Allergy, Asthma &
Immunology Research

AAIR

Review

🔓 **OPEN ACCESS**

Correspondence to
Hae-Sim Park, MD, PhD
Department of Allergy and Clinical
Immunology, Ajou University School of
Medicine, 164 World cup-ro, Yeongtong-gu,
Suwon 16499, Korea.
Tel: +82-31-219-5196
Fax: +82-31-219-5154
Email: hspark@ajou.ac.kr

†Soyoon Sim and Youngwoo Choi equally
contributed to this work.

ORCID iDs
Soyoon Sim 🔟
https://orcid.org/0000-0003-1564-3511
Youngwoo Choi 🔟
https://orcid.org/0000-0002-8384-9557
Hae-Sim Park 🔟
https://orcid.org/0000-0003-2614-0303

Disclosure
There are no financial or other issues that
might lead to conflict of interest.

Update on Inflammatory Biomarkers for Defining Asthma Phenotype

Soyoon Sim 🔟,[1†] **Youngwoo Choi** 🔟,[2†] **Hae-Sim Park** 🔟 [1*]

[1]Department of Allergy and Clinical Immunology, Ajou University School of Medicine, Suwon, Korea
[2]Department of Biomaterials Science (BK21 FOUR Program), College of Natural Resources and Life Science,
 Pusan National University, Miryang, Korea

ABSTRACT

Asthma is a chronic heterogeneous disease characterized by various symptoms and persistent airway inflammation, resulting in progressive lung function decline. Classifying asthma phenotypes/endotypes is crucial because the underlying mechanisms and long-term outcomes vary from patient to patient. Recent trials have identified several biomarkers for classifying asthma phenotypes/endotypes, and current treatments have been developed on the basis of these biomarkers. Conventional biomarkers, including immunoglobulin E, blood/sputum eosinophil counts, airway obstruction or reversibility, and fractional exhaled nitric oxide, are widely used to diagnose asthma. However, these markers have some limitations, necessitating the discovery of additional biomarkers. Therefore, this review summarizes recently suggested biomarkers for representing type 2-high (eosinophilic) vs. type 2-low (neutrophilic) asthma, non-steroidal anti-inflammatory drug-exacerbated respiratory disease, and severe asthma. Additionally, we discuss the potential benefits of these biomarkers in classifying specific phenotypes/endotypes and managing asthmatic patients.

Keywords: Asthma; biomarkers; phenotype; immunoglobulin E; eosinophils; nitric oxide; anti-inflammatory agents, non-steroidal

INTRODUCTION

A clinical diagnosis of asthma is based on typical symptoms, including shortness of breath, chest tightness, wheezing, and reversible airway obstruction and inflammation. Extensive efforts have been made to classify asthma phenotypes/endotypes because of the clinical and pathophysiological complexities of asthma.[1] In addition, ongoing large-scale genetic, molecular, and clinical studies have led to more targeted approaches in asthma diagnosis and treatment. In particular, asthmatic patients should be precisely diagnosed according to their specific phenotypes/endotypes, which might provide clinicians with guidelines for individualized treatment. Therefore, identifying and validating relevant biomarkers helps predict asthma phenotypes/endotypes as indicators of biological processes or clinical responses to specific therapeutic interventions.[2] Despite such efforts, further exploration is needed to discover more relevant biomarkers from various human sources (*e.g.*, peripheral blood, sputum, urine, exhaled gas, or bronchial biopsies), which can be influenced by several factors, including environmental exposure, comorbidities, and medication use. Although some limitations remain unsolved, identifying biomarkers associated with various immune responses is crucial for understanding the mechanisms of chronic airway inflammation, which is intimately

Generated by Xmlinkpress

mediated by immune cells and structural tissues in asthmatic airways. Therefore, this review focuses mainly on recent knowledge about inflammatory biomarkers based on the mechanisms of airway inflammation in asthma according to disease phenotypes/endotypes.

TYPE-2 (T2)-HIGH ASTHMA

Asthma can be classified into T2-high and T2-low asthma based on immunologic basis of airway inflammation. T2-high asthma is characterized by enhanced releases of T2 cytokines, including interleukin (IL)-4, IL-5, and IL-13, which induce eosinophilic airway inflammation with increased levels of serum immunoglobulin (Ig) E and fractional exhaled nitric oxide (FeNO). These cytokines contribute to the differentiation of T-helper cell type 2 (Th2) cells, activation of major effector cells (eosinophils and mast cells), and mucus production in structural cells.[3] Moreover, recent evidence has highlighted the role of innate immune responses in asthma severity. For example, epithelial cell-derived cytokines, such as IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), have been shown to promote T2 inflammation by activating group 2 innate lymphoid cells (ILC2s). The T2-high asthma phenotype accounts for 60% of patients with severe asthma (SA), suggesting that both adaptive and innate immune responses are essential for promoting T2 airway inflammation and disease severity in asthmatic patients.[4,5]

Eosinophils are the main effector cells driving T2 immune responses in asthmatic airways. According to the European Respiratory Society/American Thoracic Society, sputum eosinophil counts ≥ 2% or 3% are widely accepted as a biomarker for determining the eosinophilic asthma (EA), while the cut-off values of peripheral blood eosinophil counts (BEC) ≥ 150 or 300 cells/μL are recommended for classifying the eosinophilic phenotype or SA.[6]

