Biomarkers for Severe Asthma: Lessons From Longitudinal Cohort Studies

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ABSTRACT

Severe asthma (SA) is a heterogeneous disease characterized by uncontrolled symptoms, frequent exacerbations, and lung function decline. The discovery of phenotypes and endotypes of SA significantly improves our understanding of its pathophysiology and allows the advent of biologics blocking multiple molecular targets. The advances have mainly been made in type 2-high asthma associated with elevated type 2 inflammatory biomarkers such as immunoglobulin E (IgE), interleukins (IL)-4, IL-5, and IL-13. Previous clinical trials have demonstrated that type 2 biomarkers, including blood/sputum eosinophils and the fraction of exhaled nitric oxide (FeNO), were correlated to severe airway inflammation, persistent symptoms, frequent exacerbations, and the clinical efficacy of these biomarkers in predicting treatment outcomes of type 2-targeting biologics. However, it is well known that type 2 inflammation is partially attributable to the pathogenesis of SA. Although some recent studies have suggested that type 2-low and mixed phenotypes of asthma are important contributors to the heterogeneity of SA, many questions about these non-type 2 asthma phenotypes remain to be solved. Consequently, many efforts to investigate and find novel biomarkers for SA have also been made in their methods. Many cross-sectional experimental studies in large-scale cohorts and randomized clinical trials have proved their value in understanding SA. More recently, real-world cohort studies have been in the limelight for SA research, which is unbiased and expected to give us an answer to the unmet needs of the heterogeneity of SA.

Keywords: Asthma; cohort; severe asthma; biomarkers; eosinophil; neutrophil; biologics; leukotriene; therapeutics

INTRODUCTION

The prevalence of severe asthma (SA) accounts for a small proportion of total asthma prevalence (about 5% to 10% of total asthmatics), but it has recently been increasing. SA is a challenge to patients, physicians, and especially society, since the health care cost for SA constitutes more than 50% of that for asthma.¹,²

A high level of heterogeneity exists in SA despite more efforts to understand SA. The early clinical practice and research of asthma described some patients as having a refractory,
resistant, difficult-to-treat, or severe form of asthma. Recently, the European Respiratory Society and American Thoracic Society has announced an expanded definition of SA either as requiring high-dose inhaled corticosteroids (ICS) plus a second controller with or without systemic corticosteroids to achieve asthma control or as having suboptimal control despite this therapy as well as suffering from frequent asthma exacerbations (AEs). SA shows high heterogeneity, which is not only related to airway inflammation and responsiveness to treatment, but also reflective of other factors such as airway hyperresponsiveness (AHR), fixed airway obstruction, comorbidities, and psycho-behavioral problems.

A biomarker is defined as an indicator of biological and pathogenic processes or pharmacological responses to therapeutic intervention. Biomarkers can be used for various purposes such as 1) diagnosis of disease, 2) evaluation of disease course and severity, or 3) monitoring of clinical response to treatment. Furthermore, they may provide clinical insight into the underlying pathophysiology of highly heterogeneous diseases such as SA. Currently, all the clinically available biomarkers for SA represent type 2 airway inflammation. The introduction of sputum sample analysis allowed for inflammatory phenotyping, and an important breakthrough has been achieved in understanding SA pathophysiology. Increased sputum eosinophil counts are associated with higher airway inflammation levels, poorer asthma controls, and more frequent AEs. However, we need an expensive instrument and a well-trained technician to perform sputum analysis, which could also be laborious for patients. Blood total eosinophil count (TEC) and FeNO level are surrogate biomarkers for type 2 airway inflammation and have been shown to predict severe AE as well as responsiveness to ICS and novel biologics targeting type 2 inflammation. Previous randomized clinical trials (RCTs) of severe eosinophilic asthma have demonstrated that the use of type 2-targeting biologics (omalizumab, mepolizumab, reslizumab, benralizumab, and dupilumab) leads to significant reductions in AEs and oral corticosteroid (OCS) usage, and improves lung function. In these studies, omalizumab could reduce AEs and recover forced expiratory volume in 1 second (FEV1)% predicted levels in moderate-to-severe asthmatics with higher serum total IgE levels (30 to 1,500 IU/mL) and positive results to allergy skin testing, while mepolizumab, reslizumab, and benralizumab were beneficial for improving FEV1(%) and asthma control in asthmatics with blood eosinophilia (≥300 cells/μL). Biomarkers for clinical responsiveness to dupilumab therapy in SA is still under investigation. It has been suggested that patients with higher type 2 biomarkers (TEC, FeNO, and IgE) showed a better clinical response to the treatment; however, these biomarkers overlapped with those for other types of biologics. Therefore, it needs to be clarified which of type 2 biomarkers are best and the ideal cutoff value of each biomarker. The following questions about SA should be answered: some patients with type 2-high biomarkers show resistance to anti-inflammatory treatment; and type 2 biomarkers do not always reflect distinct clinical characteristics of SA such as AEs and impaired lung function. Only weak correlations between eosinophilic inflammation and AHR/ fixed airway obstruction have been documented by experimental and clinical studies, and they have recently been considered due to airway structural cells (airway epithelial cells and smooth muscle cells) rather than eosinophilic airway inflammation. Therefore, biomarkers for predicting the treatment-refractory group need to be clarified. In addition, leukotrienes (LTs) and prostaglandins (PGs) that are the main products of arachidonic acid metabolism exert pro- and anti-inflammatory effects on the bronchoconstriction and activation of inflammatory cells (eosinophils) enhancing airway inflammation. LTE4 is the last stable product of arachidonic acid metabolism; high urinary levels of LTE4/PGD2 metabolites are related to lung function decline, which is suggested as a potential biomarker for type 2-high SA.
Type 2-low SA is far behind type 2-high SA in our understanding of potential biomarkers and therapeutics. Type 2-low SA is characterized by onset age of asthma, symptom severity, airway remodeling, resistance to anti-inflammatory treatment, and obesity. A previous study has demonstrated that the number of airway neutrophils is increased during AE, supporting the importance of type 2-low asthma as an endotype of SA. However, there has been an issue as to whether true type 2-low asthma exists because ICSs could eliminate eosinophils in the airway which is more likely in asthmatics who depend on high-dose ICSs and systemic corticosteroids. Although the exact pathogenesis of type 2-low asthma is not well understood with relevant biomarkers, several mechanisms underlying type 2-low SA include: 1) non-type 2 inflammation associated with type 1 (IFN-mediated) or type 3 (IL-17-mediated) immune responses, 2) inflammation related to obesity and metabolic dysfunction, and 3) pauci-granulocytic inflammation. In addition, mixed endotypes, such as type 1/type 2 and type 2/type 3 asthma, have been reported to be related to steroid insensitivity, suggesting that type 2-high and type 2-low asthma may not be mutually exclusive among endotypes.

