Review

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Contemporary Biomarkers in Severe Asthma Management

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Contemporary asthma management requires a proactive and personalized approach, integrating precise diagnosis with individualized treatment strategies. The incorporation of biological therapies for severe asthma into standard clinical practice underscores the importance of specific patient selection, outcome prediction, and continuous monitoring of these costly and prolonged treatments. Biomarkers, functioning as crucial indicators, have significantly impacted asthma by facilitating disease identification, predicting severity and prognosis, and assessing treatment effectiveness. This comprehensive review aims to thoroughly explore the diverse biomarkers associated with asthma, elucidating their roles in disease identification, severity prediction, and treatment response assessment. By delving into the intricate molecular and genetic aspects of asthma susceptibility, the review seeks to provide a nuanced understanding of the factors that influence the onset and severity of asthma. The review covers a spectrum of molecular factors that influence the susceptibility and severity of asthma. It explores the roles of specific biomarkers such as galectin-3, periostin, fractional exhaled nitric oxide, and eosinophils in airway remodeling and inflammation. Genetic factors like filaggrin mutations and chitinase-3-like protein 1 (CHI3L1 or YKL-40) variations are also examined. The review delves into proteomics, revealing unique plasma protein signatures linked to severe asthma and chronic obstructive pulmonary disease. It thoroughly examines the impact of the microbiome on asthma development, persistence, and severity, considering gut and lung microbial dysbiosis. Additionally, it highlights the potential of probiotics in managing allergic respiratory diseases and investigates the preventative effects of early exposure to pets in childhood. This review is a valuable resource for clinicians, researchers, and healthcare professionals in this field. It highlights the crucial role of biomarkers in asthma and offers a comprehensive understanding of genetics, proteomics, and the microbiome, enriching our comprehension of asthma management and preventive measures.

Keywords: biomarkers; asthma; severe asthma; children

Asthma in Pediatric Age

Asthma is a chronic inflammatory condition that affects the airways, causing reversible airflow restrictions and increased airway sensitivity. Globally, an estimated 5–10% of individuals with asthma experience severe symptoms, requiring the use of oral corticosteroids or other controller medications alongside high doses of inhaled corticosteroids to achieve and maintain control [1].

Severe asthma presents a complex pathophysiology. With advancing understanding of the condition, diagnostic methods have evolved. Conventional diagnostic categories have been replaced by definitions based on specific traits (phenotypes) or underlying causes (endotypes or endophenotypes). The distinction between phenotypes and endotypes is crucial as it enables personalized and targeted treatment strategies that align with the specific airway inflammation mechanisms [2,3].

This highlights the importance of exploring the phenotypes and endotypes of the condition to facilitate a more individualized approach to its treatment [4]. When assessing asthma, clinical factors including the age of onset, spirometry, presence or absence of atopy, and other exposures are essential factors. However, these factors often do not fully characterize patients with severe disease or predict their response to treatment accurately. Biomarkers provide objective measurements that help identify specific biological processes or characteristics associated with a disease. In the context of asthma, biomarkers hold promise in providing more effective personalized treatment strategies. They aid in identifying the underlying drivers of the disease and predict individual responses to different treatments. Additionally, biomarkers facilitate the monitoring of disease progression and treatment efficacy, providing real-time feedback on the effectiveness of therapies and enabling neces-

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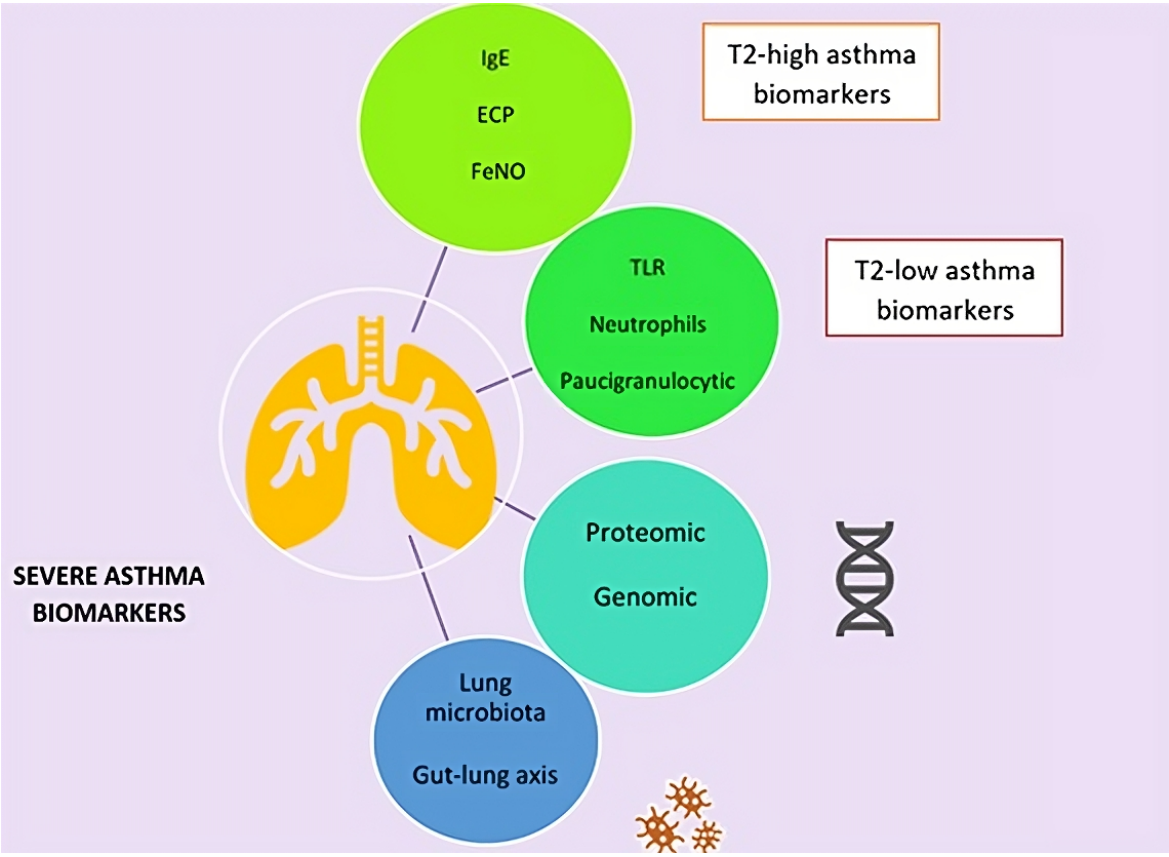