BEC is an easily measurable and minimally invasive biomarker for the EA phenotype (sputum eosinophil counts ≥ 2% or 3%), demonstrating good diagnostic accuracy with a mean area under the curve (AUC) of 0.79 in receiver operating characteristic curve analysis.[7] The cut-off values for determining EA in adult asthmatics vary widely: 188 cells/μL (AUC: 0.89), 210 (AUC: 0.70), 220 (AUC: 0.79), 260 (AUC: 0.90), 270 (AUC: 0.89), 300 (AUC: 0.69), and 330 (AUC: 0.82) (**Table 1**).[8-15] Notably, BEC is significantly associated with high exacerbation frequencies and lung function decline, indicating that it remains a useful biomarker for identifying T2-high asthma, and predicting disease severity. In addition, BEC is variable according to current symptoms and anti-inflammatory medications, including corticosteroids and T2-biologics in each asthmatic. Therefore, monitoring changes in BEC is more informative for evaluating T2-inflammation status. Taken together, BEC is a clinically relevant biomarker for diagnosing T2-high asthma and monitoring clinical outcomes and therapeutic responses in asthmatic patients.

Table 1. Diagnostic accuracy of blood eosinophil count and serum total/free IgE for adult asthma

Biomarker	Phenotype (definition)	Mean AUC (Min. to Max.)	Study subjects	References
Blood eosinophil count	Eosinophilic asthma (sputum eosinophilia ≥ 2% or 3%)	0.79 (0.69–0.90)	Adult asthmatics	8-14
Serum total IgE	Eosinophilic asthma (sputum eosinophilia ≥ 2 %)	0.64	Adult asthmatics	8
	T2-high asthma (Blood eosinophil count ≥ 150 cells/uL and/or sputum eosinophilia ≥ 2% and/or FeNO ≥ 20 ppb)	0.57	Adult asthmatics	16
Serum free IgE	T2-high asthma (Blood eosinophil count ≥ 150 cells/uL and/or sputum eosinophilia ≥ 2% and/or FeNO ≥ 20 ppb)	0.73	Adult asthmatics	16

AUC, area under curve; IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide.

The role of IgE is well-established in T2-high asthma, as it mediates early and late allergic responses after allergen exposure. The cross-linking of IgE with mast cells and basophils induces degranulation, with an explosive release of histamines, leukotrienes, prostaglandins, and cytokines, which cause immediate bronchoconstriction and mucus secretion. The formation of IgE is intimately regulated by IL-4 and IL-13 in B cells, suggesting a close association between IgE and the T2-high phenotype of asthma. Thus, increased serum total IgE levels are common in asthmatic patients. Moreover, significantly higher levels of serum total IgE have been reported in patients with T2-high asthma than in those with T2-low asthma, suggesting its potential as serum biomarkers for the T2-high/eosinophilic phenotype, with the cut-off values of 91 IU/mL (AUC: 0.64) and 120 IU/mL (AUC: 0.57) in adult asthmatics (**Table 1**).[8,16] Therefore, anti-IgE therapy (omalizumab) is approved for treating allergic SA by reducing asthma exacerbations (AEs) and improving asthma control. In addition, more favorable outcomes were noted in patients with T2-high asthma characterized by high total IgE (≥ 100 IU/mL) and allergen-specific IgE levels (≥ 0.35 kU/L), together with high BEC and FeNO values.[17,18] Despite these data, recent studies have reported that total IgE levels are not an independent biomarker for selecting biologics or predicting therapeutic responses, as they could be elevated due to increased levels of omalizumab-IgE complexes after omalizumab treatment.[19,20] Alternatively, serum-free IgE has been suggested as a more reliable biomarker for predicting T2-high asthma (**Table 1**) and monitoring therapeutic responses to anti-IgE therapy in adult asthmatics, although further validation studies are needed.[16,21]

FeNO is the least invasive biomarker for classifying T2-high asthma and predicting therapeutic responses to steroids or T2-biologics in asthmatic patients.[22] The expression of inducible nitric oxide synthase (iNOS; a main source of NO) is increased in airway epithelial cells (AECs) and airway smooth muscle cells (ASMCs) of asthmatic airways, which are affected by T2 cytokines (IL-4/IL-13). Moreover, increased NO facilitates the activation and recruitment of lymphocytes, mast cells, basophils, and eosinophils. Thus, anti-IL-4/IL-13 receptors (dupilumab) and anti-IL-13 antibodies (lebrikizumab) can decrease FeNO levels, improving lung functions and reducing severe AEs in patients with T2-high asthma.[23,24] However, the role of IL-5 in activating iNOS has not been verified, indicating that both FeNO and BEC should be considered to differentiate T2-high asthma from T2-low asthma.[25] Therefore, the GINA recommends FeNO ≥ 20 ppb for classifying T2 airway inflammation in asthmatic patients. It remains an acceptable biomarker for diagnosing and assessing the severity of T2-high asthma, although variable cut-off values (AUC range: 0.77–0.88) are debated in previous publications across various cohorts, including adult asthmatics,[8,9,11,13] smoker vs. non-smoker asthmatics,[26-28] or those with severe asthma (**Table 2**).[29] FeNO values also fluctuate according to the severity of airway inflammation and medications used. Taken together, FeNO is a useful biomarker for classifying the T2-high phenotype and monitoring therapeutic responses in long-term management of asthma.

