

Predictive Biomarkers for Asthma Therapy

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Abstract

Purpose of Review Asthma is a heterogeneous disease characterized by multiple phenotypes. Treatment of patients with severe disease can be challenging. Predictive biomarkers are measurable characteristics that reflect the underlying pathophysiology of asthma and can identify patients that are likely to respond to a given therapy. This review discusses current knowledge regarding predictive biomarkers in asthma.

Recent Findings Recent trials evaluating biologic therapies targeting IgE, IL-5, IL-13, and IL-4 have utilized predictive biomarkers to identify patients who might benefit from treatment. Other work has suggested that using composite biomarkers may offer enhanced predictive capabilities in tailoring asthma therapy.

Summary Multiple biomarkers including sputum eosinophil count, blood eosinophil count, fractional concentration of nitric oxide in exhaled breath (FeNO), and serum periostin have been used to identify which patients will respond to targeted asthma medications. Further work is needed to integrate predictive biomarkers into clinical practice.

Keywords Asthma · Therapy · Biomarkers · Phenotypes · Endotypes · Biologics

Introduction

Asthma is a heterogeneous disease characterized by airway inflammation resulting in expiratory airflow limitation that varies in intensity over time. Symptoms include wheezing, shortness of breath, chest tightness, and cough [1•]. Worldwide, the rate of physician-diagnosed asthma is 4.3%, but its prevalence by country ranges from 0.2 to 21% [2]. In the USA, surveillance data collected between 2000 and 2010 showed that the prevalence of asthma reached a maximum of 8.4% of the general population, or 25.7 million people [3].

Within the population of patients with asthma, a subset has severe disease. Although these patients represent a minority of patients with asthma, they are at higher risk of morbidity and mortality and account for a disproportionate amount of resource utilization within the healthcare system [4•]. According to ATS/ERS severe asthma guidelines, patients with severe asthma are those who require high-dose inhaled corticosteroid plus a second controller medication or oral corticosteroids to prevent their asthma from becoming uncontrolled or have uncontrolled disease despite these interventions [4•]. Due to the burden of disease faced by these patients, they are the focus for the development of novel targeted therapies.

One approach towards improving treatment of severe asthma is to identify subpopulations that will respond to targeted treatments [5]. This has led to the description of different phenotypes of asthma, which are defined as “the visible characteristics of an organism resulting from the interaction between its genetic makeup and the environment.” Different phenotypic categories include those defined by clinical

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characteristics, inflammatory patterns, or triggers for disease [6]. Current treatment guidelines identify some asthma phenotypes including allergic asthma, non-allergic asthma, late-onset asthma, asthma with fixed airflow limitation, and asthma with obesity [1•]. Many of these and other phenotypes are identified based on clinical characteristics.

More recently, efforts to determine the pathophysiologic underpinnings of these phenotypes have led to interest in defining endotypes of asthma, which are subtypes of disease “defined functionally and pathologically by a molecular mechanism or treatment response” [7]. This model attempts to connect pathologic mechanistic pathways and disease presentation in hopes to better establish individualized therapies. Biomarkers can help identify and characterize these endotypes and are the focus of this review.

Characteristics of Biomarkers

With the clinical and pathophysiologic heterogeneity inherent in asthma, clinicians and researchers have looked to biomarkers to identify patients more likely to specific therapeutic interventions. The Biomarkers Definition Working Group, supported by the National Institute of Health (NIH), defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.” Possible applications of biomarkers include the diagnosis, staging, and prognostication of different disease processes. Another role for biomarkers is characterizing therapeutic interventions either by monitoring response to therapy or predicting which patients might respond favorably to a therapy [8]. Unfortunately, in recent years there has been some confusion about the terminology surrounding biomarkers, particularly regarding the use of biomarkers as surrogate endpoints in clinical trials [9]. In 2015, the Federal Drug Administration (FDA) and the NIH created a Biomarker Working Group to clarify the definition of these terms to promote unambiguous communication in the scientific literature. The glossary created from their efforts established subcategories of biomarkers including diagnostic biomarkers, monitoring biomarkers, pharmacodynamic/response biomarkers, predictive biomarkers, prognostic biomarkers, safety biomarkers, and susceptibility/risk biomarkers.

A predictive biomarker is defined as one “used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent.” One use of predictive biomarkers is therefore choosing the optimal therapy for a given patient. These biomarkers also have important applications in clinical trials, ideally in situations where patients are randomized to a treatment or placebo arm, and then stratified by biomarker status. This trial design

can accurately determine whether a biomarker is indeed predictive [10].

