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Review

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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 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).[3,4] 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.[5] Increased sputum eosinophil counts are associated with higher airway inflammation levels, poorer asthma controls, and more frequent AEs.[6,7] 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.[8,9] 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.[10,11] 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.[10,11] 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.[12] 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.[13] 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. LTE_4 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.[14,15]

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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.[16,17] 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.[5] 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.[18] 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.[19] 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.[20,21]

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.[22,23] It is widely accepted that SA comprises 2 predominant endotypes based on the inflammatory pathway involved: type 2-high and type 2-low SA.[24] 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.[5,22,25,26] However, there is no correlation between TEC and eosinophil counts in bronchoalveolar lavage fluid in SA.[27] 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.[23,28] 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.[29,30]

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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	Enhance adhesion and migration of epithelial cells, mucus production, and eosinophils tissue infiltration	Serum
EDN	+	+	+	Associated with EET Correlation with the severity Higher in SA than NSA	Contribute to inflammatory response in SA	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.

Periostin is a well-known matrix protein highly expressed in epithelial cells and fibroblasts.[31] 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.[31-33] 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-β1).[31] Periostin is also known as a systemic biomarker for predicting favorable responses to ICSs.[31-33] 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-β1 level, suggesting that serum periostin is a useful biomarker for SA and eosinophilic asthma in adult asthmatics.[34] 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.[35,36] EDN is a granular protein released from activated eosinophils.[37] 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.[35,36] 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.[38] 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.[39,40]

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.[41] 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.[41] 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

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sputum neutrophil counts, which emphasizes the importance of eosinophilic inflammation as the primary mechanism of SA.[42] In addition, no differences were found between smokers and ex-smokers in inflammatory markers, lung functions, or asthma symptoms.[42] 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.[43] 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-ß family is composed of 3 isoforms (TGF-ß1, 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.[44] TGF-β is secreted by almost all immune cells including fibroblasts, endothelial cells, vascular/airway smooth muscle (ASM) cells, and airway epithelial cells.[44] After released under various conditions, TGF-ß exerts both anti- and pro-apoptotic effects in airway epithelial cells to induce myofibroblast differentiation and collagen production, contributing to airway remodeling in asthma.[44] SA displays high TGF-β1-mRNA positivity in eosinophils obtained from bronchial biopsies.[22,44] The serum TGF-β1 level is significantly higher in SA than in non-severe asthma (NSA), and it is correlated with TEC.[45] A higher level of TGF-ß1 in lung biopsy tissues is associated with airway remodeling and lung function decline.[44,46] 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 PC_{20} methacholine value.[47] All of these results suggest that high levels of serum TGF-ß1 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.[48] The recruitment of neutrophils is mediated by Th17 through releasing IL-17 which decreases FEV1(%) levels, steroid responsiveness, and increases AHR in SA.[22,35] Activated neutrophils release reactive oxidative stress, which contributes to the pathogenesis of neutrophilic asthma by inducing inflammatory pathways in airway epithelial cells.[49] 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.[50] Specifically, NETs down-regulate the expression of the tight junction protein of epithelial cells and lead to cell death and detachment.[50] 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-

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Table 2. Neutrophil-related biomarkers from cross-sectional studies for <1 year in adult asthmatic cohorts

Biomarkers	ICS/steroids	AEs prediction	Correlation to airway remodeling	Correlation in severe asthma	Function	Source
Sputum neutrophils	+	+	–	–	Neutrophilic airway inflammation	Sputum
IL-17	+	+	ND	Uncontrolled status of asthma	Recruitment of neutrophils Increase neutrophils survival	Serum
Ceramide/S1P	+	+	ND	Increased blood neutrophil counts	Recruit inflammatory cells into the airway	Serum
CHI3L1	+	+	+	Associated with severity Higher in SA than NSA	ND	Serum
S100A9	ND	ND	ND	Increased in neutrophilic asthma compared to pauci-granulocytic asthma	Initiate and amplify the neutrophilic inflammation	Serum

ICS, inhaled corticosteroids; AE, asthma exacerbation; IL, interleukin; ND, no data; CH3L1, chitinase-3-like protein 1; SA, severe asthma; NSA, non-severe asthma.