Table 2. Diagnostic accuracy of FeNO for adult asthma

Biomarker	Phenotype (definition)	Mean AUC (Min. to Max.)	Study subjects	References
FeNO	Eosinophilic asthma (sputum eosinophilia ≥ 2% or 3%)	0.77 (0.72–0.79)	Adult asthmatics	8,9,11,13
		0.88	Smokers with asthma	26
		0.78 (0.77–0.79)	Non-smokers with asthma	27,28
		0.80	Severe asthmatics	29

AUC, area under curve; FeNO, fractional exhaled nitric oxide; EA, eosinophilic asthma; MGA, mixed granulocytic asthma.

T2 cytokines are key mediators responsible for developing and promoting T2/eosinophilic airway inflammation in asthmatics. Among them, IL-4 and IL-13 share binding receptors and downstream activities that drive Th2 airway inflammation and subsequent tissue remodeling (*e.g.*, differentiation/proliferation of Th2 cells, IgE production in B cells, activation/ degranulation of mast cells, goblet cell hyperplasia, mucus hypersecretion, and subepithelial fibrosis in AECs, and contraction of ASMCs).[3] Conversely, IL-5 promotes eosinophilic inflammation accompanied by maturation, differentiation, migration, recruitment, and activation of eosinophils in asthmatic airways. These cytokines are considered T2 signature biomarkers to determine the T2-high/eosinophilic phenotype in asthmatic patients. Despite their importance, their practical use as biomarkers is limited due to difficulties in obtaining and processing each cytokine expression from subjects. Therefore, BEC and FeNO are being used as reasonable biomarkers for selecting and monitoring current T2 biologics (dupilumab, reslizumab, mepolizumab, benralizumab, and tezepelumab) to improve clinical outcomes in patients with uncontrolled T2-high asthma.[7,22] Additionally, combination effects of these biomarkers need to be investigated.

Periostin is an extracellular matrix protein released from AECs and fibroblasts when stimulated by T2 cytokines (IL-4 and IL-13) and is localized in the thickened basement membrane of AECs in asthmatic patients. Consequently, it is considered a surrogate biomarker for T2 airway inflammation and tissue remodeling. There have been a few studies reporting that high serum periostin levels may predict favorable responses to T2-biologic agents (anti-IL-13 or anti-IgE antibodies) in adult asthmatics.[30,31] In particular, asthmatic patients with high serum periostin levels had blood/sputum eosinophilia, high FeNO levels, increased airway hyperresponsiveness (AHR), and high rates of aspirin intolerance, which are key characteristics of severe T2-high asthma uncontrolled by high-dose inhaled glucocorticoids. Serum periostin is an acceptable biomarker for determining T2-high/ eosinophilic phenotype (AUC range: 0.69–0.84)[15,27,32,33] or reflecting disease severity (AUC range: 0.70–0.79)[34,35] in adult asthmatics (**Table 3**). However, little is known about whether it may change according to clinical outcomes. Taken together, serum periostin can be a useful biomarker for representing the severity of T2-high asthma. Further investigations are needed to validate its role as a therapeutic marker for current T2-biologics.

Alarmins, which are epithelium-derived cytokines (IL-25, IL-33, and TSLP) released from AECs in response to external stimuli and epithelial damage, orchestrate both innate and adaptive immune responses that enhance T2 airway inflammation. Alarmins activate dendritic cells and ILC2s, resulting in Th2 cell differentiation and increased T2 cytokine release.[4] They are also involved in the maturation, proliferation, development, and infiltration of eosinophils into the airway, potentially leading to persistent airway eosinophilia, severe AEs, and steroid resistance in asthmatic patients.[36,37] However, these alarmins are not practical biomarkers as they are difficult to obtain and measure in human samples.[38]

Oleoylethanolamide (OEA), an endogenously generated cannabinoid, exerts immunomodulatory effects by binding to cannabinoid receptors (CB1 and CB2 receptors) on immune cells, such as eosinophils and monocytes. Recently, higher serum OEA levels

Table 3. Diagnostic accuracy of serum periostin for adult asthma

Biomarker	Phenotype (definition)	Mean AUC (Min. to Max.)	Study subjects	References
Serum periostin	Eosinophilic asthma (sputum eosinophilia ≥ 3%)	0.69 (0.69–0.70)	Patients with EA and MGA	15,32,33
		0.84	Non-smokers with asthma	27

AUC, area under curve; EA, eosinophilic asthma; MGA, mixed granulocytic asthma.

were reported in patients with SA or non-steroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease (N-ERD). Moreover, OEA was found to induce IL-33 secretion in AECs, which in turn increases T2 cytokine levels by activating ILC2s and eosinophils, thus promoting T2 airway inflammation.[39,40] Especially, surface CB2 receptor expression on eosinophils was significantly higher in patients with EA than in those with non-EA. Finally, the blockade of CB2 receptors decreased the release of alarmins and T2 cytokines as well as the recruitment of eosinophils and ILC2s to the airways, indicating that serum OEA levels may be a novel biomarker for classifying T2-high asthma, which is related to ILC2 activation and reduced steroid responsiveness.[41]

T2-LOW ASTHMA

Although the pathogenesis of T2-low asthma is not fully understood to date, it is believed to be associated with neutrophilic airway inflammation driven by the IL-17 pathway, which forms a clinically distinct feature without prominent T2 cytokine signatures. Moreover, increased IL-17 levels are related to asthma severity with steroid resistance by promoting neutrophil infiltration.[42] Although airway neutrophilia can be observed without airway eosinophilia, more cases present a mixed granulocytic asthma (MGA), characterized by both airway eosinophilia and neutrophilia. Moreover, it has been suggested that significant changes in sphingolipid metabolism could enhance the T2-low phenotype by promoting airway neutrophilia and M1 macrophage polarization.[43,44]