An ideal biomarker should be sensitive, specific, easy to measure, easy to reproduce, and provide biologically relevant information [11]. Other ideal characteristics include being inexpensive, available throughout different treatment settings, and measurable in patients with and without the disease in question [12]. In asthma, biologic biomarkers can be measured in the sputum, peripheral blood, exhaled air, bronchoalveolar lavage (BAL) fluid, biopsied bronchial epithelium, saliva, and urine [13].

T2 Biomarkers

The most comprehensively described endotype in asthma is the T2 high inflammatory endotype. Multiple studies over the last few years identified this group of patients which constitute more than 50% of the severe asthma population. For many years, clinicians have noted that some but not all patients have allergies [14]. Further work suggested that patients with severe asthma have different patterns of inflammatory cells present in their airways. A 1999 study evaluated 34 patients with severe asthma and compared them to patients without asthma or with well-controlled asthma. All patients underwent bronchoscopy with endobronchial biopsy and BAL. Spirometry data, clinical characteristics, symptoms, and medical history were collected. Within the severe asthma group, a subgroup of patients had high eosinophil counts in the BAL fluid and endobronchial biopsies. Those patients with eosinophilia were more likely to have a history of intubation compared with severe asthmatics without airway eosinophilia. The eosinophilic patients also had thicker airway basement membranes, an indicator of airway remodeling secondary to asthma [15•]. It was proposed that the type 2 helper T (T_H2) cells played a pathogenic role in the patients with airway eosinophilia. T_H2 cells produce cytokines including IL-5, IL-4, and IL-13, which trigger eosinophil chemotaxis and have a number of other effects including promoting airway remodeling, increasing bronchial hyperreactivity, and causing goblet cell metaplasia [16]. A 2009 study lent support to this hypothesis. It obtained airway samples from patients with asthma and healthy volunteers and evaluated this tissue using molecular phenotyping. Specifically, the analysis evaluated epithelial cell expression of the genes POSTN, CLCA1, and SERPINB2, which prior studies suggested were up-regulated after exposure to IL-13. The study also evaluated cytokine expression. Results found two clusters of inflammation, one with higher levels of biomarkers involved in the T2 pathway and one with normal levels. Patients with cytokine and gene expression consistent with activation of the T2 pathway had higher serum IgE, blood eosinophilia, and BAL eosinophilia; they also had a more robust response to inhaled corticosteroid (ICS)

administration [17]. This and other studies helped define the T2 endotype of asthma. More recently, T2 biomarkers have been used to identify patients with the T2 endotype and evaluate their response to therapies targeting this pathway. Examples of such biomarkers include sputum eosinophils, blood eosinophils, fractional concentration of nitric oxide in exhaled breath (FeNO), serum IgE, periostin, DPP-4, IL-13, and cysteinyl leukotrienes. Table 1 provides a summary of the characteristics of these biomarkers.

Sputum Eosinophil Count

Quantifying the inflammatory cell pattern present in the sputum of asthmatics provides valuable information which relates to endotypes of asthma. Sputum can show a number of patterns including eosinophilic, neutrophilic, paucigranulocytic, or mixed granulocytic [18]. To obtain samples for sputum analysis, patients nebulize 3% saline for 20 min. The sputum expectorated over this period is centrifuged, stained, and analyzed by quantifying the number of different cell types [19]. As far back as the 1950s, researchers showed that sputum eosinophilia predicts the response to corticosteroid therapy [20]. Table 2 provides a summary of biomarkers which have been validated as predictors of response to various therapies. A 2007 Cochrane meta-analysis supported using sputum eosinophil counts to guide corticosteroid therapy in asthma based on an analysis of three studies [21•]. Targeting a sputum eosinophil level in adult asthmatics of 1–3% [22], less than 2% [23], or less than 4% [24] reduced exacerbation rates as compared to usual care. Current treatment guidelines for severe asthma recommend using sputum eosinophil counts to guide corticosteroid treatment in centers that have experience with this laboratory technique [4].