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.[49] Chitinase-3-like protein 1 (CHI3L1) or YKL40 is demonstrated as one of the neutrophilic asthma biomarkers.[43,51] 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.[51] 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.[5] 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%.[52] 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.[53] 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.[42,52] In addition, high TEC and blood neutrophil count distinguished severe asthmatics from non-severe asthmatics in the longitudinal study. The

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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.[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.[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.[15] Chronic rhinosinusitis (CRS) with or without nasal polyps accompanied by prominent eosinophilic inflammation is often associated with NERD.[15] Moreover, the baseline urinary LTE_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.[55,56] Therefore, an increased urinary LTE_4 level is consistently found and considered the most reliable biomarker for the diagnosis of NERD.[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.[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.[39,40] Taken together, a higher urinary LTE4 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.[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).[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.[60]

Omalizumab is the first biologic approved by the Food and Drug Administration (FDA) for the treatment of SA.[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

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basophils.[62,63] In previous clinical trials, omalizumab showed clinical efficacy by reducing AE and OCS use as well as by improving quality of life.[19,61] 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.[60] 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.[64] 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.[65] The usefulness of eosinophilic biomarkers (high blood/sputum eosinophil count and FeNO level) for predicting favorable responses to omalizumab remains controversial.[66]

Mepolizumab is an anti-IL-5 antibody that has been approved as an add-on treatment of SA.[61] Mepolizumab not only inactivates and eliminates eosinophils, but also down-regulates IL-5 receptor expression on eosinophil membranes.[67] 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.[68] Mepolizumab is considered a reference to biomarker-based stratification for the prediction of therapeutic responsiveness.[69] 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.[70] 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.[69,71] 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.[72,73]

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.[61] 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.[61] 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.[74-76] 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.[77] The TEC level (>300 cells/μL) is a useful biomarker for predicting favorable responsiveness to benralizumab therapy.[69]

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.[78] Previous RCTs have shown clinical efficacy of dupilumab in terms of reduced annual AE rates, improved lung function, and better asthma control.[19] FeNO level and TEC are used as selective biomarkers for predicting dupilumab responsiveness in uncontrolled, moderate-to-severe asthmatics.[79] However, in a randomized, double-blind study, an increase in TEC (>300 cells/μL) did not predict clinical response to dupilumab.[80] 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

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Table 3. Biomarkers for severe asthma from longitudinal studies in adult asthmatic cohorts

Population	Subjects	Methods	Findings
Korean	1,679 asthmatics Followed up: every 3 months for 1 yr	Trajectory cluster analysis of FEV1	Persistent airflow obstruction may be related to non-atopy, a low IgE level, and older age accompanied by neutrophilic inflammation and low baseline FEV1 levels
Korean	567 severe and 1,337 non-severe adult asthmatics for up to 10 yr	Longitudinal outcome model	Higher blood eosinophils/neutrophils, decrease in FEV1, higher AEs Potential biomarkers: increased platelets/basophil counts and higher blood urea nitrogen/uric acid levels

FEV1, the forced expiratory volume in one second; AE, asthma exacerbation.

Table 4. Summary of approved biologics and related biomarkers

Biologics	Target	Biomarkers	Clinical outcome	Airway remodeling outcomes	Reference
Omalizumab	IgE	Serum IgE Positive skin prick tests	Decrease AE and ICS requirement Improve QOL	Decrease the airway thickness and production of TNF-α/TGF-β/IL-4	59
Mepolizumab	IL-5	TEC ≥ 150/μL	Reduce AE and improve lung function and QOL	Reduced the expression of 3 extracellular matrix proteins Decrease TGF-β1 level in BAL fluid	69, 71
Reslizumab	IL-5	TEC ≥ 400/μL	Reduce AE and improve lung function and QOL	ND	72, 73
Benralizumab	IL-5Rα (IL-5)	TEC ≥ 300/μL	Reduce AE and TEC Improve FEV1% and symptoms	Reduced ASM mass Decrease tissues myofibroblasts, and airway expression of TGFβ1	24, 61, 69, 77
Dupilumab	IL4Rα (IL-4 and IL-13)	ND	Reducing AE and improve the lung function/asthma control	ND	79, 80

IgE, immunoglobulin E; AE, asthma exacerbation; ICS, inhaled corticosteroids; QOL, quality of life; TNF-α: tumor necrosis factor alpha; TGF-β: transforming growth factor; IL, interleukin; TEC, blood total eosinophil count; ASM, airway smooth muscle cells; ND, no data.

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.[27,81] 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.[35] Serum EDN levels are significantly elevated in SA than in NSA and showed a good positive correlation to TEC.[35] 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.[38] EDN also induces production of matrix metalloproteinase 9 in CRS, and is suggested to integrate airway inflammation and airway remodeling.[82] Furthermore, our experimental study has shown that the serum EDN level has positive correlations with EET formation as well as with TEC.[36,39] *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.[40] 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.[24,59] First, low Th2 phenotype is a well-known phenotype of SA characterized by predominant neutrophil recruitment into

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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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