Previous studies have provided scientific and clinical insights into understanding of SA, and made great strides toward precision treatment. The methodology of SA research has changed from cross-sectional experimental and clinical studies to RCTs, large-scale cohort studies and most recently, real-world longitudinal cohort studies based on big data analyses. This review summarizes potential biomarkers for SA investigated in cross-sectional and longitudinal cohort studies of adult asthmatics, clinical phenotypes/endotypes, biomarkers for nonsteroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease (NERD) (which is a distinct clinical phenotype of SA) and therapeutic approaches to SA based on recently reported biomarkers.

### POTENTIAL BIOMARKERS SUGGESTED IN CROSS-SECTIONAL COHORTS STUDIES

Many studies have aimed to develop biomarkers for asthma classification and treatment targeting SA. It is widely accepted that SA comprises 2 predominant endotypes based on the inflammatory pathway involved: type 2-high and type 2-low SA. Type 2-high SA is related to prominent eosinophilic airway inflammation and recurrent AE, while type 2-low SA is related to airway neutrophilia, pauci-granular inflammation, or obesity-related asthma.

#### Biomarkers related to eosinophil activation

As shown in Table 1, sputum eosinophilia (≥ 3%) is currently considered a reliable criterion for eosinophilic airway inflammation; TEC (≥ 300 cells/µL in adult asthmatics) has been considered a potential, less-invasive, easy-to-conduct biomarker for eosinophilic inflammation. However, there is no correlation between TEC and eosinophil counts in bronchoalveolar lavage fluid in SA. Moreover, the measurement of sputum eosinophils is not commonly employed in clinical practice, and it is neither reliable nor reproducible. In addition to sputum and blood eosinophil counts, the FeNO (generated by the synthesis of nitric oxide under IL-13 stimulation) level has been used to assess type 2 airway inflammation, since it is noninvasive and easy-to-perform as standardized by the international societies. A high FeNO level is associated with airway eosinophilia, corticosteroid responsiveness, and prediction of AE in asthmatics. However, a high FeNO level is of little clinical benefit for the management of SA because structural changes in epithelial cells and airway mucosa may also increase FeNO levels.
Periostin is a well-known matrix protein highly expressed in epithelial cells and fibroblasts. Under stimulation of IL-4 and IL-13, periostin is released to promote adhesion and migration of epithelial cells, mucus production, eosinophil infiltration into the tissue, and subepithelial fibrosis. The serum periostin level is significantly higher in adult asthmatics than in normal controls and is positively correlated with TEC, serum total IgE, eosinophil cationic protein (ECP), and transforming growth factor-beta (TGF-β). Periostin is also known as a systemic biomarker for predicting favorable responses to ICSs. In addition, the serum periostin level was higher in severe asthmatics than in nonsevere asthmatics and asthmatics with a higher serum periostin level also had a higher serum TGF-β level, suggesting that serum periostin is a useful biomarker for SA and eosinophilic asthma in adult asthmatics. However, since serum periostin is not useful for differentiating any phenotypes/endotypes of severe asthmatics, further investigations are required to identify additional biomarkers.

Recently, serum eosinophil-derived neurotoxin (EDN) has been suggested as a potential biomarker for SA, especially in severe eosinophilic asthma. EDN is a granular protein released from activated eosinophils. A higher serum EDN level was noted in patients with SA and in uncontrolled asthmatics; a positive correlation was noted between TEC and the serum EDN level. Although the serum ECP level has been suggested to reflect eosinophil activation in previous studies, the serum EDN level could be superior to the serum ECP level in reflecting asthma severity, asthma control status, and eosinophil activation as well as being more reliable and cost-effective. Recent studies have demonstrated that the count of higher eosinophil extracellular trap (EET)-forming eosinophils was an experimental biomarker for SA, since EETs could activate innate lymphoid type 2 cells and enhance type 2 airway inflammation. Increased EET formation was correlated with the serum EDN level in patients with SA, suggesting that the serum EDN level could be a useful biomarker for SA.

These biomarkers have been validated in the 3 cohorts of SA. The first cohort was the National Heart, Lung, and Blood Institute’s Severe Asthma Research Program (SARP) in the US population, where severe asthmatics tended to be less atopic as assessed by their skin tests than mild or moderate asthmatics. However, the type 2 biomarkers TEC, serum IgE, and FeNO could not differentiate among phenotypes/endotypes in severe asthmatics. The FeNO level was not associated with disease severity, TEC, serum total IgE level, or OCS use in that cohort. In addition, when classified the phenotypes of SA according to the age of asthma onset (early vs. late), there were no significant differences in TEC, serum total IgE level, or FeNO level between the 2 phenotypes of SA. The second is the U-BIOPRED cohort in the European population where severe asthmatics showed higher sputum eosinophil and blood neutrophil counts than non-severe asthmatics, although no difference was noted in

Table 1. Eosinophil-related biomarkers from cross-sectional studies for <1 year in adult asthmatic cohorts

| Biomarkers | ICS/steroids | AEs prediction | Correlation to airway remodeling | Correlation in SA | Function | Source |
|------------|--------------|----------------|----------------------------------|------------------|---------|--------|
| Sputum eosinophil | + | + | – | – | Increase airway inflammation | Sputum |
| Blood eosinophil | + | + | – | – | Increase airway inflammation | Blood |
| FeNO | + | + | – | – | Type 2 inflammation | Exhaled breath |
| Periostin | + | + | + | + | Associated with persistent airflow limitation Higher in SA than NSA | Serum |
| EDN | + | + | + | + | Associated with EET Correlation with the severity Higher in SA than NSA | Serum |

ICS, inhaled corticosteroids; AE, asthma exacerbation; SA, severe asthma; FeNO, fractional exhaled nitric oxide; NSA, non-severe asthma; EDN, eosinophil derived neurotoxin; EET, eosinophil extracellular trap.