Subsequent work has also identified sputum eosinophilia not only as a validated biomarker for corticosteroid therapy, but also as a biomarker for newer biologic therapies. An early trial for mepolizumab, an anti-interleukin-5 (IL-5) monoclonal antibody, noted that patients in the active treatment arm receiving this medication had a reduction in sputum eosinophilia at the end of the study period [25]. Subsequent mepolizumab trials enrolled asthmatics based on their sputum eosinophilia and evaluated the efficacy of the medication in these patients. One trial that included patients with sputum eosinophilia of 3% or more at one time point within the prior 2 years despite high-dose ICS treatment found that mepolizumab therapy reduced exacerbations and improved quality of life measures [26]. Mepolizumab also benefited asthmatics with sputum eosinophilia requiring chronic oral prednisone [27]. Having > 3% sputum eosinophilia was an inclusion criterion for a large clinical trial reported in 2012, which showed a treatment benefit with mepolizumab [28]. Reslizumab, a different anti-IL-5 therapy, showed improved quality of life and a trend towards decreased exacerbation in patients with sputum eosinophilia of greater than 3% [29]. A trial of dupilumab, a targeted therapy against IL-4R-alpha that modulates the IL-4/IL-13 pathway, enrolled patients with sputum eosinophilia or blood eosinophilia and found that this medication improved asthma control and lung function [30]. A trial for fevipiprant, an antagonist of the prostaglandin-D2 receptor (DP2), enrolled patients with a sputum eosinophil count of at least 2%; although it did not show improvements in spirometry or quality of life with drug administration, it did show a reduction in sputum eosinophilia in treated patients [31].

Unfortunately, despite its use as a biomarker in many clinical trials, the use of sputum eosinophil count has limitations. It is a technically challenging test, and even in controlled settings, not all asthmatics can provide adequate samples [22]. Fortunately,

Table 1 Characteristics of major biomarkers

Biomarker	Advantages	Disadvantages
Sputum eosinophils	• Use has been validated as a method of guiding inhaled corticosteroid therapy • Predictive of a response to therapy in many biologic therapies	• Not all patients can provide adequate samples • Analysis can be technically challenging and might not be universally available
Blood eosinophils	• Easy to measure • Correlate with sputum eosinophilia and poor asthma control • Predict exacerbations	• Varying cutoffs are used to determine predictive characteristics • Can be elevated due to other causes, such as parasitic infection
FeNO	• High levels indicate risks for exacerbation and underlying airway inflammation	• Multiple confounders • Measurement requires specialized equipment
Serum IgE	• Easy to measure • Identifies patients who may be candidates for Anti-IgE therapy	• Various levels have been used as cutoffs for inclusion in trials • Level is not predictive of response to Anti-IgE • Outperformed by other markers of T2 inflammation and allergen specific IgE
Serum periostin	• Marker of IL-13 activity and T2 airway inflammation	• Diseases other than asthma can increase its level • Not widely available

Table 2 Biomarkers predictive of response to different asthma therapies

Target	Drug	Sputum Eos	Blood Eos	FeNO	IgE ^a	Periostin	DPP-4	IL-13	Leukotriene E4
	Glucocorticoid	*		*					
IgE	Omalizumab		*	*	*	*			
IL-5	Mepolizumab	*	*	*					
IL-5	Reslizumab	*	*						
IL-5	Benralizumab		*	*					
IL-4 / IL13	Dupilumab		*	*		*			
IL-13	Lebrikizumab			*		*			
IL-13	Tralokinumab					*	*	*	
DP2	Fevipirant	*							
LT	Leukotriene receptor antagonists								*

^a Determines eligibility for omalizumab but level is not predictive of response

there are other biomarkers that correlate with sputum eosinophilia. Perhaps the best validated biomarker which correlates with sputum eosinophilia is blood eosinophilia: various studies have shown an area under the receiver operating curve (AUC) for detecting sputum eosinophilia to range from 0.8–0.9 [32•, 33, 34]. The AUC for FeNO was approximately 0.8 [32•, 33]. IgE showed a poorer correlation, with an AUC of only 0.69 [32•]. However, a recent meta-analysis evaluating the predictive capability of biomarkers for predicting sputum eosinophilia casts some doubts on the ability of any one alternate biomarker to detect sputum or airway epithelium eosinophilia. The AUCs found in their study were lower, with a value of 0.78 for blood eosinophilia, 0.75 for FENO, and 0.65 for IgE. The authors comment that 81% of studies included showed a risk for bias, which is partly due to the inclusion of a number of studies that were not randomized controlled trials [35]. Nonetheless, as evidenced by the design of recent clinical trials which will be described in the following sections, markers other than sputum eosinophilia are gaining prominence.