Fig. 1. Biomarkers in severe asthma management. The picture is original by the author using the functions of Word 2021 (Microsoft, Redmond, WA, USA). IgE, Immunoglobulin E; T2, Type 2; ECP, eosinophil cationic protein; FeNO, fractional exhaled nitric oxide; TLR, toll-like receptor.

sary adjustments. This is especially crucial in severe asthma cases, where traditional therapies may not be sufficient, and alternative approaches are required (Fig. 1).

Asthma Phenotypes and Endotypes

Based on the clinical characteristics and pathophysiological aspects of asthma, various phenotypes and endotypes have been identified [5]. Asthma phenotypes are generally categorized as either Type 2 (T2) high or T2 low (non-T2) diseases, depending on clinical manifestations such as age of onset and atopic comorbidities, along with the presence or absence of markers indicating underlying airway inflammation (Fig. 2).

T2 asthma is the most well-defined subtype of asthma, characterized by distinct immunopathology, related biomarkers, and targeted treatments [6]. This type of asthma is primarily marked by airway eosinophilic inflammation, often classified as either eosinophilic or non-eosinophilic [7]. The eosinophilic inflammation is driven predominantly by T2 immune responses regulated by T2-helper (Th2) cells and Group 2 innate lymphoid cells (ILC-2), along with their associated Th2 cytokines: interleukin (IL)-4, IL-5, and IL-13. These cytokines interact with allergen-specific B cells, facilitating Immunoglobulin E (IgE) class switching. Subsequently, IgE binds to the surface of mast cells and basophils, triggering the release of histamine, leukotrienes, and cytokines that induce bronchial inflammation [8]. IL-5, IL-4, and IL-13 are key cytokines in the eosinophilic inflammatory process, as they regulate the eosinophilic migration, activation, and survival within the asthmatic airway [9]. Many proposed asthma biomarkers are linked to these T2 inflammatory pathways. Eosinophilic inflammation, observed in both allergic and nonallergic individuals with asthma, is orchestrated by various inflammatory cells. These cells include not only those associated with adaptive immunity, like Th2 cells but also those linked to innate immunity, such as Group 2 innate lymphoid cells (ILC-2). The presence of eosinophils plays a crucial role in defining asthma phenotypes [10,11] and stems from inflammation associated with Type 2 (T2) pathways. Numerous potential asthma biomarkers have been linked to inflammatory pathways related to T2 responses.

Biomarkers Reflecting T2 Airway Inflammation

A biomarker, a quantifiable indicator of a biological state or disease, is typically assessed in biological flu-