Numerous investigations have sought to identify non-T2 cytokines as therapeutic targets for modulating neutrophil infiltration in T2-low asthma. Especially, IL-8 and IL-17 are involved in the recruitment and activation of neutrophils. IL-8 is primarily released from AECs in response to lipopolysaccharide or T1 cytokines. On the contrary, IL-17 is secreted from various immune cells, such as Th17 cells, ILC1s, and ILC3s, contributing to airway remodeling by enhancing hyperplasia of goblet cells and ASMCs. Both cytokines are strongly correlated with airway neutrophilia and asthma severity, characterized by accelerated lung function decline and steroid resistance.[42,45] Moreover, increased serum levels or sputum expression of IL-8 and IL-17 have been reported in patients with neutrophilic asthma (NA) or paucigranulocytic asthma (PGA).[46,47] Therefore, blocking these cytokines from binding to their receptors could be a novel intervention in asthma treatment. Recently, antagonists of CXCR2, a high-affinity receptor of IL-8, were shown to reduce blood/sputum neutrophilia and improve asthma control in patients with SA, suggesting that CXCR2 antagonists could serve as an add-on therapy for patients with T2-low asthma.[48] However, blocking IL-17 did not improve neutrophilic inflammation or asthma control in clinical trials in patients with SA,[49] indicating that the potential of serum/sputum IL-8 or IL-17 as biomarkers for T2-low asthma remains controversial.

IL-1β is an innate immune cytokine mainly released from macrophages via activation of the nucleotide-binding domain leucine-rich repeat pyrin domain-containing 3 (NLRP3) inflammasome. Especially, the NLRP3/IL-1β pathway is associated with airway neutrophilia, steroid resistance, and asthma severity by promoting Th17 cell differentiation and IL-17 production. Thus, regulating NLPR3 activation could potentially improve neutrophilic airway inflammation and AHR in asthmatic patients.[50,51] Patients with NA exhibited increased expression of IL-1β in blood monocytes as well as sputum macrophages/neutrophils. Recently, the deficiency of ovarian tumor protease deubiquitinase with linear linkage

specificity (otulin) was found to drive NLRP3 activation and IL-1β overproduction in patients with NA, suggesting that otulin could be a biomarker or therapeutic target for NA in adult asthmatics.[52] Thus, the NLRP3 inflammasome and related signaling enzymes could be potential biomarkers for T2-low asthma, particularly NA.

S100 calcium-binding protein A9 (S100A9) is an alarmin released from activated neutrophils, macrophages, and AECs. S100A9 initiates and amplifies neutrophil infiltration and airway inflammation by activating Toll-like receptor 4 and increasing IL-8 production. Higher serum/sputum levels of S100A9 were reported in patients with NA compared to those with EA or PGA.[53] Furthermore, high serum S100A9 levels are associated with AHR, lung function decline, and blood/sputum neutrophilia, suggesting its potential as a biomarker for identifying NA or SA phenotypes in adult asthmatics.[54] Therefore, S100A9 may be a promising biomarker for differentiating T2-low asthma from T2-high asthma in asthmatic patients, although further investigations are needed to confirm its role as a monitoring marker in NA.

N-ERD

N-ERD, characterized by eosinophilic inflammation in lower airway mucosa, is found in patients with asthma as well as in those with chronic rhinosinusitis with nasal polyps. It is considered an independent phenotype because of its unique pathophysiology involving dysregulation of arachidonic acid metabolism, which produces lipid mediators, such as cysteinyl leukotrienes (CysLTs), even in the absence of cyclooxygenase-1 (COX-1) inhibitor. Both aspirin and NSAIDs block COX-1 and induce immediate hypersensitivity reactions with T2/eosinophilic inflammation.[55] To date, while oral or lysine aspirin challenges are commonly used to diagnose N-ERD, reliable biomarkers (except for leukotriene metabolites) have yet to be validated.

The overproduction of CysLTs, accompanied by persistent eosinophilic inflammation and impaired lung function, is a key feature of N-ERD. It reflects the upregulation of the 5-lipoxygenase (5-LO) pathway, resulting in the consecutive conversion of CysLTs to LTD4 and finally to LTE4.[55] LTE4 is produced by eosinophils, neutrophils, mast cells, and basophils and acts as a strong bronchoconstrictor as well as a chemoattractant and activator of eosinophils and mast cells via binding to CysLT1 and CysLT2 receptors, exacerbating T2 inflammation. This process presents a more severe phenotype/endotype of N-ERD. Thus, high concentrations of serum or urinary LTE4 (uLTE4), the stable end product of CysLTs, could be a useful biomarker for differentiating patients with N-ERD from those with aspirin-tolerant asthma (ATA), which has been replicated in various cohorts. The measurement of LTE4 in urine (AUC range: 0.80–0.87)[56-58] or serum (AUC: 0.65)[59] is a noninvasive, sensitive, and reliable method for diagnosing N-ERD, albeit without standardized cut-off values (**Table 4**). Although uLTE4 levels were not fully suppressed by pharmacologic treatments such as inhaled corticosteroids and leukotriene modifier,[60] T2-biologics such as omalizumab significantly reduced uLTE4 levels

Table 4. Diagnostic accuracy of urine/serum LTE4 for N-ERD

Biomarker	Phenotype	Mean AUC (Min. to Max.)	Study subjects	References
Urine LTE4	N-ERD (evaluated by HPLC-MS)	0.82 (0.80–0.83)	Adult asthmatics	56,57
	N-ERD (evaluated by ELISA)	0.87 (0.86–0.87)	Adult asthmatics	56,58
Serum LTE4	N-ERD (evaluated by UHPLC/Q-TOF MS)	0.65	Patients with N-ERD vs. those with ATA	59