Blood Eosinophil Count

Blood eosinophilia, either using the percent of blood eosinophils or the absolute blood eosinophil count, is one of the most commonly used predictive biomarkers for the T2 high asthma phenotype. This test is easy to perform and readily available at most medical centers. An early study showed that there was an inverse relationship between blood eosinophil count and the forced expiratory volume in one second (FEV1); in this study, a cutoff of 350 cells per microliter (cells/ μ L) was considered significant, a level which was three standard deviations above the mean [36]. The inverse relationship was confirmed in a subsequent study [37]. A 2013 large-scale study of patient-reported data collected between 2007 and 2010 in the USA correlated blood eosinophilia with self-reported

history of asthma, wheezing, and asthma attacks. It demonstrated a dose-response relationship, with patients with high eosinophil counts more likely to have a history of asthma compared with those with intermediate levels of blood eosinophilia [38]. Data from the UK showed that asthma patients with blood eosinophilia levels greater than 400 cells/ μ L had more severe exacerbations and were more likely to have poor asthma control [39].

In recent years, the use of blood eosinophilia as a predictive biomarker has increased in popularity in clinical trials. Along with sputum eosinophilia and elevated FeNO, a mepolizumab trial showed decreased exacerbations with treatment using blood eosinophilia > 300 cells/ μ L as an inclusion criteria [28]. Subsequent mepolizumab trials with encouraging results used blood eosinophilia as a sole biomarker indicating eosinophilic asthma when assessing patients for trial inclusion [40, 41]. Two trials evaluating reslizumab required a blood eosinophil count of > 400 cells/ μ L for inclusion, and both showed improved FEV1 and asthma-related quality of life with treatment [42, 43]. Another trial 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/ μ L [44]. Trials for benralizumab, an anti-IL-5 receptor monoclonal antibody, stratified patients by eosinophil counts of 300 cells/ μ L and demonstrated that patients with eosinophilia responded to the medication [45–47]. In an evaluation of benralizumab in asthmatics on chronic oral glucocorticoids, patients with blood eosinophil counts of at least 150 cells/ μ L were included, and treatment showed reductions in exacerbation rates and oral steroid dose [48]. 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 pathway [30]. Post-hoc analyses of trials evaluating omalizumab, a monoclonal antibody blocking circulating IgE, demonstrated that T2 biomarkers including blood

eosinophilia were predictors of clinical response (reduction of exacerbation) [49]. A post-marketing study evaluating the effectiveness of omalizumab in patients with uncontrolled asthma and normal lung function demonstrated a decrease in exacerbations only in the subgroup of patients with blood eosinophilia [50]. Another small study evaluating patients who received omalizumab for a year showed that those who had baseline elevation in blood eosinophil count had improvements in asthma-related quality of life [51].

Although the blood eosinophil count is easy to obtain and correlates well with sputum eosinophilia [32, 33, 34], one caveat in its use is that the optimal cutoff has yet to be established. Clinical trials have used levels ranging from 150 [41, 48] to 400 [42] as inclusion criteria. Furthermore, blood eosinophil levels may be elevated due to co-existing conditions, especially in countries with high prevalence of parasitic infestations, thus limiting its use as a predictive biomarker.

FeNO

FeNO is measured using a noninvasive technique which provides information about the inflammatory state of the airways [52]. Nitric oxide is created by nitric oxide synthase 2 in the respiratory epithelial cells and serves as an intracellular messenger, inflammatory mediator, and vasodilator [53]. A level of less than 25 parts per billion (ppb) is normal in adults, and a level greater than 50 ppb is elevated [52]. Patients with asthma tend to have higher FeNO levels compared with healthy controls, with lower FeNO levels seen in asthmatics on ICS therapy [54]. FeNO levels correlate with airway hyperresponsiveness [55] and are predictive of the presence of asthma [38] as well as the risk of exacerbation [38]. Levels also correlate with sputum eosinophilia [32, 33]. Similar to the sputum eosinophil count, FeNO levels have been used to tailor ICS therapy [56]. Two meta-analyses showed that using FeNO levels to guide therapy in patients with asthma resulted in a reduced exacerbation rate [57, 58].