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sputum neutrophil counts, which emphasizes the importance of eosinophilic inflammation as the primary mechanism of SA. In addition, no differences were found between smokers and ex-smokers in inflammatory markers, lung functions, or asthma symptoms. The third is the Wessex Severe Asthma Cohort showing that 3 type 2 biomarkers (periostin, FeNO level, and TEC) were not useful for differentiating among phenotypes/endotypes of SA. Since current type 2 biomarkers in SA could not be used to predict asthma control status and to differentiate among phenotypes of SA, additional biomarkers need to be validated and applied in clinical practice.

The TGF-β family is composed of 3 isoforms (TGF-β1, 2, and 3) which have multifunctional regulators in epithelial and endothelial barrier functions, immune cell recruitment, platelet aggregation, and apoptosis as well as the differentiation and proliferation of cells. TGF-β is secreted by almost all immune cells including fibroblasts, endothelial cells, vascular/airway smooth muscle (ASM) cells, and airway epithelial cells. After released under various conditions, TGF-β exerts both anti- and pro-apoptotic effects in airway epithelial cells to induce myofibroblast differentiation and collagen production, contributing to airway remodeling in asthma. SA displays high TGF-β1-mRNA positivity in eosinophils obtained from bronchial biopsies. The serum TGF-β1 level is significantly higher in SA than in non-severe asthma (NSA), and it is correlated with TEC. A higher level of TGF-β1 in lung biopsy tissues is associated with airway remodeling and lung function decline. Moreover, periostin is suggested as a factor for inducing TGF-β1 release. Negative correlations were found between periostin and FEV1% as well as between TGF-β1 and PC20 methacholine value. All of these results suggest that high levels of serum TGF-β1 and periostin could be potential biomarkers for SA, although further validation studies are needed to determine their clinical implications in SA.

Taken together, current available type 2 biomarkers (TEC, sputum eosinophil count, and FeNO level) are considered to reflect the degree of eosinophilic airway inflammation in adult asthmatics, while serum periostin is considered a biomarker for SA, with some limitations to reflect asthma control status or eosinophil activation levels. Serum EDN appears to be a more suitable biomarker for predicting the phenotypes of eosinophilic asthma, SA, and control status of asthma in adult asthmatics. Additional studies are needed to validate the benefits of serum EDN for predicting long-term clinical outcomes and selecting right biologics for right patients with SA.

**Biomarkers related to neutrophil activation**

**Table 2** summarizes biomarkers related to neutrophil activation. Along with type 2 eosinophilic inflammation, SA often presents neutrophilic inflammation in airway mucosa (defined as sputum neutrophilia of ≥61%–65%), which is characterized by steroid resistance. The recruitment of neutrophils is mediated by Th17 through releasing IL-17 which decreases FEV1(%) levels, steroid responsiveness, and increases AHR in SA. Activated neutrophils release reactive oxidative stress, which contributes to the pathogenesis of neutrophilic asthma by inducing inflammatory pathways in airway epithelial cells. Importantly, the formation of neutrophil extracellular traps (NETs) in peripheral neutrophils is thought to be one of the major mechanisms for damaging airway epithelial cells and increasing eosinophil degranulation. Specifically, NETs down-regulate the expression of the tight junction protein of epithelial cells and lead to cell death and detachment. Additionally, NETs enhance type 2 inflammation by stimulating eosinophils to release EDN. Patients with SA have a higher level of NETs than those with NSA. In addition, the activation of neutrophil-
and platelet-adherent eosinophils are associated with the disequilibrium of ceramide/S1P (sphingosine-1-phosphate) which is thought to further recruit inflammatory cells (eosinophils and neutrophils) into asthmatic airways. Chitinase-3-like protein 1 (CHI3L1) or YKL40 is demonstrated as one of the neutrophilic asthma biomarkers. Indeed, serum YKL40 is correlated with sputum neutrophils, myeloperoxidase, IL-8, and IL-6 as well as induces epithelial mesenchymal transition and subepithelial fibrosis through activating focal adhesion kinase and mitogen-activated protein kinase signaling pathways. Hence, YLK-40 has been suggested as a blood-based biomarker for airway inflammation/remodeling in neutrophilic asthma; however, it was not replicated in our cohort of adult asthmatics. Recent studies have demonstrated 2 phenotypes based on sputum inflammatory cell profiles: neutrophil-dominant and pauci-granular types. The S100A9 level was significantly higher in patients with neutrophil-dominant type, and the serum folliculin level (a potential biomarker for epithelial cell activation) was higher in patients with pau-granular type compared to the other 3 types. To the best of our knowledge, since there have been few biomarkers for predicting these 2 phenotypes, further studies are needed to overcome unmet needs in clinical practice: 1) these patients usually have a poor response to corticosteroids, 2) currently available biologics targeting eosinophilic asthma are not suitable for these patients, and 3) there have been no specific biomarkers for the diagnosis and phenotyping of these patients.

