type 2 asthma.[50] The measurement of FeNO is used clinically as a biomarker of airway inflammation related to IL-4- and IL-13-mediated pathways.[51]

Higher levels of FeNO are related to severe, early-onset, allergic and eosinophilic asthma and can therefore help identify patients with these phenotypes. However, FeNO has only moderate predictive accuracy for sputum eosinophilia, with an estimated sensitivity of 66%.[52]

Measurement of FeNO is a simple, well-tolerated and non-invasive method of assessing airway inflammation. Nitric oxide is a mediator produced in cells by nitric oxide synthases (NOS).[34,53] Inducible NOS is responsible for nitric oxide production in the airways, and FeNO levels are correlated with inducible NOS expression in the airway epithelium. In patients with asthma, FeNO is elevated and correlates with other markers of disease activity, including airway hyperresponsiveness, bronchodilator response and symptoms.[34]

FeNO is also a biomarker for monitoring treatment responses or adherence to inhaled corticosteroids. FeNO is therefore often measured as a point of care test in the clinical setting, through commercially available device systems, to phenotype patients with airway inflammation or adjust inhaled corticosteroid therapy.[54,55]

Two meta-analyses showed that using FeNO levels to guide therapy in patients with asthma resulted in a reduced exacerbation rate. FeNO has been used less often as a predictive biomarker in recent clinical trials compared with blood or sputum eosinophil count.[56]

A trial for mepolizumab included a FeNO of greater than 50 parts per billion (ppb) as a marker of eosinophilic asthma and an inclusion criterion.[57] FeNO proved to be a good predictor of response to lebrikizumab but had limited value in dupilumab studies where eosinophils were the most predictive biomarker (QUEST clinical trial post-hoc analysis).[58]

A study published by Hearn *et al.*,[59] 2021, showed benralizumab cohort and not the mepolizumab cohort reduced FeNO. Benralizumab cohort also depleted the IL-5-expressing basophils, which are responsible for IL-13 production.

Taken together, this suggests that depletion of cells expressing IL-5R may be driving the FeNO reduction. The clinical effectiveness of mepolizumab and benralizumab is independent of the baseline FeNO level.

Inhaled corticosteroid therapy typically suppresses FeNO levels, and thus measuring it serially can be useful as a marker of compliance among asthmatics.[60] However, despite its capabilities, the use of FeNO has some limitations. Normal values vary by age, height and according to the type of analyser used. Other confounding factors include smoking, atopic status and the use of anti-inflammatory medications. According to the ATS recommendations, FeNO < 25 ppb in adults indicates that eosinophilic inflammation is less likely, and FeNO >50 ppb indicates that eosinophilic inflammation is likely. The value of a low FeNO in excluding airway eosinophilia is greater than the value of a high FeNO in predicting it. The current guidelines for the treatment of severe asthma use FeNO optionally as a guiding tool in the selection of therapy.[60]

IMMUNOGLOBULIN E

The discovery of IgE in 1966 brought a change. The era shifted into the investigation of genetics, functions and clinical applications of this immunoglobulin. It was established that IgE has unique properties as it can induce rapid pathological responses and it can act as a highly sensitive immunological amplifier. It became well established that IgE levels are increased in patients affected by atopic conditions and that IgE provides the critical link between the antigen recognition role of the adaptive immune system and the effector functions of mast cells and basophils at mucosal and cutaneous sites of environmental exposure. These functions have made IgE an attractive target for pharmacological intervention, with IgE blockade having clinical potential across many different therapy areas. Despite focusing on IgE for several years, there has been relatively little consideration of pathways outside that of mast cells and the acute phase reaction.[61]

In the early 1990s, the discovery of Th2 lymphocytes and their role in controlling IgE production and in the late phase of allergic inflammation reduced the biological importance of IgE antibodies. Thus, traditionally, IgE antibodies were believed to be responsible for the classic "early phase" of an allergic reaction and considered to have only a minor (peripheral) role in the "late phase" reaction. During this period, IgE lost some "popularity" and was only considered of importance from a diagnostic perspective to confirm forms of allergic asthma through the skin and *in vitro* testing. Total IgE did not have any diagnostic significance. Despite decreased interest in IgE antibodies, several studies achieved decidedly important results regarding not only the biological role of IgE but also the therapeutic effects of IgE-blocking mAbs.[62]

This was the turning point in defining the biological role of IgE and, in 2003, the introduction of omalizumab, a humanised mAb that selectively binds to IgE, for the treatment of moderate-to-severe persistent allergic asthma, marked a milestone in both mAb and anti-IgE therapy for asthma.[63,64]