FeNO has been used less often as a predictive biomarker in recent clinical trials compared with blood or sputum eosinophil count. A trial for mepolizumab included a FeNO of greater than 50 ppb as a marker of eosinophilic asthma and an inclusion criteria [28]. Analysis of patients receiving benralizumab showed that patients with an elevated FeNO had a positive response to therapy [45]. Patients with a baseline elevation in FeNO also had a significant reduction in asthma exacerbations with omalizumab therapy [49]. FeNO was also a good predictor of response to novel therapies targeting the IL-13 pathway (dupilumab and lebrikizumab) [30, 59].

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 analyzer used. Other confounding factors include smoking, atopic status, and

the use of anti-inflammatory medications [61, 62]. The current guidelines for the treatment of severe asthma do not recommend the use of FeNO for guiding asthma therapy [4].

Serum IgE

IgE is an immunoglobulin which mediates type 1 hypersensitivity reactions and anaphylaxis; it also plays a key role in the pathogenesis of asthma. It binds to IgE receptors on mast cells and basophils, producing cytokines that mediate T2 responses. IL-4 and IL-13 contribute to IgE class switch, which in turn increases IgE levels [63]. Researchers have correlated elevated levels of IgE with asthma, even in patients considered atopic based on skin prick testing [64]. Longitudinal data show that asthmatic subjects have an inverse relationship between IgE and FEV1/FVC ratio [65].

With the development of omalizumab, a recombinant human anti-IgE antibody that binds to circulating IgE at the IgE receptor binding site and blocks further activation, trials have used IgE as a biomarker to identify patients for inclusion in clinical trials of this medication. For example, a large phase III study reported in 2001 included 525 patients with asthma and IgE levels ranging from 30 to 700 IU/mL. Medication administration resulted in reduced exacerbation rates and improved quality of life scores [66]. GINA guidelines now recommend considering omalizumab therapy in patients with severe allergic asthma and elevated IgE levels as an add-on therapy [1]. A 2014 Cochrane review on the use of omalizumab in the treatment of asthma questions whether there is a clear threshold level of IgE for optimal efficacy. The authors note a wide spread in the mean serum IgE levels of patients included in clinical trials, ranging from 141.5 to 508.1 IU/mL [67].

Analysis of pooled clinical trial data also questions whether IgE is indeed the ideal predictive biomarker for omalizumab. A study published in 2005 subdivided patients into two groups based on whether their serum IgE level was above or below the median. Although both groups showed decreased exacerbation rates, there was no significant difference in rates of exacerbations between the two groups [68]. A subsequent pooled analysis showed treatment efficacy across all levels of IgE, with no significant linear relationship between IgE and treatment effect. As mentioned previously, other studies have demonstrated that patients with high levels of blood eosinophils, periostin, and FeNO have a more pronounced reduction in asthma exacerbations with omalizumab therapy [49, 51].

Serum Periostin

Periostin is an extracellular matrix protein encoded by the gene POSTN which researchers initially identified as a product of an osteoblast cell line [69]. It was recognized as being important in the pathophysiology of asthma after studies evaluated the consequences of IL-4 and IL-13 signaling on human

bronchial epithelial cells. Levels of periostin increased with exposure of the cell cultures to these cytokines [70]. Later studies found that it correlated with eosinophilic airway inflammation [71]. Although the mechanism by which periostin contributes to the course of asthma is unclear, some work suggests that it is involved in promoting subepithelial fibrosis and thus airway remodeling [69]. Clinical studies in patients with asthma associate high baseline periostin level with lower lung function [72, 73].

As a predictive biomarker, periostin has been applied primarily to the anti-IL-13 monoclonal antibody therapies. A 2011 clinical trial evaluating lebrikizumab in patients with asthma stratified patients as having high or low baseline levels of periostin, and found that the subgroup of patients with high periostin had more robust improvements in lung function [74]. Although a trial evaluating tralokinumab did not show decreased exacerbations in all patients with asthma, patients with periostin levels higher than the median had reductions in exacerbations and improvements in lung function [75]. While a pooled analysis of lebrikizumab found a reduction in exacerbation rates by 60% in a periostin high group [59], two large randomized controlled trials found inconsistent reductions in exacerbations in patients with high periostin levels questioning its potential use as a predictive biomarker [76]. Outside of the realm of anti-IL-13 therapy, two studies showed predictive capabilities of periostin for omalizumab therapy [49, 51].

Periostin has great potential as a biomarker due to its clinical stability over time [77]. Unfortunately, like eosinophils, periostin can be elevated due to other disease states other than asthma: examples include atopic dermatitis, allergic rhinitis, scleroderma, cancer, and cardiovascular disease [78]. Furthermore, the exact cutoff levels have not been determined and several confounders may interfere with its level.