N-ERD, non-steroidal anti-inflammatory drug-exacerbated respiratory disease; AUC, area under curve; HPLC, high-performance liquid chromatography; MS, mass spectrometry; ELISA, enzyme-linked immunosorbent assay; LTE4, leukotriene E4; UHPLC, ultra-high-performance liquid chromatography; Q-TOF, quadrupole time-of-flight; ATA, aspirin-tolerant asthma; HC, healthy control.

and improved clinical outcomes in patients with N-ERD.[61] However, further development of a practical marker is urgent, as the measurement of uLTE4 is not easy in clinical practice. Taken together, uLTE4 is a relevant diagnostic marker for the phenotype of N-ERD and may become a therapeutic biomarker in response to T2-biologics targeting mast cells.

In contrast to the enhanced 5-LO pathway, a reduction in COX-2 expression followed by increased prostaglandin D2 (PGD2) and decreased PGE2 synthesis is also a key feature of N-ERD. PGD2, mainly released from eosinophils and mast cells, induces proinflammatory responses and bronchoconstriction by binding with the chemoattractant receptor-homologous molecule expressed on Th2 cells. In contrast, diminished PGE2 production in AECs or ASMCs of patients with N-ERD cannot suppress the activation or degranulation of eosinophils and mast cells, which are critical for the pathogenesis of N-ERD.[55] Moreover, PGE2 not only inhibits Th2 cell differentiation but also promotes the proliferation of regulatory T cells, ultimately resolving T2 inflammation.[62] In addition, PGE2 prevents bronchospasm and fibrotic remodeling in the airway by binding to the PGE2 receptor 1–4.[55] Several studies have shown significantly increased PGD2 and decreased PGE2 levels in the urine, sputum, or blood of patients with N-ERD. However, the application of PGD2 or PGE2 levels for diagnosing NERD remains controversial, as it is very difficult to measure them in human samples. Further studies are required to identify additional biomarkers.

Genome-wide association studies in asthma have identified several genetic variants associated with asthma susceptibility and lung function decline, including dipeptidyl peptidase 10 (DPP10). DPP10 is localized in AECs and immune cells, such as eosinophils, neutrophils, and peripheral blood mononuclear cells. Higher levels of serum DPP10 were reported in patients with N-ERD than in those with ATA or HCs. Furthermore, DPP10 contributes to eosinophilic inflammation and fibrotic remodeling by inducing the extracellular matrix and collagen deposition in asthmatic airways, resulting in lung function decline in patients with N-ERD. Therefore, serum DPP10 levels can be used as diagnostic biomarkers for N-ERD (AUC: 0.79),[63,64] although further validation is needed.

SA

SA is characterized by uncontrolled symptoms and frequent AEs, even with regular anti-inflammatory treatments including steroids. However, recent longitudinal studies have improved our understanding of the pathogenic mechanisms of SA and identified multiple target molecules.[65]

Enhanced eosinophilic inflammation is a key feature of SA. Eosinophil-derived neurotoxin (EDN), a granule protein from eosinophils, has served as a surrogate biomarker that mirrors eosinophil activation/degranulation and disease severity in asthmatic patients. Serum EDN levels are strongly correlated with high blood/sputum eosinophil counts and low FEV1 (%) values, which are main clinical features of SA. Serum EDN has been highlighted as a representative biomarker for SA or poor control status, which is more indicative than BECs or eosinophil-cationic protein in adult asthmatics. In addition, it can be detected in urine and sputum samples in relation to the degree of eosinophilic inflammation and asthma control status.[60] Serum EDN levels significantly decreased after anti-IL-5 antibody treatment, suggesting its role as monitoring biomarker[66]; however, additional replication studies are needed according to treatment duration and various T2-biologics.

Extracellular traps released from eosinophils (EETs) could be a target molecule for diagnosing and treating SA. EETs are involved in the progression of T2 airway inflammation and tissue remodeling in patients with severe EA. Activated eosinophils release EETs, a whole complex of DNA fibers and granule proteins, that act as an innate immune defense mechanism against bacterial or viral infection. EETs induce epithelial damage and cell detachment, which exacerbate T2 inflammation, especially in patients with EA.[67,68] Increased proportions of EET-forming eosinophils in patients with SA contribute to low FEV1 (%) values and high serum EDN levels. Thus, regulating EET formation may be a novel therapeutic target for SA, requiring additional treatment. EETs also induce the release of TSLP and IL-33 in AECs, promoting ILC2 activation and T2 cytokine production, which are resistant to steroid treatment.[69] Considering that EETs activate both innate and T2 immune responses in asthmatic airways, they could serve as both diagnostic and therapeutic biomarkers for SA, especially in severe EA. It is not easy to detect EETs in real-world practice; further validation studies whether any serum biomarker including serum EDN may represent EET formation in patients with SA.

CONCLUSION

Although several reliable biomarkers for T2-high asthma or SA have been reported, there remain unmet needs for identifying relevant biomarkers for T2-low asthma, MGA, or PGA. Further investigation into molecular and inflammatory mechanisms of asthma could help identify and validate local/systemic biomarkers specific to each phenotype/endotype and biomarkers useful for predicting therapeutic responses, ultimately improving clinical outcomes in the long-term management of asthma.