### POTENTIAL BIOMARKERS SUGGESTED IN LONGITUDINAL COHORTS STUDIES

There have been a limited number of longitudinal cohort studies to identify biomarkers for SA. A recent longitudinal study of asthmatics who were followed up every 3 months for 1 year identified 3 trajectories in terms of variability in FEV1% predicted or FEV1%. Patients with the persistently low FEV1(%) trajectory showed older age, male sex, presence of smoking history, less atopy, absence of comorbid rhinitis, and variations in asthma control status. Patients with lower baseline lung functions showed greater variability in FEV1% during a 1-year follow-up period. Neither TEC, sputum eosinophil/neutrophil (%), nor serum total IgE level affected this trajectory unlike the remaining trajectories. We analyzed a longitudinal cohort of SA patients during up to 10 years of follow-up period in a real-world practice setting. SA was characterized by female predominance, older age with late asthma onset, higher body mass index, lower baseline FEV1% and FEV1/FVC, and less atopy, which is in line with the results of previous studies. In addition, high TEC and blood neutrophil count distinguished severe asthmatics from non-severe asthmatics in the longitudinal study.
in-depth analyses confirmed that a higher frequency of AEs was related to a lower FEV1/FVC and a higher degree of AHR as documented in previous cohort studies.\textsuperscript{54} Moreover, there were no differences in FEV1\% decrease or the frequency of AEs between severe eosinophilic and severe non-eosinophilic asthmatics, implying that factors other than eosinophilic inflammation (possibly airway epithelial cell damage or ASM hypertrophy) could be involved in SA.\textsuperscript{53} We implemented a prediction model of SA comprising 17 demographic and clinical variables using the machine learning technique, and replicated high blood eosinophil/neutrophil counts, low FEV1\%, and less atopy to house dust mites as significant predictors. In addition, new potential biomarkers (higher platelet/blood uric acid level) have been suggested. Taken together, further translational studies based on real-world longitudinal outcome models will provide useful biomarkers for predicting long-term clinical outcomes of SA and representing heterogeneity in the phenotype/endotype of SA.

**BIOMARKERS FOR NERD**

NERD is a unique phenotype of SA characterized by moderate-to-severe asthma symptoms with frequent AE and hospitalization.\textsuperscript{15} Chronic rhinosinusitis (CRS) with or without nasal polyps accompanied by prominent eosinophilic inflammation is often associated with NERD.\textsuperscript{15} Moreover, the baseline urinary LTE\textsubscript{4} level is higher in NERD patients than in ASA/NSAID-tolerant asthmatic patients, although NERD patients have maintained anti-asthmatic medications including ICSs and leukotriene receptor antagonists.\textsuperscript{55,56} Therefore, an increased urinary LTE\textsubscript{4} level is consistently found and considered the most reliable biomarker for the diagnosis of NERD.\textsuperscript{55,57} Recent studies have classified 302 NERD patients into 4 subtypes based on 3 representative clinical characteristics (urticaria, CRS, and atopy) using the 2-step cluster analysis in a Korean cohort.\textsuperscript{58} Subtypes 1/2 had higher TEC, frequent AEs, and even higher medication requirements, such as higher doses of ICS/OCS, than subtypes 3/4. In addition, higher serum total IgE levels can be used to differentiate between subtypes 1 and 2.\textsuperscript{39,40} Taken together, a higher urinary LTE\textsubscript{4} level is a consistent biomarker for NERD. Further studies on biomarkers for predicting long-term outcomes of NERD are warranted to select right patients for right biologics.

**THERAPEUTIC APPROACHES BASED ON BIOMARKERS**

Biologics are used in patients with uncontrolled asthma who have been treated with high-dose ICS plus LABA or OCS to maintain good control status.\textsuperscript{19,59} The majority of currently available biologics for the treatment of SA (omalizumab, mepolizumab, reslizumab, benralizumab, and dupilumab) alleviate type 2 inflammation by blocking different inflammatory molecules. The most appropriate biologic for each patient should be selected on the basis of clinical characteristics (atopy, TEC, sputum eosinophil count, and FeNO level) and comorbid conditions (CRS, atopic dermatitis, urticaria, and obesity).\textsuperscript{19} These type 2-targeting biologics proved to be efficacious against allergic inflammation in SA. However, evidence of their immunomodulatory effects on airway remodeling is limited and should be accumulated from further studies.\textsuperscript{60}

Omalizumab is the first biologic approved by the Food and Drug Administration (FDA) for the treatment of SA.\textsuperscript{61} It hinders binding of IgE, a key mediator in the upstream allergic inflammation, to its receptors (FcεRI and FcεRII) expressed on the surface of mast cells and...
In previous clinical trials, omalizumab showed clinical efficacy by reducing AE and OCS use as well as by improving quality of life. In addition, a recent study has reported that IgE in patients with allergic asthma is related to ASM proliferation as well as deposition of type I collagen and fibronectin. Another study has demonstrated that airway wall thickness as measured by high-resolution computed tomography (CT) was reduced 16 weeks after omalizumab therapy in patients with SA. Omalizumab can significantly decrease the levels of TNF-α, TGF-β1, and IL-4 in bronchial epithelial cells after stimulating with allergens and IL1β. Moreover, serum IgE level and atopic status have been demonstrated to be the selective biomarkers for omalizumab therapy. The usefulness of eosinophilic biomarkers (high blood/sputum eosinophil count and FeNO level) for predicting favorable responses to omalizumab remains controversial.