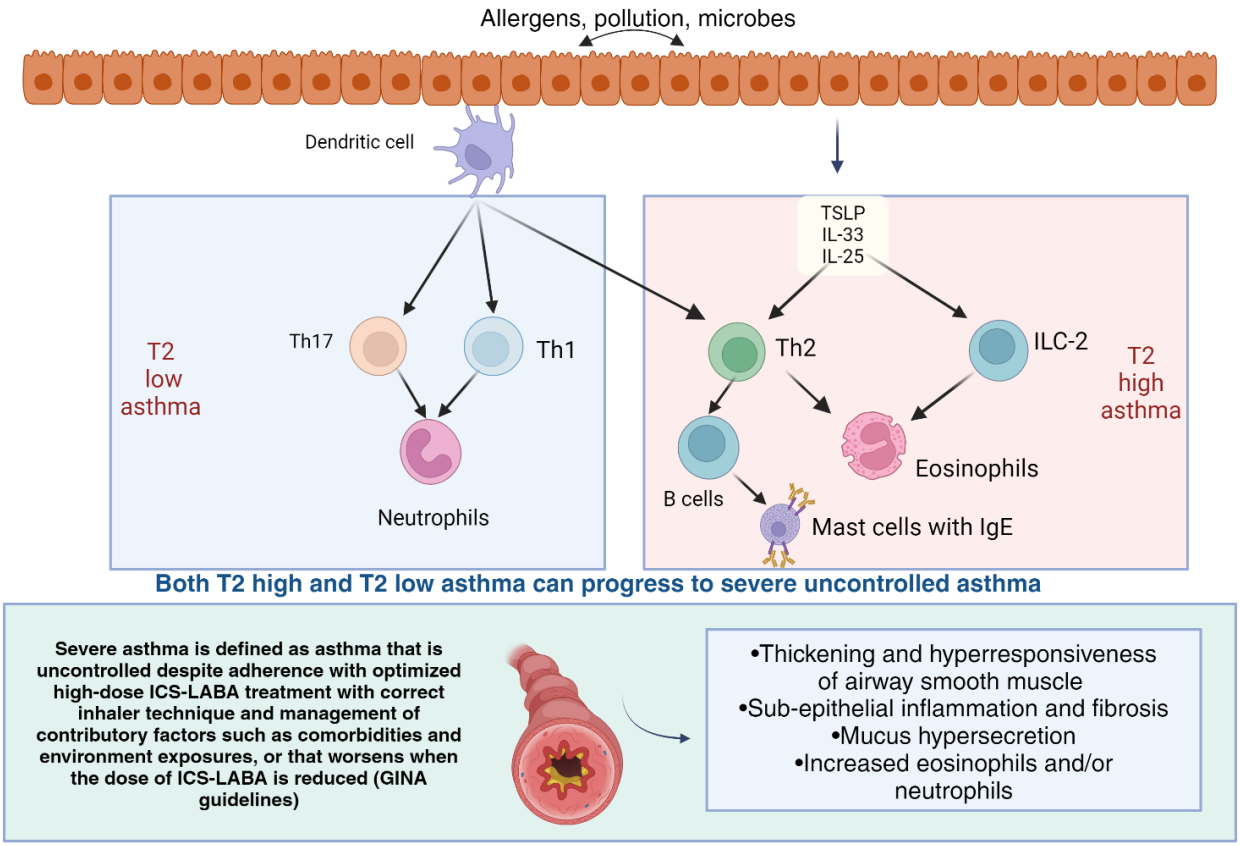

Fig. 2. T2 high and T2 low asthma's pathogenesis. Severe asthma characteristics. Figure was created by the author using https://www.biorender.com/. Th2, T2-helper; IL, interleukin; ILC-2, Group 2 innate lymphoid cells; TSLP, thymic stromal lymphopoietin; ICS, Inhaled corticosteroids.

ids such as urine, sputum, blood, or exhaled air. Asthma biomarkers can offer diagnostic value by reflecting the clinical presentation and underlying airway inflammation. They may also have prognostic insights by identifying individuals at risk of unfavorable outcomes, such as asthma exacerbations, or predictive information to determine patients likely to respond to specific treatments. In blood, biomarkers of T2 airway inflammation include serum IgE, serum periostin, and eosinophil cationic protein (ECP). Exhaled biomarkers encompass fractional exhaled nitric oxide (FeNO).

Sputum Eosinophils

Sputum induction is a minimally invasive procedure compared to bronchial brushing, biopsy, or bronchoalveolar lavage (BAL). During this procedure, hypertonic saline is inhaled to stimulate sputum production, enabling the identification of different types of inflammation based on cell composition. Inflammation can be classified as paucigranulocytic, neutrophilic, or eosinophilic [12,13]. Eosinophilia in sputum is defined by an eosinophil proportion of 3%, utilizing reference values from healthy individuals [14]. Research has shown an inverse correlation between sputum eosinophilia and forced expiratory volume in one second (FEV1) [15], airway hyperresponsiveness [16], and an increased risk of exacerbations [17]. Eosinophilia in the sputum can help predict a patient's response to treatment. However, its fluctuating counts over time and the high cost and time-consuming nature of the procedure limit its widespread application to research settings.