Other T2 Biomarkers

DPP-4, or serum dipeptidyl peptidase-4, emerged as a possible predictive biomarker based on a tralokinumab trial published in 2015. In this study, researchers found that IL-13 induced production of DPP-4 in airway epithelial cells and showed that patients with high serum levels had improvements in lung function and quality of life with tralokinumab therapy [75]. Many cells in the body produce this enzyme, which can stimulate or inhibit the immune system in different situations. Overall, results are conflicting about whether levels are up-regulated or down-regulated in asthma [79].

Studies have evaluated levels of IL-13 expression directly in the sputum and bronchial epithelial cells in patients with severe asthma and found that these patients had elevated levels of this marker compared with healthy controls. Asthmatics with high IL-13 also had worse quality of life scores [80]. A trial evaluating tralokinumab found that a subgroup of patients with detectable levels of IL-13 in the sputum had improvements in

asthma-related quality of life scores and FEV1 after 13 weeks of treatment, whereas the general study population did not show significant responses to treatment [81].

A final predictive T2 biomarker for asthma are the cysteinyl leukotrienes. These are biologically active fatty acids derived from arachidonic acid that can cause airway smooth muscle contraction, bronchoconstriction, eosinophil chemotaxis, and stimulation of the T2 cytokine cascade [82]. Investigations showed higher sputum cysteinyl leukotriene concentrations in patients with asthma as compared with healthy controls [83] and found a positive relationship between urinary cysteinyl leukotriene levels and sputum eosinophilia [84]. In studies of leukotriene receptor antagonists, asthmatic children and adults with high urinary leukotriene E4 were more likely to respond to this class of medication [85, 86].

Non-T2 Biomarkers

Despite the wealth of predictive biomarkers evaluating the T2 high asthma pathways, fewer biomarkers have been evaluated for patients with T2 low asthma. Non-eosinophilic asthma phenotypes represent a sizeable portion of patients with asthma. Eosinophil-negative patients represented 41% of patients in one study evaluating the inflammatory patterns of severe asthmatics [15•]. A study of 995 patients with asthma performed serial sputum induction and divided patients into one of three groups: persistently eosinophilic, intermittently eosinophilic, and non-eosinophilic. Forty-seven % of patients fell into this latter category. In general, these patients had less airway responsiveness to the administration of corticosteroid therapy, tended to have higher body mass indexes (BMI), and were older. A cluster analysis of the non-eosinophilic patients identified three subgroups, including 1) older overweight females with low IgE, low lung function, and elevated sputum neutrophils, 2) young females with normal BMI and moderate to severe bronchial-hyperresponsiveness, and 3) young males with low FEV1 and evidence of fungal sensitization [87]. A different cluster analysis which evaluated 856 patients that previously had detailed phenotypic characterization performed found five clusters of patients. Three groups had characteristics indicating T2 activation, including a history of atopy and elevations of IgE. However, the subgroup with the most severe asthma and highest healthcare utilization did not have elevated T2 biomarkers, and instead had higher sputum neutrophil counts; this group had late-onset disease and more irreversible changes in lung function. Similar to the other study, these researchers also identified a subgroup of patients who were older, female, and overweight [88]. This cluster was also noted by a subgroup analysis performed in 2008. This study found these patients to have statistically significant elevations in sputum neutrophils [89]. Based on these analyses, non-eosinophilic asthma represents a significant portion of

patients with asthma, and although phenotypic assessment is ongoing and showing some consistent results, endotypic characterization is still lacking. Only a small number of biomarkers including sputum neutrophils, blood neutrophils, and IL-17 expression have been evaluated for their abilities to predict response to asthma therapies.

Sputum Neutrophils

The presence of sputum or blood neutrophilia is one candidate predictive biomarker for non-T2 asthma. One study evaluating sputum inflammatory patterns in patients with asthma showed that 20% had sputum neutrophil percentages of > 61% [18]. Another analysis showed that a similar proportion, 21%, of asthmatics had sputum neutrophilia [87]. Sputum neutrophilia has been linked to severe asthma in a number of studies. A study from 1999 evaluating patients with severe asthma showed that these patients had higher sputum neutrophil counts as compared with patients with mild asthma or healthy controls [15•]. Work reported in 2006 found an inverse correlation between sputum neutrophilia and FEV1 [18]. Multiple cluster analyses found high sputum neutrophil counts in severe asthma subphenotypes [87–89, 90•].