Mepolizumab is an anti-IL-5 antibody that has been approved as an add-on treatment of SA. Mepolizumab not only inactivates and eliminates eosinophils, but also down-regulates IL-5 receptor expression on eosinophil membranes. A previous study on the effects of airway remodeling markers in bronchial biopsies of 24 atopic asthmatics has documented that mepolizumab therapy could reduce the expression of 3 extracellular matrix proteins (tenascin, lumican, and procollagen III) in the reticular basement membrane, TGF-β1 mRNA expression in eosinophils, and TGF-β1 expression in BAL fluid. Mepolizumab is considered a reference to biomarker-based stratification for the prediction of therapeutic responsiveness. The DREAM study has suggested that TEC (≥300 cells/µL), but not FeNO level, could be used as a good biomarker for predicting clinical responses to mepolizumab in SA. Therefore, further studies using the threshold of TEC > 300 cells/µL at re-randomization or TEC >150 cells/µL during the optimization phase are warranted. Similarly, reslizumab, an anti-IL-5 antibody, has been demonstrated to improve lung function and QoL-related metrics and to reduce AE in patients with TEC (>400 cells/µL) and sputum eosinophilia.

Benralizumab is a monoclonal antibody against IL-5 receptor alpha and the most recently approved IL-5-targeting agent by the FDA to treat adult patients with SA. Benralizumab binds to IL-5 receptor alpha, thereby inactivating eosinophils and inducing eosinophil apoptosis by antibody-dependent cell-mediated cytotoxicity, along with natural killer cells. Along with other IL-5-targeting agents, benralizumab showed its clinical efficacy in severe, uncontrolled eosinophilic asthma by reducing the number of AEs and OCS requirements, as well as by improving lung function (FEV1), as shown in previous RCTs. Another study assessing the effects of benralizumab on airway remodeling has shown that benralizumab reduce ASM mass, the number of tissues myofibroblasts, and airway expression of TGF-β1. The TEC level (>300 cells/µL) is a useful biomarker for predicting favorable responsiveness to benralizumab therapy.

Dupilumab is a monoclonal antibody to IL-4 receptor alpha that inhibits the binding of IL-4 and IL-13 to down-regulate their intracellular signaling. Previous RCTs have shown clinical efficacy of dupilumab in terms of reduced annual AE rates, improved lung function, and better asthma control. FeNO level and TEC are used as selective biomarkers for predicting dupilumab responsiveness in uncontrolled, moderate-to-severe asthmatics. However, in a randomized, double-blind study, an increase in TEC (>300 cells/µL) did not predict clinical response to dupilumab. Biomarkers for predicting clinical response of dupilumab remain to be studied.

There have been remarkable advances in the treatment of SA using biomarkers for predicting favorable responses to biologics as summarized in Table 3. Severe asthmatics with high...
serum total and specific IgE levels or positive results to skin prick tests for common inhalant allergens are expected to have good clinical responses to omalizumab. For anti-IL-5 and anti-IL-5Rα antibodies, a TEC of at least 300 cells/µL has been used to predict favorable clinical responses. However, previous reports have indicated that TEC could not be used to predict eosinophilic asthma in SA.

A few potential type 2 biomarkers have been suggested to predict responders to type 2 biologics as summarized in Table 4. Since these results are based on RCTs which are relatively short-term studies and potentially biased, long-term real-world studies are needed to identify biomarkers best representing phenotypes/endotypes of SA. Our recent study has suggested the serum EDN level as a biomarker for SA. Serum EDN levels are significantly elevated in SA than in NSA and showed a good positive correlation to TEC. Another study has demonstrated that higher serum EDN levels are found in children at the acute phase than at the stable phase of asthma, and that the serum EDN level predicts the severity of asthma, while the serum ECP level and TEC do not.

In vivo experiments have shown that EET could increase AHR and type 2 cytokine levels in BAL fluid, which were suppressed by anti-IL-13 antibody treatment, but not by anti-IL-5 antibody treatment. These findings suggest that anti-IL-4R antibody treatment could suppress EET-mediated AHR and type 2 inflammation in patients with high EET-forming eosinophils, because blocking of the IL-4/IL-13 pathway could suppress the cross-talk between inflammatory cells and airway epithelial cells in the airways. Further clinical evidence needs to be accumulated.

Although recent studies have focused on identifying endotypes of SA to predict clinical response to biologics, the endotypes remain to be elucidated. First, low Th2 phenotype is a well-known phenotype of SA characterized by predominant neutrophil recruitment into
the airways and resistance to ICSs. Non-type 2-targeting agents are still under investigation due to lack of predictive biomarkers. Moreover, co-existence of and interactions between type 2-high and type 2-low inflammatory pathways have been reported, which results in more complicated pathophysiology of SA.59 Secondly, blood and sputum eosinophilia reflect clinical responses to anti-IL-5 and anti-IL-5 receptor antibodies as mentioned above. However, recent studies have shown that increased TEC and sputum eosinophil number rarely reflect the degree of airway eosinophilia in SA.27,81 Thirdly, anti-IgE and anti-IL-5/5R antibodies are well known as selective biomarkers for allergic status and eosinophilia, respectively, but both antibodies are biomarkers for an overlap between allergic and nonallergic eosinophilic asthma. Therefore, anti-IgE (omalizumab) or anti-IL-5 antibodies (mepolizumab, reslizumab, and benralizumab) should be determined at admission. Moreover, further clinical trials are warranted to compare these antibodies in a real-world clinical setting.

CONCLUSION

Clinical evidence to date has suggested several type 2 biomarkers for SA (blood/sputum eosinophils, FeNO, serum periostin, and serum EDN), but they are insufficient to evaluate asthma severity. Although the potential biomarkers for non-type 2 asthma have been evaluated in large-scale RCTs, further multi-dimensional analyses of real-world clinical databases using phenotype/endotype classification as well as cross-sectional/longitudinal outcome models can identify and validate biomarkers for SA.