ACKNOWLEDGMENTS

REFERENCES

1. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012;18:716-25. **PUBMED** | **CROSSREF**
2. Guida G, Bagnasco D, Carriero V, Bertolini F, Ricciardolo FLM, Nicola S, et al. Critical evaluation of asthma biomarkers in clinical practice. Front Med (Lausanne) 2022;9:969243. **PUBMED** | **CROSSREF**
3. Robinson D, Humbert M, Buhl R, Cruz AA, Inoue H, Korom S, et al. Revisiting type 2-high and type 2-low airway inflammation in asthma: current knowledge and therapeutic implications. Clin Exp Allergy 2017;47:161-75. **PUBMED** | **CROSSREF**
4. Gauvreau GM, Bergeron C, Boulet LP, Cockcroft DW, Côté A, Davis BE, et al. Sounding the alarmins-the role of alarmin cytokines in asthma. Allergy 2023;78:402-17. **PUBMED** | **CROSSREF**
5. Garg D, Que LG, Ingram JL. Effects of biological therapies on patients with type-2 high asthma and comorbid obesity. Front Pharmacol 2024;14:1315540. **PUBMED** | **CROSSREF**
6. Khurana S, Bush A, Holguin F. Management of severe asthma: summary of the European Respiratory Society/American Thoracic Society task force report. Breathe (Sheff) 2020;16:200058. **PUBMED** | **CROSSREF**
7. Korevaar DA, Westerhof GA, Wang J, Cohen JF, Spijker R, Sterk PJ, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. Lancet Respir Med 2015;3:290-300. **PUBMED** | **CROSSREF**

8. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Pascual RM, et al. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. J Allergy Clin Immunol 2013;132:72-80.e12. **PUBMED** | **CROSSREF**

9. Soma T, Iemura H, Naito E, Miyauchi S, Uchida Y, Nakagome K, et al. Implication of fraction of exhaled nitric oxide and blood eosinophil count in severe asthma. Allergol Int 2018;67S:S3-11. **PUBMED** | **CROSSREF**

10. Liang Z, Zhao H, Lv Y, Li R, Dong H, Liu L, et al. Moderate accuracy of peripheral eosinophil count for predicting eosinophilic phenotype in steroid-naïve non-atopic adult asthmatics. Intern Med 2012;51:717-22. **PUBMED** | **CROSSREF**

11. Schleich FN, Manise M, Sele J, Henket M, Seidel L, Louis R. Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. BMC Pulm Med 2013;13:11. **PUBMED** | **CROSSREF**

12. Zhang XY, Simpson JL, Powell H, Yang IA, Upham JW, Reynolds PN, et al. Full blood count parameters for the detection of asthma inflammatory phenotypes. Clin Exp Allergy 2014;44:1137-45. **PUBMED** | **CROSSREF**

13. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Bel EH, et al. External validation of blood eosinophils, FE_{NO} and serum periostin as surrogates for sputum eosinophils in asthma. Thorax 2015;70:115-20. **PUBMED** | **CROSSREF**

14. Schleich F, Brusselle G, Louis R, Vandenplas O, Michils A, Pilette C, et al. Heterogeneity of phenotypes in severe asthmatics. Respir Med 2014;108:1723-32. **PUBMED** | **CROSSREF**

15. Simpson JL, Yang IA, Upham JW, Reynolds PN, Hodge S, James AL, et al. Periostin levels and eosinophilic inflammation in poorly-controlled asthma. BMC Pulm Med 2016;16:67. **PUBMED** | **CROSSREF**

16. Woo SD, Yang EM, Jang J, Lee Y, Shin YS, Ye YM, et al. Serum-free immunoglobulin E: a useful biomarker of atopy and type 2 asthma in adults with asthma. Ann Allergy Asthma Immunol 2021;127:109-115.e1. **PUBMED** | **CROSSREF**

17. 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;55:1900588. **PUBMED** | **CROSSREF**

18. Soong W, Yoo B, Pazwash H, Holweg CTJ, Casale TB. Omalizumab response in patients with asthma by number and type of allergen. Ann Allergy Asthma Immunol 2021;127:223-31. **PUBMED** | **CROSSREF**

19. Sánchez J, Morales E, Santamaria LC, Acevedo AM, Calle A, Olivares M, et al. IgE, blood eosinophils and FeNO are not enough for choosing a monoclonal therapy among the approved options in patients with type 2 severe asthma. World Allergy Organ J 2021;14:100520. **PUBMED** | **CROSSREF**

20. Gon Y, Ito R, Maruoka S, Mizumura K, Kozu Y, Hiranuma H, et al. Long-term course of serum total and free IgE levels in severe asthma patients treated with omalizumab. Allergol Int 2018;67:283-5. **PUBMED** | **CROSSREF**

21. Ye YM, Park JW, Kim SH, Cho YS, Lee SY, Lee SY, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of YH35324, a novel long-acting high-affinity IgE_{Trap}-Fc protein in subjects with atopy: results from the first-in-human study. Int Immunopharmacol 2024;130:111706. **PUBMED** | **CROSSREF**

22. Maniscalco M, Fuschillo S, Mormile I, Detoraki A, Sarnelli G, Paulis A, et al. Exhaled nitric oxide as biomarker of type 2 diseases. Cells 2023;12:2518. **PUBMED** | **CROSSREF**

23. 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**

24. Yatera K, Mukae H. Possible pathogenic roles of nitric oxide in asthma. Respir Investig 2019;57:295-7. **PUBMED** | **CROSSREF**