Fractional Exhaled Nitric Oxide (FeNO)

The fractional exhaled nitric oxide (FeNO) is a non-invasive method for assessing bronchial inflammation. It indicates that nitric oxide production in the airways is related to inducible nitric oxide synthases (NOS). FeNO levels are increased in patients with asthma [18]. In asthma, the bronchial airway is usually rich in Nitric oxide (NO), which is produced by inducible nitric oxide synthases (iNOS) under the influence of the T2 inflammatory cytokines IL-4 and IL-13 [18]. FeNO is also correlated with other indicators of disease activity such as symptoms [19], bronchodilator response [20], and airway hyperresponsiveness [21]. The correlation between FeNO and various indicators, such as sputum eosinophil count [22], blood eosinophil count [23], eosinophils in bronchoalveolar lavage fluid [24], and

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bronchial biopsies [25], suggests that FeNO is a specific marker of eosinophilic airway inflammation. However, a precise correlation between FeNO and sputum eosinophilia remains unknown. According to the guidelines of the American Thoracic Society (ATS), in adults, eosinophilic inflammation is less likely if FeNO is below 25 parts per billion and more likely if it exceeds 50 parts per billion [26]. Besides its elevation in asthma, FeNO levels can predict exacerbation risk [27], loss of asthma control [28], and the likelihood of exacerbations [29]. Notably, FeNO levels are similar in individuals with severe and non-severe asthma. Nonetheless, Dweik *et al*. [30] found that patients with high FeNO and severe asthma exhibited the most severe phenotype, characterized by the highest airway reactivity, the greatest airflow limitation, and the highest number of intensive care unit admissions.

Serum IgE

Allergen-specific IgE is a specific type of antibody produced by the immune system in response to common allergens. In individuals with atopy, this antibody triggers allergic reactions by binding to the allergen, leading to an inflammatory response that causes symptoms such as sneezing, itching, and swelling [31]. The presence of allergen-specific IgE is a hallmark of atopic asthma [32], a subtype of asthma that is closely associated with allergies. Study has shown that the levels of allergen-specific IgE can predict the onset and severity of asthma [33]. High levels of allergen-specific IgE are associated with an increased risk of developing asthma, while low levels are associated with a lower risk. Moreover, elevated total IgE levels are also linked to asthma. Total IgE represents the overall amount of IgE antibodies in the blood and can be measured through immunoassays in clinical laboratories. Elevated total IgE levels have been linked to reduced lung function in asthmatic patients [34]. It is suggested that total IgE can act as an indicator of airway inflammation, reflecting the activity of immune cells in the airways. In summary, allergen-specific IgE and total IgE are biomarkers linked to both asthma and atopy. Assessing these antibodies through immunoassays can provide valuable insights into the nature and severity of asthma, aiding in its diagnosis and management. Despite robust evidence from early clinical studies of omalizumab demonstrating immunological changes associated with anti-IgE therapies [35], the role of IgE as a biomarker for allergic inflammation in asthma remains incompletely understood. However, total IgE levels have been proposed as a biomarker for monitoring IgE production and guiding treatment on an individual basis [36]. Moreover, study has shown that the therapeutic effectiveness of omalizumab is consistent in patients with baseline IgE levels between 30 and 700 UI/mL [37]. Consequently, IgE cannot be reliably used as a predictive biomarker for treatment response to omalizumab.

Blood Eosinophils

Blood eosinophils are easier to measure than sputum eosinophils, making them a focus in studying eosinophilic airway inflammation. A meta-analysis demonstrated a composite area under the curve of 0.78 for diagnosing this type of inflammation [34]. However, blood eosinophils do not exhibit as strong an association with Th2 airway inflammation as sputum eosinophils. The count of blood eosinophils is inversely related to FEV1 and is linked to symptom scores and airway hyperresponsiveness [38]. Higher blood eosinophil counts have been associated with a significant risk of aggravation and mortality [39]. Blood eosinophil counts can be used as an outcome metric and an additional biomarker for characterizing patients in clinical studies. These counts are easily obtained through tests and can help evaluate the effectiveness of therapeutic interventions. The definition of a high blood eosinophil cut point remains a topic of debate, ranging from >150 to >300 cells/mL. Baseline blood eosinophil count (BEC) has been identified as a predictor of the reduction in severe exacerbation rates in patients with severe eosinophilic asthma treated with Inhaled corticosteroids (ICS) [40]. Additionally, BEC values between 150 and 400 cells/μL have been used to predict the response to monoclonal antibody (mAb) treatments targeting IL-5 and its receptor IL-5R [41]. Study on the anti-IL5 mAb reslizumab has shown significant reductions in asthma exacerbation rates [42] and improvements in lung function among patients with persistent asthma and BEC >400 cells/μL.

Serum Periostin

Periostin, a matricellular protein belonging to the fasciclin family, is an emerging biomarker of the T2 inflammatory response. It is secreted by lung fibroblasts following stimulation by the type 2 cytokines IL-4 and/or IL-13 [43]. Periostin plays a role in subepithelial fibrosis in individuals with bronchial asthma, suggesting its involvement in airway remodeling during the chronic stage of the disease [44].