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REFERENCES

1. Song WJ, Lee JH, Kang Y, Joung WJ, Chung KF. Future risks in patients with severe asthma. Allergy Asthma Immunol Res 2019;11:763-78. PUBMED | CROSSREF
2. Lee E, Kim A, Ye YM, Choi SE, Park HS. Increasing prevalence and mortality of asthma with age in Korea, 2002–2015: a nationwide, population-based Study. Allergy Asthma Immunol Res 2020;12:467-84. PUBMED | CROSSREF
3. Holguin F, Cardet JC, Chung KF, Diver S, Ferreira DS, Fitzpatrick A, et al. Management of severe asthma: a European Respiratory Society/American Thoracic Society guideline. Eur Respir J 2020;55.e55. PUBMED | CROSSREF
4. Kim BK, Park SY, Ban GY, Kim MA, Lee JH, An J, et al. Evaluation and management of difficult-to-treat and severe asthma: an expert opinion from the Korean academy of asthma, allergy and clinical immunology, the working group on severe asthma. Allergy Asthma Immunol Res 2020;12:910-33. PUBMED | CROSSREF
5. Hur GY, Ye YM, Yang E, Park HS. Serum potential biomarkers according to sputum inflammatory cell profiles in adult asthmatics. Korean J Intern Med 2020;35:988-97. PUBMED | CROSSREF
6. Pizzichini MM, Popov TA, Efthimiadis A, Hussack P, Evans S, Pizzichini E, et al. Spontaneous and induced sputum to measure indices of airway inflammation in asthma. Am J Respir Crit Care Med 1996;154:866-9. PUBMED | CROSSREF
7. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.
PUBMED | CROSSREF
8. Hanania NA, Alpan O, Hamilos DL, Condepi JJ, Reyes-Rivera I, Zhu J, et al. Omalizumab in severe allergic asthma inadequately controlled with standard therapy: a randomized trial. Ann Intern Med 2011;154:573-82.
PUBMED | CROSSREF
9. Szefler SJ, Wenzel S, Brown R, Erzurum SC, Fahy JV, Hamilton RG, et al. Asthma outcomes: biomarkers. J Allergy Clin Immunol 2012;129 Suppl:S9-23.
PUBMED | CROSSREF
10. Park HS, Lee SH, Lee SY, Kim MK, Lee BI, Werkström V, et al. Efficacy and safety of benralizumab for Korean patients with severe, uncontrolled eosinophilic asthma. Allergy Asthma Immunol Res 2019;11:508-18.
PUBMED | CROSSREF
11. McGregor MC, Krings JG, Nair P, Castro M. Role of biologics in asthma. Am J Respir Crit Care Med 2019;199:433-45.
PUBMED | CROSSREF
12. Shah SP, Grunwell J, Shih J, Stephenson S, Fitzpatrick AM. Exploring the utility of noninvasive type 2 inflammatory markers for prediction of severe asthma exacerbations in children and adolescents. J Allergy Clin Immunol Pract 2019;7:2624-2633.e2.
PUBMED | CROSSREF
13. Mansur AH, Srivastava S, Sahal A. Disconnect of type 2 biomarkers in severe asthma; dominated by FeNO as a predictor of exacerbations and perioestin as predictor of reduced lung function. Respir Med 2018;143:31-8.
PUBMED | CROSSREF
14. Kolmert J, Gómez C, Balgoma D, Sjödin M, Bood J, Konradsen JR, et al. Urinary leukotriene E4 and prostaglandin D2 metabolites increase in adult and childhood severe asthma characterized by type-2 inflammation. Am J Respir Crit Care Med 2020;203:57-53.
PUBMED | CROSSREF
15. Woo SD, Luu QQ, Park HS. NSAID-exacerbated respiratory disease (NERD): from pathogenesis to improved care. Front Pharmacol 2020;11:1147-47.
PUBMED | CROSSREF
16. Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, et al. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. J Allergy Clin Immunol 2010;125:1028-1036.e13.
PUBMED | CROSSREF
17. Holguin F, Bleecker ER, Busse WW, Calhoun WJ, Castro M, Erzurum SC, et al. Obesity and asthma: an association modified by age of asthma onset. J Allergy Clin Immunol 2011;127:1486-1493.e2.
PUBMED | CROSSREF
18. Hanratty CE, Matthews JG, Arron JR, Choy DF, Pavord ID, Bradding P, et al. A randomised pragmatic trial of corticosteroid optimization in severe asthma using a composite biomarker algorithm to adjust corticosteroid dose versus standard care: study protocol for a randomised trial. Trials 2018;19:5.
PUBMED | CROSSREF
19. Akar-Ghibril N, Casale T, Custovic A, Phipatanakul W. Allergic endotypes and phenotypes of asthma. J Allergy Clin Immunol Pract 2020;8:429-40.
PUBMED | CROSSREF
20. Agache I, Strasser DS, Pierlot GM, Farine H, Izuhara K, Akdis CA. Monitoring inflammatory heterogeneity with multiple biomarkers for multidimensional endotyping of asthma. J Allergy Clin Immunol 2018;141:442-5.
PUBMED | CROSSREF
21. Peters MC, Ringel L, Dyjack N, Herrin R, Woodruff PG, Ríos C, et al. A transcriptomic method to determine airway immune dysfunction in T2-high and T2-low asthma. Am J Respir Crit Care Med 2019;199:465-77.
PUBMED | CROSSREF
22. Chiappori A, De Ferrari L, Folli C, Mauri P, Riccio AM, Canonica GW. Biomarkers and severe asthma: a critical appraisal. Clin Mol Allergy 2015;13:20.
PUBMED | CROSSREF
23. Pavord ID, Afzalnia S, Muenzies-Gow A, Heaney LG. The current and future role of biomarkers in type 2 cytokine-mediated asthma management. Clin Exp Allergy 2017;47:148-60.
PUBMED | CROSSREF

https://e-aair.org
24. Bakakos A, Loukides S, Usmani OS, Bakakos P. Biologics in severe asthma: the overlap endotype - opportunities and challenges. Expert Opin Biol Ther 2020;20:1427-34.

25. Tran TN, Khatry DB, Ke X, Ward CK, Gossage D. High blood eosinophil count is associated with more frequent asthma attacks in asthma patients. Ann Allergy Asthma Immunol 2014;113:19-24.

26. Horn BR, Robin ED, Theodore J, Van Kessel A. Total eosinophil counts in the management of bronchial asthma. N Engl J Med 1975;292:1152-5.