Investigators have proposed macrolide antibiotics as a possible therapeutic intervention for patients with non-eosinophilic asthma. These medications have anti-inflammatory effects in addition to their antibiotic effects, and in other lung diseases have been shown to decrease airway neutrophil counts [91]. A small randomized trial evaluating clarithromycin use in patients with refractory asthma showed reductions in airway neutrophil counts and improvements in quality of life measures in patients undergoing active treatment. A subgroup analysis of asthmatics with sputum neutrophilia of > 61% showed that they had greater improvements in quality of life scores compared with those without sputum neutrophilia [91]. Another trial evaluating azithromycin did not evaluate sputum neutrophil counts, but did show that patients with severe non-eosinophilic asthma based on low FeNO and blood eosinophilia levels had fewer exacerbations with active treatment [92]. A recent study published in 2017 showed reduced exacerbations and improved quality of life in patients who took azithromycin three times weekly for 48 weeks; improvements were noted both in patients with non-eosinophilic asthma and eosinophilic asthma [93].

Although blood neutrophil count is not a well validated surrogate of sputum neutrophilia [34], an elevated blood neutrophil count of at least $2.7 \times 10^9/L$ was used as an inclusion criteria for a recent randomized trial evaluating a CXCR2 antagonist, ACD5096. Prior work suggested that activation of CXCR2 resulted in increased airway neutrophilia, thus contributing to the pathogenesis of non-eosinophilic asthma. Unfortunately, this trial did not

demonstrate any clinical improvements with the administration of this medication [94].

IL-17

Researchers identified a subset of T cells, the Th17 cells, as possibly being important to the pathogenesis of asthma. One study showed that levels of IL-17, a cytokine secreted by Th17 cells, was higher in the bronchial epithelial cells of patients with asthma compared with healthy controls [95]. Subsequent work linking IL-17 with severe asthma suggested that IL-17 is linked to airway neutrophil activity, and hypothesized that activation of this pathway could play a role in steroid insensitivity [96]. Unfortunately, a trial evaluating the safety and efficacy of brodalumab, an anti-IL-17 receptor monoclonal antibody, did not show any improvements with administration. This trial enrolled patients with moderate to severe asthma on chronic inhaled corticosteroids, but did not use any biomarkers to enrich the study population for those with non-eosinophilic asthma [97].

Emerging Techniques for Biomarker Identification

Identifying and developing new biomarkers which could outperform or add clinical insight to the current repertoire is an area of active investigation. Some researchers are looking to novel techniques to discover new biomarkers, including methods such as exhaled breath analysis, sputum gene expression analysis, microRNA evaluation, and microbiome assessment.

Analyzing exhaled breath condensate (EBC) offers a non-invasive method of sampling the airway environment. It analyzes both volatile and nonvolatile compounds by saturating exhaled breath with water vapor and collecting the condensed material [98]. Clinical practice guidelines exist which allow for standardized collection techniques [99]. Examples of compounds collected in EBC include nitric oxide products, hydrogen peroxide, leukotrienes, and cytokines. Certain components correlate with asthma diagnosis, asthma severity, and other biomarkers; however, no components have been used to predict responses to therapy [98]. Some studies suggest that some markers, such as hydrogen peroxide, can decrease in response to asthma treatment [100]. Unfortunately, EBC still has some hurdles to overcome before it becomes widely used. It can be difficult to determine the concentration of a given component due to variable dilution for nonvolatile components [98]. Additionally, samples obtained from EBC do not correlate well with samples obtained by BAL [101]. This could be because the two techniques sample material from different parts of the airway, with BAL obtaining fluid from the alveoli and EBC collecting material from larger airways; regardless of the cause, this fact could be a hindrance in

applying the substantial research performed to date using BAL fluid to EBC analysis.

Another strategy for evaluating exhaled breath is to examine exhaled volatile organic compounds (VOC). In the setting of inflammation, oxidative stress results in reactive oxygen species that degrade lipids and create these compounds. Two different techniques can measure exhaled VOCs, including gas chromatography and the “eNose” technique; the latter method uses a collection of cross-reactive sensors that interact with VOCs to analyze the breath, similar to how the human nose functions. A meta-analysis from 2016 suggests that evaluation of exhaled VOCs can help to make the diagnosis of asthma [102]. In children, measurement of VOCs by gas chromatography coupled with mass spectrometry could predict the risk for exacerbation [103]. In one small study of adult patients, the eNose technique could identify patients with asthma, predict which patients would lose asthma control upon withdrawal of steroid therapy, and predict which patients would respond to oral corticosteroid treatment [104]. Although it is simple to obtain samples from patients using this method, barriers to implementation of this technology include issues with contamination from the ambient environment, standardization of the technique, and validation of the measurement strategies.