In various clinical studies, serum periostin has been linked to fixed airflow restriction [45,46]. A multivariate analysis of well-controlled asthmatics undergoing ICS treatment revealed a significant correlation between high serum periostin levels and fixed airflow limitation [47]. This suggests that serum periostin could serve as a valuable biomarker for the development of airflow restriction in asthmatic patients using inhaled corticosteroids [48]. Treatment with ICS notably improved airflow limitation, reduced the proportion of sputum eosinophils, and considerably decreased serum periostin levels [48]. On the other hand, Ono *et al*. [49] utilized an Enzyme-Linked Immunosorbent Assay (ELISA) approach to investigate protein cleavage products and their relation to periostin levels in sputum. They

showed that poorer lung function and IL-13 in sputum were related to periostin in sputum but not in serum, and oral corticosteroid therapy reduced periostin in sputum but not in serum [49]. Regarding the correlation between serum periostin and eosinophilia in sputum, there is still inconsistent data. In a study by Jia *et al*. [50], it was shown that serum periostin could effectively predict eosinophilic airway inflammation. However, in another study, periostin was unable to differentiate between eosinophilic and non-eosinophilic airway inflammation [51].

Determining periostin levels could help identify patients with severe asthma who would benefit from treatment with biologic drugs. In a randomized trial, it was observed that patients with uncontrolled asthma who had higher blood periostin levels prior to therapy experienced greater improvement in lung function when treated with lebrikizumab compared to those with lower levels [52]. Similarly, in patients with elevated periostin levels before treatment, tralokinumab, an anti-IL13 monoclonal antibody, significantly decreased the occurrence of asthma flare-ups, improved lung function, and lessened symptoms [53].

Periostin dosage has been correlated with the risk of developing asthma in specific populations. Indeed, the serum periostin level during hospitalization for bronchiolitis was found to be associated with the risk of developing asthma by the age of 6 years. This insight stems from a prospective multicenter cohort study conducted on infants with severe bronchiolitis, particularly in those predisposed to allergies [54]. However, the absence of verified predicted values and established evaluation methods limits the widespread use of periostin [55].

Biomarkers in non-T2 Asthma

Type 2 low asthma is characterized by the presence of either neutrophilic, paucigranulocytic, or mixed granulocytic inflammation [56,57], and by the absence of signs and markers of type 2 inflammation [58]. The frequency of the T2-low or non-T2 asthma endotype is lower than that of the T2-high asthma endotype, especially in children [7]. Research has shown that obesity, smoking, and poorly understood underlying smooth muscle abnormalities are associated with T2-low asthma [59].

The main characteristic of neutrophilic asthma is the high prevalence of sputum neutrophil. Currently, there is no established threshold for diagnosing neutrophilic asthma and various thresholds ranging from >40% to 76% neutrophils have been used [60–62]. In children, a cut-off of ≥5% neutrophils in bronchial lavage fluid is used to diagnose neutrophil-predominant severe asthma [63]. Analysis of sputum cultures from patients with neutrophilic asthma revealed an increased presence of *Moraxella catarrhalis* and *Hemophilus influenzae*, indicating chronic bacterial colonization [64]. Additionally, Steinke *et al*. [65] found that children with isolated neutrophilia showed the highest prevalence of pathogenic bacteria. Children with respiratory pathogen were found to have higher percentages of neutrophils and BAL cells. The pathobiology of T2-low asthma is further supported by an increase of toll-like receptors (TLRs), specifically TLR2 and TLR4, along with several pro-inflammatory cytokines, as revealed by sputum analysis [56]. When TLRs are activated, there is a shift towards the activation of T helper 1 (Th1) and T helper 17 (Th17) cells, leading to the release of IFN-γ, Tumor necrosis factor-alpha (TNF-α), IL-8, IL-17A, IL-17F, and IL-22. This activation sets off a positive feedback loop that promotes further recruitment and neutrophilic inflammation, particularly when recruited neutrophils secrete IL-8. The cytokine pattern found in BAL samples from children with refractory neutrophilic asthma indicates a combined Th17/Th1/Th2 response [65]. Specifically, there is an association with cytokines and chemokines involved in Th17 differentiation (IL-6), expression (IL-17), and neutrophil chemotaxis (chemokine (C-X-C motif) ligand 8 (CXCL8), GCSF, CXCL10, and TNF-α). Metalloproteinase 9 (MMP9), which is implicated in inflammation and airway remodeling in asthmatic individuals, serves as another potential marker [66]. The presence of neutrophils in sputum is a prognostic marker associated with breathlessness, an early decrease in FEV1, and incomplete reversibility of airflow obstruction [67]. Paugranulocytic asthma presents with normal sputum levels of both eosinophils and neutrophils [68]. While animal models have suggested potential pathways of pathogenesis, the exact pathogenesis of PGA is still unknown. One potential contributing factor is the dissociation between airway inflammation and obstruction, which may result from a variety of processes but is primarily attributed to structural alterations in the airways, such as the hypertrophy of the airway smooth muscle (ASM) [68]. Another possible mechanism includes the neurogenic mechanisms associated with taste and olfactory receptors on lung cells or parasympathetic nerves [69,70].