27. Ullmann N, Bossley CJ, Fleming L, Silvestri M, Bush A, Saglani S. Blood eosinophil counts rarely reflect airway eosinophilia in children with severe asthma. Allergy 2013;68:402-6.

28. Dweik RA, Sorkness RL, Wenzel S, Hammel J, Curran-Everett D, Comhair SA, et al. Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. Am J Respir Crit Care Med 2010;181:1033-41.

29. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 2014;43:343-73.

30. Hamid Q. Gross pathology and histopathology of asthma. J Allergy Clin Immunol 2003;111:431-2.

31. Matsumoto H. Serum periostin: a novel biomarker for asthma management. Allergol Int 2014;63:153-60.

32. Blanchard C, Mingler MK, McBride M, Putnam PE, Collins MH, Chang G, et al. Periostin facilitates eosinophil tissue infiltration in allergic lung and esophageal responses. Mucosal Immunol 2008;1:289-96.

33. Sehra S, Yao W, Nguyen ET, Ahyi AN, Tuana FM, Ahlfeld SK, et al. Periostin regulates goblet cell metaplasia in a model of allergic airway inflammation. J Immunol 2011;186:4959-66.

34. Kim SH, Uuganbayar U, Trinh HK, Pham DL, Kim N, Kim M, et al. Evaluation of neutrophil activation status according to the phenotypes of adult asthma. Allergy Asthma Immunol Res 2019;11:381-93.

35. Lee Y, Lee JH, Yang EM, Kwon E, Jung CG, Kim SC, et al. Serum levels of eosinophil-derived neurotoxin: a biomarker for asthma severity in adult asthmatics. Allergy Asthma Immunol Res 2019;11:394-405.

36. An J, Lee JH, Sim JH, Song WJ, Kwon HS, Cho YS, et al. Serum eosinophil-derived neurotoxin better reflect asthma control status than blood eosinophil counts. J Allergy Clin Immunol Pract 2020;8:2681-2688.e1.

37. Acharya KR, Ackerman SJ. Eosinophil granule proteins: form and function. J Biol Chem 2014;289:17406-15.

38. Kim CK, Callaway Z, Fletcher R, Koh YY. Eosinophil-derived neurotoxin in childhood asthma: correlation with disease severity. J Asthma 2010;47:568-73.

39. Choi Y, Le Pham D, Lee DH, Lee SH, Kim SH, Park HS. Biological function of eosinophil extracellular traps in patients with severe eosinophilic asthma. Exp Mol Med 2018;50:104-04.

40. Choi Y, Sim S, Park HS. Distinct functions of eosinophils in severe asthma with type 2 phenotype: clinical implications. Korean J Intern Med 2020;35:823-33.

41. Moore WC, Bleeker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, et al. Characterization of the severe asthma phenotype by the national heart, lung, and blood institute’s severe asthma research program. J Allergy Clin Immunol 2007;119:405-43.

42. Shaw DE, Sousa AR, Fowler SJ, Fleming LJ, Roberts G, Corfield J, et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. Eur Respir J 2015;46:1308-21.

43. Hinks TS, Brown T, Lau LC, Rupani H, Barber C, Elliott S, et al. Multidimensional endotyping in patients with severe asthma reveals inflammatory heterogeneity in matrix metalloproteinases and chitinase 3-like protein 1. J Allergy Clin Immunol 2016;138:61-75.
44. Al-Alawi M, Hassan T, Chotirmall SH. Transforming growth factor β and severe asthma: a perfect storm. Respir Med 2014;108:1409-23.
45. Ulambayar B, Lee SH, Yang EM, Ye YM, Park HS. Association between epithelial cytokines and clinical phenotypes of elderly asthma. Allergy Asthma Immunol Res 2019;11:79-89.
46. Aubert JD, Dalal BI, Bai TR, Roberts CR, Hayashi S, Hogg JC. Transforming growth factor beta 1 gene expression in human airways. Thorax 1994;49:225-32.
47. Choi Y, Lee DH, Lee JH, Shin YS, Kim SH, Park HS. Immunomodulatory function of surfactant protein D in eosinophilic asthma. Allergy 2019;74:192-5.
48. Israel E, Reddel HK. Severe and difficult-to-treat asthma in adults. N Engl J Med 2017;377:965-76.
49. Kim SH, Jung HW, Kim M, Moon JY, Ban GY, Kim SJ, et al. Ceramide/sphingosine-1-phosphate imbalance is associated with distinct inflammatory phenotypes of uncontrolled asthma. Allergy 2020;75:1991-2004.
50. Pham DL, Ban GY, Kim SH, Shin YS, Ye YM, Chwae YJ, et al. Neutrophil autophagy and extracellular DNA traps contribute to airway inflammation in severe asthma. Clin Exp Allergy 2017;47:57-70.
51. Yang Y, Jia M, Ou Y, Adcock I, Yao X. Airway epithelial cell damage in asthma: mechanisms and biomarkers. Authorea. Forthcoming 2020.
52. Kim JH, Chang HS, Shin SW, Baek DG, Son JH, Park CS, et al. Lung function trajectory types in never-smoking adults with asthma: clinical features and inflammatory patterns. Allergy Asthma Immunol Res 2018;10:614-27.
53. Lee Y, Park YI, Kim CS, Lee EY, Lee HY, Woo SD, et al. Longitudinal outcomes of severe asthma: real-world evidence of multidimensional analyses. J Allergy Clin Immunol Pract. Forthcoming 2020.
54. Thomas ET, Guppy M, Straus SE, Bell KJ, Glasziou P. Rate of normal lung function decline in ageing adults: a systematic review of prospective cohort studies. BMJ Open 2019;9:e028150.
55. Park H, Choi Y, Jung CG, Park HS. Potential biomarkers for NSAID-exacerbated respiratory disease. Mediators Inflamm 2017;2017:8160148-48.
56. Hagan JB, Laidlaw TM, Divekar R, O’Brien EK, Kita H, Volcheck GW, et al. Urinary leukotriene E4 to determine aspirin intolerance in asthma: a systematic review and meta-analysis. J Allergy Clin Immunol Pract 2017;5:990-997.e1.
57. Daffern PJ, Muilenburg D, Hugli TE, Stevenson DD. Association of urinary leukotriene E4 excretion during aspirin challenges with severity of respiratory responses. J Allergy Clin Immunol 1999;104:559-64.
58. Lee HY, Ye YM, Kim SH, Ban GY, Kim SC, Kim JH, et al. Identification of phenotypic clusters of nonsteroidal anti-inflammatory drugs exacerbated respiratory disease. Allergy 2017;72:616-26.
59. Chung KF, Adcock IM. Precision medicine for the discovery of treatable mechanisms in severe asthma. Allergy 2019;74:1649-59.
60. Kardas G, Kuna P, Panek M. Biological therapies of severe asthma and their possible effects on airway remodeling. Front Immunol 2020;11:1134.
61. Rogliani P, Calzetta L, Matera MG, Laitano R, Ritondo BL, Hanania NA, et al. Severe asthma and biological therapy: when, which, and for whom. Pulm Ther 2020;6:47-66.
62. Shields RJ, Werther WR, Zioncheck K, O’Connell L, Klassen T, Fendly B, et al. Anti-IgE monoclonal antibodies that inhibit allergen-specific histamine release. Int Arch Allergy Immunol 1995;107:412-3.
63. Humbert M, Busse W, Hanania NA, Lowe P, Canvin J, Erpenbeck VJ, et al. Omalizumab in asthma: an update on recent developments. J Allergy Clin Immunol Pract 2014;2:525-36.e1.
64. Hoshino M, Ohtawa J. Effects of adding omalizumab, an anti-immunoglobulin E antibody, on airway wall thickening in asthma. Respiration 2012;83:520-8.
PUBMED | CROSSREF