25. Menzies-Gow A, Mansur AH, Brightling CE. Clinical utility of fractional exhaled nitric oxide in severe asthma management. Eur Respir J 2020;55:1901633. **PUBMED** | **CROSSREF**

26. Hillas G, Kostikas K, Mantzouranis K, Bessa V, Kontogianni K, Papadaki G, et al. Exhaled nitric oxide and exhaled breath condensate pH as predictors of sputum cell counts in optimally treated asthmatic smokers. Respirology 2011;16:811-8. **PUBMED** | **CROSSREF**

27. Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, et al. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. J Allergy Clin Immunol 2012;130:647-654.e10. **PUBMED** | **CROSSREF**

28. Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavord ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. Clin Exp Allergy 2005;35:1175-9. **PUBMED** | **CROSSREF**

29. Tseliou E, Bessa V, Hillas G, Delimpoura V, Papadaki G, Roussos C, et al. Exhaled nitric oxide and exhaled breath condensate pH in severe refractory asthma. Chest 2010;138:107-13. **PUBMED** | **CROSSREF**

30. Hanania NA, Wenzel S, Rosén K, Hsieh HJ, Mosesova S, Choy DF, et al. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. Am J Respir Crit Care Med 2013;187:804-11. PUBMED | CROSSREF

31. Corren J, Lemanske RF Jr, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med 2011;365:1088-98. PUBMED | CROSSREF

32. 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

33. Yormaz B, Menevse E, Cetin N, Esin Celik Z, Bakir H, Tulek B, et al. Diagnostic value of thymus and activation-regulated chemokine and of periostin in eosinophilic asthma: a prospective study. Allergy Asthma Proc 2021;42:e30-9. PUBMED | CROSSREF

34. Sunadome H, Matsumoto H, Tohda Y, Horiguchi T, Kita H, Kuwabara K, et al. Assessment of serum periostin level as a predictor of requirement for intensive treatment for type-2 inflammation in asthmatics in future: a follow-up study of the KiHAC cohort. Allergol Int 2021;70:252-4. PUBMED | CROSSREF

35. Mansur AH, Srivastava S, Sahal A. Disconnect of type 2 biomarkers in severe asthma; dominated by FeNO as a predictor of exacerbations and periostin as predictor of reduced lung function. Respir Med 2018;143:31-8. PUBMED | CROSSREF

36. Johnston LK, Bryce PJ. Understanding interleukin 33 and its roles in eosinophil development. Front Med (Lausanne) 2017;4:51. PUBMED | CROSSREF

37. Cheung PF, Wong CK, Ip WK, Lam CW. IL-25 regulates the expression of adhesion molecules on eosinophils: mechanism of eosinophilia in allergic inflammation. Allergy 2006;61:878-85. PUBMED | CROSSREF

38. Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM, et al. Tezepelumab in adults with uncontrolled asthma. N Engl J Med 2017;377:936-46. PUBMED | CROSSREF

39. Reinke SN, Gallart-Ayala H, Gómez C, Checa A, Fauland A, Naz S, et al. Metabolomics analysis identifies different metabotypes of asthma severity. Eur Respir J 2017;49:1601740. PUBMED | CROSSREF

40. Kwon EK, Choi Y, Yoon IH, Won HK, Sim S, Lee HR, et al. Oleoylethanolamide induces eosinophilic airway inflammation in bronchial asthma. Exp Mol Med 2021;53:1036-45. PUBMED | CROSSREF

41. Kwon EK, Choi Y, Sim S, Ye YM, Shin YS, Park HS, et al. Cannabinoid receptor 2 as a regulator of inflammation induced oleoylethanolamide in eosinophilic asthma. J Allergy Clin Immunol 2024;153:998-1009.e9. PUBMED | CROSSREF

42. Fitzpatrick AM, Chipps BE, Holguin F, Woodruff PG. T2-"low" asthma: overview and management strategies. J Allergy Clin Immunol Pract 2020;8:452-63. PUBMED | CROSSREF

43. Choi Y, Kim M, Kim SJ, Yoo HJ, Kim SH, Park HS. Metabolic shift favoring C18:0 ceramide accumulation in obese asthma. Allergy 2020;75:2858-66. PUBMED | CROSSREF

44. Sim S, Choi Y, Park HS. Potential metabolic biomarkers in adult asthmatics. Metabolites 2021;11:430. PUBMED | CROSSREF

45. Pease JE, Sabroe I. The role of interleukin-8 and its receptors in inflammatory lung disease: implications for therapy. Am J Respir Med 2002;1:19-25. PUBMED | CROSSREF

46. Fogli LK, Sundrud MS, Goel S, Bajwa S, Jensen K, Derudder E, et al. T cell-derived IL-17 mediates epithelial changes in the airway and drives pulmonary neutrophilia. J Immunol 2013;191:3100-11. PUBMED | CROSSREF

47. Ntontsi P, Loukides S, Bakakos P, Kostikas K, Papatheodorou G, Papathanassiou E, et al. Clinical, functional and inflammatory characteristics in patients with paucigranulocytic stable asthma: comparison with different sputum phenotypes. Allergy 2017;72:1761-7. PUBMED | CROSSREF

48. O'Byrne PM, Metev H, Puu M, Richter K, Keen C, Uddin M, et al. Efficacy and safety of a CXCR2 antagonist, AZD5069, in patients with uncontrolled persistent asthma: a randomised, double-blind, placebo-controlled trial. Lancet Respir Med 2016;4:797-806. PUBMED | CROSSREF