SERUM PERIOSTIN

Periostin is an extracellular matrix protein induced by IL-4 and IL-13 in the airway epithelial cells and lung fibroblasts and has a role in accelerating eosinophil tissue infiltration.[63] Serum periostin has become another potential candidate as a biomarker of Th2-high asthma. Elevated levels correlate with airway eosinophilia and decline in FEV1 and predict response to ICS.[64]

This protein is significantly increased in patients with a high composite airway eosinophil score (based on sputum and biopsy eosinophil levels) but the overlap among the different groups is high.[64,65]

Also, 20–30% of adult asthmatics are cigarette smokers and therefore have poorer disease control, corticosteroid insensitivity, and non-eosinophilic/Th2-low airways inflammation compared with never smokers with asthma. A study by Thomson NC *et al*[67] compared serum periostin levels in smokers and never smokers with asthma and in healthy controls. A notable finding was that although smokers with asthma had significantly lower serum periostin levels than asthmatic never smokers, approximately 40% of smokers with asthma had serum periostin levels above the group median, suggesting that subtyping of periostin high smokers with asthma may guide targeted anti-Th2 interventions in this difficult patient group.[67,68]

Kanemitsu Y[70] *et al* showed that elevated serum periostin has previously correlated with accelerated lung function decline in asthma. This is further supported by another bronchial biopsy study by Kanemitsu Y[71] *et al* in asthmatics that demonstrated both periostin and osteopontin (another extracellular matrix protein) to be associated with a long-standing decline in pulmonary function.[69,70] In addition, in steroid-naive asthmatics, ICS led to decreased serum periostin, improved airflow limitation, decreased sputum eosinophils and reduced airway wall thickness. Thus, collectively, these results strengthen the case for serum periostin as a biomarker for targeting not only eosinophilic inflammation but also the development of airway remodeling in asthma. Sputum periostin measurement may represent a more organ-targeted asthma-specific biomarker than serum periostin and has confirmed an association between sputum periostin levels and eosinophilic inflammation and airflow obstruction.[71]

Studies have shown periostin was the best predictor of a high composite eosinophil score when compared with FeNO, blood eosinophilia and total serum IgE. However, a more recent study conducted by Pavlidis S[73] *et al* could not reproduce this finding. Furthermore, lebrikizumab trials have attempted to use periostin as a biomarker with varying results of statistical significance and whether periostin use can be expanded to predict response to therapy remains unclear.[71,72]

TSLP

TSLP is an epithelial cell-derived cytokine that is produced in response to proinflammatory stimuli, leading to inflammation driven by type 2 helper T (Th2) cells. TSLP thus may contribute to the mechanisms of airway remodeling in asthma and is a potential target for asthma management. It may also be used as an immunological tool in the differential diagnosis of obstructive lung disease. Tezepelumab is a human monoclonal antibody that binds specifically to TSLP, blocking it from interacting with its receptor. Phase II and Phase III studies showed that when this potential first-in-class medicine was added to SOC, there was a reduction in annualised exacerbation rate.

Tezepelumab has shown a potential role across a broad population of patients with severe asthma, regardless of the type of inflammation[73-75]

We are currently in the era of precision medicine, where precision covers management as well as diagnosis of diseases. From the conventional definition of severe asthma to the inclusion of contemporary phenotypes, we certainly are making huge strides in our understanding of severe asthma. But currently in clinical practice, confirming the diagnosis and predicting response to therapy remain the need of the hour. This is where biomarkers play a very important role. It remains debatable whether the concept of a single diagnostic biomarker is plausible; however, one can certainly agree that the standardisation of existing biomarkers (e.g., exhaled breath analysis) is critical to practice today. These molecular markers can also help us in answering a few critical questions such as who would be at a greater risk for exacerbation, selection, and titration of targeted treatment and response to treatment. The existing biomarkers from omics technologies have been of great support, giving a new approach to understanding molecular details of asthma but one of the hurdles, apart from the cost, is the overlapping information that we get from various different biomarkers (blood/sputum as well as breath analysis). The identification and daily use of standardised and defined biomarkers remain a long and winding journey but perhaps a suitable alternative will be a definition of different panels of asthma with clarity on different biomarkers that can be used depending on the eligibility of patients.[76-80]

CONCLUSION

The evolving data supporting the heterogeneity of severe asthma necessitate the development, refinement and utilisation of biomarkers to better delineate asthma phenotypes, predict treatment response and monitor response to therapy. Multiple potential biomarkers in sputum, exhaled breath and blood have been identified for T2 asthma. Further study is needed to better clarify their role, individually or in combination, in the diagnosis and treatment of severe asthma but so far three readily applicable biomarkers (blood eosinophils, FeNO and IgE) have been extensively studied, which remain to have clinical significance. Future clinical trials of novel asthma therapies should include the use of biomarkers in their design, which may lead to a more stratified approach to therapy and improve outcomes.

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