Evaluating sputum gene expression is another way of collecting information about possible biomarkers. A study published in 2014 obtained sputum gene expression profiles from patients with eosinophilic asthma, neutrophilic asthma, and paucigranulocytic asthma. After analyzing 277 genes across the samples collected, six genes which had variable expression between the three six groups were identified. Each group had an expression “signature” which was unique. The differential expression of these three six genes could predict responsiveness to ICS better than the presence of sputum eosinophilia [105]. While this strategy is far from becoming mainstream, it offers a more precise way to represent the micro-environment of the lung compared to simple inflammatory cell counts.

Micro RNAs, which are RNA that work on the post-transcriptional level to regulate physiologic processes, can be measured in the blood, sputum, or saliva. Over 100 miRNA have differential expression in asthmatics versus healthy controls [106]. The ease of collection of this specimen makes it an interesting target for further research.

A final novel technique for biomarker assessment is to analyze the microbiome of the lung. A recent study evaluated healthy controls, atopic patients without asthma, and steroid-naïve patients with atopic asthma and performed microbiome assessment of bronchial brushings. Each of the groups had a unique profile of microorganisms [107]. While further work is needed to evaluate

whether this has any clinical utility, it represents a unique approach to the assessment of asthma.

Composite Biomarkers

Some researchers posit that the approach to increased biomarker accuracy is not to develop new techniques, but instead to combine known biomarkers to increase their predictive capabilities. Asthma endotypes represent complex biologic networks, and perhaps one specific marker in isolation cannot adequately encompass these intricacies.

One recent study identified asthmatic patients with T2 inflammation based on airway mucosal expression of CCL26, periostin, or a multigene measure of IL-13 activity. Next, they measured more easily attainable biomarkers and determined if a combination of these biomarkers that could predict the type 2 endotype. In their analysis, a combination of FeNO, blood eosinophil count, serum CCL26 expression, and serum CCL17 expression had a 100% positive predictive value for identifying the T2 endotype [108•].

A number of other investigations have looked at combinations of commonly measured biomarkers in efforts to increase their predictive capabilities. A combination of blood eosinophil count and FeNO in particular has been evaluated in a few different studies; this combination was shown to be more predictive of sputum eosinophil count compared to the capability of either measurement in isolation [32•, 87] and was also associated with poor lung function and higher measures of asthma severity [109]. Using a composite of periostin and FeNO predicted lung function decline and risk of exacerbation in a Japanese population [109]. A composite of FeNO and exhaled VOC analysis predicted lung function in another investigation [110]. An ongoing study in the UK is identifying patients with severe asthma and optimizing their corticosteroid therapy based on a composite biomarker profile [5•].

Future Directions

Future work in asthma needs to further elucidate asthma endotypes based on phenotypic analyses to aid in the development of predictive biomarkers and to offer personalized asthma management strategies [111]. In particular, development of more discrete non-T2 endotypes is critically important because this population has a high severity of disease and fewer directed treatment options [112]. There are still opportunities to subgroup T2 high populations, and this topic merits attention as well [112].

Conclusions

Using biomarkers to predict which patients will respond to targeted asthma therapeutics has great clinical significance due to the heterogeneity of asthma. In particular, patients with severe asthma can benefit from the development of these clinical tools to obtain better control of their disease. A variety of biomarkers have been used clinically to predict the response to steroid therapy, and in the clinical trial setting to identify patients that will respond to biologic therapies. Further work is needed to validate these markers and ensure that they are readily available to clinicians in multiple practice settings.

Compliance with Ethical Standards

Conflict of Interest Dr. Hanania has received honoraria from serving on advisory boards or as a consultant with Novartis, Boehringer Ingelheim, GlaxoSmithKline, Astra Zeneca, and Roche. His institution has received grant support for research from GSK, Boehringer Ingelheim, Cheisi, Astra Zeneca, and Roche. Dr. Parulekar reports personal fees from AstraZeneca. Dr. Medrek declares no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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