65. Huang YC, Leyko B, Frieri M. Effects of omalizumab and budesonide on markers of inflammation in human bronchial epithelial cells. Ann Allergy Asthma Immunol 2005;95:443-51.
PUBMED | CROSSREF

66. Casale TB, Luskin AT, Busse W, Zeiger RS, Trzaskoma B, Yang M, et al. Omalizumab effectiveness by biomarker status in patients with asthma: evidence from PROSPERO, a prospective real-world study. J Allergy Clin Immunol Pract 2019;7:156-164.e1.
PUBMED | CROSSREF

67. Krings JG, McGregor MC, Bacharier LB, Castro M. Biologics for severe asthma: treatment-specific effects are important in choosing a specific agent. J Allergy Clin Immunol Pract 2019;7:1379-92.
PUBMED | CROSSREF

68. Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. J Clin Invest 2003;112:1029-36.
PUBMED | CROSSREF

69. Fricker M, Heaney LG, Upham JW. Can biomarkers help us hit targets in difficult-to-treat asthma? Respir Med 2017;22:430-42.
PUBMED | CROSSREF

70. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 2012;380:651-9.
PUBMED | CROSSREF

71. Wan XC, Woodruff PG. Biomarkers in severe asthma. Immunol Allergy Clin North Am 2016;36:547-57.
PUBMED | CROSSREF

72. Castro M, Zangrilli J, Wechsler ME, Bateman ED, Brusselle GG, Bardin P, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. Lancet Respir Med 2015;3:355-66.
PUBMED | CROSSREF

73. Castro M, Mathur S, Hargrave F, Boulet LP, Xie F, Young J, et al. Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. Am J Respir Crit Care Med 2011;184:1125-32.
PUBMED | CROSSREF

74. Bleecker ER, FitzGerald JM, Chanez P, Papi A, Weinstein SF, Barker P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β₂-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. Lancet 2016;388:2115-27.
PUBMED | CROSSREF

75. FitzGerald JM, Bleecker ER, Nair P, Korn S, Ohta K, Lommatzsch M, et al. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet 2016;388:2128-41.
PUBMED | CROSSREF

76. Nair P, Wenzel S, Rabe KF, Bourdin A, Lugogo NL, Kuna P, et al. Oral glucocorticoid-sparing effect of benralizumab in severe asthma. N Engl J Med 2017;376:2448-58.
PUBMED | CROSSREF

77. Laviolette M, Gossage DL, Gauvreau G, Leigh R, Olivenstein R, Katial R, et al. Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. J Allergy Clin Immunol 2013;132:1086-1096.e5.
PUBMED | CROSSREF

78. Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, et al. Dupilumab in persistent asthma with elevated eosinophil levels. N Engl J Med 2013;368:2455-66.
PUBMED | CROSSREF

79. Wenzel SE, Pavord I, Zhang B, Maroni J, Rowe P, Hamilton JD, et al. Type 2 biomarkers associated with dupilumab efficacy in patients with uncontrolled, moderate-to-severe asthma enrolled in the phase 3 study LIBERTY asthma quest. Am J Respir Crit Care Med 2018;197:A5949.
PUBMED | CROSSREF

80. Wenzel S, Castro M, Corren J, Maspero J, Wang L, Zhang B, et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting β2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. Lancet 2016;388:31-44.
PUBMED | CROSSREF
81. Pavlidis S, Takahashi K, Ng Kee Kwong F, Xie J, Hoda U, Sun K, et al. “T2-high” in severe asthma related to blood eosinophil, exhaled nitric oxide and serum periostin. Eur Respir J 2019;53:1800938.
PUBMED | CROSSREF

82. Tsuda T, Maeda Y, Nishide M, Koyama S, Hayama Y, Nojima S, et al. Eosinophil-derived neurotoxin enhances airway remodeling in eosinophilic chronic rhinosinusitis and correlates with disease severity. Int Immunol 2019;31:33-40.
PUBMED | CROSSREF