Currently, treatment strategies for T2 low asthma include weight loss and smoking cessation, as well as the utilization of long-acting muscarinic antagonists (LAMA) and macrolides [71]. Both national and international asthma guidelines continue to recommend the use of standard asthma medications across all patients, despite the variability and heterogeneity of inflammation associated with the condition [72]. Further investigation into T2-“low” mechanisms is imperative to identify potential biomarkers and advance the development of more efficacious therapies.

Emerging Biomarkers

Advances in genomics have led to the identification of genetic biomarkers associated with both asthma susceptibility and severity. Polymorphisms in genes related to airway remodeling, inflammation, and corticosteroid responsiveness are being investigated for their potential role in predicting treatment effectiveness.

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Genomic and Proteomic Biomarkers

Genomic biomarkers associated with asthma severity provide valuable insights into the genetic factors contributing to the progression and severity of the condition [73]. Chromosome 17q21 has emerged as a key focus in current genomic epidemiological studies on asthma, harboring a multitude of genes and single nucleotide polymorphisms (SNPs) of significance [74]. Among these, genes such as Charcot-Leyden Crystal Galectin (*CLC*), EGF-like module-containing mucin-like hormone receptor-like 4 pseudogene (*EMR4P*), interleukin-5 receptor alpha (*IL-5RA*), Fibroblast Reducing Substance 1 (*FRRS1*), Histamine Receptor H4 (*HRH4*), Solute Carrier Family 29 Member 1 (*SLC29A1*), Sialic Acid-binding Ig-like Lectin 8 (*SIGLEC8*), and interleukin 1 Receptor-like 1 (*IL1RL1*) have been identified as being overexpressed in allergic conditions like asthma, dermatitis, and rhinitis [75]. Furthermore, variations in genes related to the IL-4 and IL-13 pathways, such as IL-4, IL-13, and IL4R, have been linked to asthma severity [76]. Polymorphisms within the beta-2 adrenergic receptor gene (*ADRB2*) have been associated with bronchodilator response and may influence the severity of asthma symptoms [77]. Genetic variants in the *ORMDL* sphingolipid biosynthesis regulator 3 (*ORMDL3*) gene have been linked to childhood-onset asthma and increased asthma severity [78]. Mutations in the filaggrin gene (*FLG*) have been connected to atopic dermatitis and allergic sensitization, both of which are risk factors for severe asthma. *FLG* mutations might contribute to impaired skin barrier function and increased susceptibility to allergens [79]. Variations in the human leukocyte antigen-G (*HLA-G*) gene have been linked to severe asthma. *HLA-G* is involved in immune regulation, and genetic variations within this gene could impact the immune response in asthma [80]. Elevated levels of chitinase-3-like protein 1 (*CHI3L1* or *YKL-40*), involved in inflammation and tissue remodeling, have been associated with asthma severity [81]. Genetic variants in genes encoding glutathione S-transferases (*GSTs*), such as *GSTM1* and *GSTP1*, have been linked to asthma severity. *GST* enzymes play a role in detoxification, and variations in these genes can impact antioxidant defenses [82]. Thymic stromal lymphopoietin (*TSLP*) participates in initiating allergic responses. Variants in the *TSLP* gene have been associated with asthma severity, particularly in relation to airway remodeling [83]. Mutations in genes related to the interferon pathway, such as interferon regulatory factor 1 (IRF1), have been linked to severe asthma. These genes play a role in antiviral responses and immune regulation [84]. Variations in the cadherin-related family member 3 (*CDHR3*) gene have been associated with asthma exacerbations and increased severity, particularly in individuals with early-onset asthma [85]. Proteomic profiling enables the identification of protein biomarkers associated with different asthma phenotypes to aid in the development of targeted therapies. This includes biomarkers involved in airway inflammation, remodeling, and response to therapy. In the study led by Suzuki *et al*. [86], a total of 143 networks were discovered, each characterized by enriched or modified protein sets, when comparing chronic obstructive pulmonary disease (COPD) and severe asthma ($p \leq 0.001$). Networks exhibiting increased protein levels in asthma were associated with complement and coagulation (with the representative protein PLAU) and pro-inflammatory response (Cluster of differentiation 97 (*CD97*)). Moreover, key contributors to the asthma response included pro-inflammatory mediators (TNF, IL-1β, IL-6) and transcriptional regulators (*HDAC, CAV1, MAP4k4, Wnt*, and *SOX2*) [87]. In previous studies, it has been documented that serum levels of Pregnancy-associated plasma protein-A (PAPP-A), a protease for insulin-like growth factor binding protein 4 (IGFBP-4), are higher in asthmatic individuals compared to those without asthma [88,89]. Additionally, a significant decrease in serum levels of PAPP-A was noted in patients with severe allergic asthma who were treated with the anti-IgE monoclonal antibody omalizumab [88]. In Sparreman *et al*.'s [89] study published in the European Respiratory Journal, elevated levels of 10 proteins, namely alpha-1-antichymotrypsin, apolipoprotein-E, complement component 9, complement factor I, macrophage inflammatory protein-3, interleukin-6, sphingomyelin phosphodiesterase 3, TNF receptor superfamily member 11a, transforming growth factor-β, and glutathione S-transferase, were observed in severe asthma compared to mild to moderate asthma.