49. Busse WW, Holgate S, Kerwin E, Chon Y, Feng J, Lin J, et al. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. Am J Respir Crit Care Med 2013;188:1294-302. PUBMED | CROSSREF

50. Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, et al. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. Immunity 2009;30:576-87. PUBMED | CROSSREF

51. Kim RY, Pinkerton JW, Essilfie AT, Robertson AA, Baines KJ, Brown AC, et al. Role for NLRP3 inflammasome-mediated, IL-1β-dependent responses in severe, steroid-resistant asthma. Am J Respir Crit Care Med 2017;196:283-97. PUBMED | CROSSREF

52. Quoc QL, Kim Y, Park G, Cao TBT, Choi Y, Park YH, et al. Downregulation of otulin induces inflammasome activation in neutrophilic asthma. J Allergy Clin Immunol 2024;154:557-70. PUBMED | CROSSREF

53. Lee TH, Chang HS, Bae DJ, Song HJ, Kim MS, Park JS, et al. Role of S100A9 in the development of neutrophilic inflammation in asthmatics and in a murine model. Clin Immunol 2017;183:158-66.
PUBMED | **CROSSREF**

54. Lee YG, Hong J, Lee PH, Lee J, Park SW, Kim D, et al. Serum calprotectin is a potential marker in patients with asthma. J Korean Med Sci 2020;35:e362.   **PUBMED** | **CROSSREF**

55. Rhyou HI, Nam YH, Park HS. Emerging biomarkers beyond leukotrienes for the management of nonsteroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease. Allergy Asthma Immunol Res 2022;14:153-67.   **PUBMED** | **CROSSREF**

56. Sanak M, Bochenek G, Faber J, Plutecka H, Szczeklik A. Elevated urinary leukotriene E excretion in asthma: a comparison of HPLC-mass spectrometry and ELISA. Allergy 2010;65:663-4.   **PUBMED** | **CROSSREF**

57. Comhair SA, Bochenek G, Baicker-McKee S, Wang Z, Stachura T, Sanak M, et al. The utility of biomarkers in diagnosis of aspirin exacerbated respiratory disease. Respir Res 2018;19:210.   **PUBMED** | **CROSSREF**

58. Celejewska-Wójcik N, Mastalerz L, Wójcik K, Nieckarz R, Januszek R, Hartwich P, et al. Incidence of aspirin hypersensitivity in patients with chronic rhinosinusitis and diagnostic value of urinary leukotriene E4. Pol Arch Med Wewn 2012;122:422-7.   **PUBMED** | **CROSSREF**

59. Ban GY, Cho K, Kim SH, Yoon MK, Kim JH, Lee HY, et al. Metabolomic analysis identifies potential diagnostic biomarkers for aspirin-exacerbated respiratory disease. Clin Exp Allergy 2017;47:37-47.
PUBMED | **CROSSREF**

60. Ban GY, Yang EM, Ye YM, Park HS. Association of eosinophil-derived neurotoxin levels with asthma control status in patients with aspirin-exacerbated respiratory disease. Clin Transl Allergy 2023;13:e12229.   **PUBMED** | **CROSSREF**

61. Hayashi H, Fukutomi Y, Mitsui C, Kajiwara K, Watai K, Tomita Y, et al. Omalizumab ameliorates extrarespiratory symptoms in patients with aspirin-exacerbated respiratory disease. J Allergy Clin Immunol 2023;151:1667-1672.e2.   **PUBMED** | **CROSSREF**

62. Sastre B, del Pozo V. Role of PGE2 in asthma and nonasthmatic eosinophilic bronchitis. Mediators Inflamm 2012;2012:645383.   **PUBMED** | **CROSSREF**

63. Kim SH, Choi H, Yoon MG, Ye YM, Park HS. Dipeptidyl-peptidase 10 as a genetic biomarker for the aspirin-exacerbated respiratory disease phenotype. Ann Allergy Asthma Immunol 2015;114:208-13.
PUBMED | **CROSSREF**

64. Sim S, Choi Y, Lee DH, Lee HR, Shin YS, Park HS. Contribution of dipeptidyl peptidase 10 to airway dysfunction in patients with NSAID-exacerbated respiratory disease. Clin Exp Allergy 2022;52:115-26.
PUBMED | **CROSSREF**

65. Lee Y, Quoc QL, Park HS. Biomarkers for severe asthma: lessons from longitudinal cohort studies. Allergy Asthma Immunol Res 2021;13:375-89.   **PUBMED** | **CROSSREF**

66. Jang JH, Woo SD, Lee Y, Kim CK, Shin YS, Ye YM, et al. Changes in type 2 biomarkers after anti-IL5 treatment in patients with severe eosinophilic asthma. Allergy Asthma Immunol Res 2021;13:330-8.
PUBMED | **CROSSREF**

67. Shen K, Zhang M, Zhao R, Li Y, Li C, Hou X, et al. Eosinophil extracellular traps in asthma: implications for pathogenesis and therapy. Respir Res 2023;24:231.   **PUBMED** | **CROSSREF**

68. Hussain M, Liu G. Eosinophilic asthma: pathophysiology and therapeutic horizons. Cells 2024;13:384.
PUBMED | **CROSSREF**

69. Choi Y, Kim YM, Lee HR, Mun J, Sim S, Lee DH, et al. Eosinophil extracellular traps activate type 2 innate lymphoid cells through stimulating airway epithelium in severe asthma. Allergy 2020;75:95-103.   **PUBMED** | **CROSSREF**