Although treatment with oral corticosteroids (OCS) led to a reduction in the majority of these proteins, differences between severe asthma and mild to moderate asthma persisted even after considering OCS usage [89]. A recent study identified IL18R1 and IL18R1-related molecules [Tumor Necrosis Factor Ligand Superfamily Member 1 (TNFSF1), Oncostatin M (OSM), and S100A12] as potential biomarkers for tracking uncontrolled severe asthma, notably linked to shorter survival times and decreased lung function [90]. Moreover, proteomic profiling enables the identification of distinct molecular patterns associated with different asthma phenotypes. Matrix metalloproteinase-10 (MMP10) levels were associated with eosinophilic asthma, while C-C motif chemokine ligand 4 (CCL4) levels were associated with high neutrophil concentration in individuals with asthma [91].

Microbiota

While the lower airways were traditionally considered sterile, advances in molecular techniques have enabled the identification and characterization of bacterial communities in the lungs. These microbial communities can influence the development and function of the immune system, influencing both innate and adaptive immune responses [92]. A

Table 1. T2 high, T2 low, and emerging biomarkers in severe asthma management.

Biomarker	Function	
	T2-high asthma	
Serum IgE	Antibody produced in response to exposure to common allergens, it mediates allergic reactions. Its presence is a hallmark of atopic asthma closely associated with allergies. High levels predict asthma onset and severity. Elevated total IgE is linked to reduced lung function.	
Blood eosinophils	Indicator of eosinophilic airway inflammation. There is an inverse relationship with FEV1, linked to symptoms, and airway hyperresponsiveness. Elevated counts are associated with a major risk of aggravation and mortality.	
Serum periostin	Matricellular protein linked to T2 inflammatory response. It is secreted by lung fibroblasts in response to IL-4 and/or IL-13 and is associated with subepithelial fibrosis in asthma patients, indicating involvement in airway remodeling. It is also linked to fixed airflow restriction and may be a useful biomarker for its development in asthmatic patients on ICS. Correlation with eosinophilia in sputum shows inconsistent data. Its dosage may guide patient selection for biologic drugs.	
Fractional exhaled nitric oxide (FeNO)	A non-invasive method for assessing bronchial inflammation. It is increased in asthma due to NO production related to inducible NOS under T2 inflammatory cytokines IL-4 and IL-13 mediation. It is correlated with symptoms, bronchodilator response, and airway hyperresponsiveness. It may serve as a predictor of exacerbation risk, loss of asthma control, and low compliance.	
Sputum eosinophils	Indicators of eosinophilic airway inflammation categorized as paucigranulocytic, neutrophilic, or eosinophilic. The criterion for defining sputum eosinophilia is set at an eosinophil proportion of 3%. There is an inverse correlation with FEV1, airway hyperresponsiveness, and increased risk of exacerbation. Its use is limited to research settings due to variability over time, cost, and time-consuming nature.	
	T2-low asthma	
Endotype	Characteristics	Treatment options
Neutrophilic asthma	High sputum neutrophil prevalence. No defined cut-off for diagnosis; different cut-offs used (e.g., >40% to 76% neutrophils). Associated with chronic bacterial colonization Prognostic marker linked to breathlessness, FEV1 decrease, incomplete airflow obstruction reversibility.	No specific treatment for neutrophilic asthma. Weight loss and smoking cessation are recommended. Antibiotics are used for chronic bacterial colonization.
Paucigranulocytic asthma	Normal sputum levels of both eosinophils and neutrophils. Associated with structural alterations in the airways, such as hypertrophy of airway smooth muscle. Mechanisms include neurogenic pathways related to taste and olfactory receptors.	No specific treatment. Weight loss and smoking cessation are recommended.
	Emerging biomarkers	
Biomarker	Characteristics	Associated conditions and insights
Genomic biomarkers	Genetic factors contribute to asthma susceptibility and severity. Polymorphisms in genes related to airway remodeling, inflammation, and corticosteroid responsiveness.	Chromosome 17q21 as a focal point in genomic investigations. Variations in IL-4, IL-13, IL4R linked to asthma severity. *ADRB2* gene associated with bronchodilator response.
Proteomic biomarkers	Proteins associated with different asthma phenotypes. Elevated levels in severe asthma compared to mild to moderate asthma. Potential biomarkers for tracking uncontrolled severe asthma.	Elevated serum levels of PAPP-A in individuals with asthma. Distinct molecular patterns associated with different asthma phenotypes. IL18R1-related molecules as potential biomarkers.
Microbiota biomarkers	Identification and characterization of bacterial communities in the lungs. Dysbiosis triggers inflammatory pathways, leading to bronchoconstriction and heightened bronchial responsiveness.	Balanced microbial composition characterized by *Bacteroidetes*, *Actinobacteria*, and *Firmicutes phyla*. Dysbiosis correlated with allergic airway inflammation. Existence of gut-lung axis and its impact on immune responses.

FEV1, forced expiratory volume in one second; NOS, nitric oxide synthases; *ADRB2*, beta-2 adrenergic receptor gene; PAPP-A, Pregnancy-associated plasma protein-A.

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healthy lung typically exhibits a balanced microbial composition with a prevalence of bacteria from the *Bacteroidetes*, *Actinobacteria*, and *Firmicutes phyla*. Conversely, viral respiratory infections lead to an increase in *Proteobacteria*, particularly the genera *Haemophilus* and *Moraxella*, which are more prominent in children with asthma [93]. The role of microbiota in severe asthma has become a significant focus of research, revealing the intricate interplay between the respiratory microbiome and asthma pathogenesis. Notably, environmental microbes from sources like house dust, pets, and farm animals have been implicated in the pathogenesis of asthma due to their production of bioactive molecules such as lipopolysaccharides [94]. Furthermore, respiratory microbial communities, including recently identified populations within the lungs, have been associated with allergic airway inflammation. Current evidence indicates that specific microbes, including species like *Streptococcus*, *Haemophilus*, and *Moraxella* in the airways, may impact local immune responses, thereby altering the severity and characteristics of airway inflammation. This microbial imbalance, known as dysbiosis, activates inflammatory pathways, resulting in bronchoconstriction and increased bronchial responsiveness [95]. Additionally, the gut microbiota has been implicated as a predisposing factor for asthma in both experimental models and clinical studies. A "critical window" of colonization during early infancy seems to play a significant role in shaping immune maturation and increasing susceptibility to allergic airway inflammation [96]. The mechanisms by which gut microbial communities influence lung immune responses and physiology, referred as the "gut-lung axis", are still being defined. These mechanisms involve the altered differentiation of immune cell populations crucial in asthma and the local production of metabolites that affect distant sites [97]. These findings collectively highlight a complex association between microbial communities, host immune development, and the onset of allergic airway inflammation [98]. External factors, such as a farming environment, can either positively or negatively influence the natural composition of lung microbiota, while allergens and air pollutants tend to have detrimental effects. Moreover, dysbiosis in the gut microbiota significantly impacts the development of asthma. The administration of antibiotics, antiulcer medications, and specific drugs can severely disrupt both gut and lung microbiota, resulting in dysbiosis and a decrease in microbial diversity [99]. This disruption hinders the bidirectional communication along the gut-lung axis, leading to increased sensitivity and reactivity to respiratory and food allergens. Maintaining a balanced microbiota in both the lungs and the gut is crucial for preventing inflammation and preserving optimal immune responses in the progression of asthma [100]. Exploring the microbiome composition in individuals with severe asthma may pave the way for personalized treatment approaches, including targeted microbial modulation strategies [101,102]. Moreover, microbial products and metabolites can affect the function of the airway epithelial barrier and mucus production, contributing to airway remodeling—a characteristic feature of severe asthma [103]. The crosstalk between microbiota and the host's respiratory epithelium is a dynamic process that may influence disease progression and treatment outcomes [104].

Discussion

The biomarkers mentioned are listed in Table 1.

Among these biomarkers, only a selection is used in clinical practice. FeNO is a non-invasive biomarker for assessing bronchial inflammation. In clinical practice, FeNO is often used to monitor eosinophilic inflammation in the airways and optimize asthma management. Monitoring FeNO can influence therapeutic decisions, such as adjusting the dosage of inhaled corticosteroids. Blood eosinophil count is recognized as an easily measurable biomarker. Elevated levels of blood eosinophils may indicate eosinophilic inflammation in the airways, aiding in asthma management and monitoring. Serum IgE, especially allergen-specific IgE, is identified as a biomarker associated with atopic asthma. Elevated levels may indicate an allergic response and can influence the management of allergy-related asthma. Serum IgE is essential in clinical practice for diagnosing allergies, identifying triggers, and managing conditions like atopic asthma. It guides treatment decisions, influences the use of biological therapies like omalizumab, and helps monitor treatment response. Customizing treatment plans based on IgE levels improves patient care, emphasizing its significance in the management of allergies and asthma. Additionally, serum periostin is emerging as a potential biomarker of type 2 inflammatory responses. Some studies suggest its association with airflow restriction and its potential utility in predicting treatment efficacy. Genetic polymorphisms and proteomic profiles are also highlighted as biomarkers in the study phase. However, their practical implementation may require further confirmation and validation.

Conclusions

The landscape of severe asthma management is evolving through the identification of established and emerging biomarkers that provide deeper insights into the underlying pathophysiology of the disease. Further research is needed to validate and establish the clinical utility of these emerging biomarkers. However, their potential to revolutionize asthma management is promising. These biomarkers present two key advantages. Firstly, they offer a more personalized approach to asthma treatment, allowing clinicians to customize treatment approaches based on individual inflammatory pathways. Secondly, these biomarkers have the potential to predict treatment responses, optimizing therapeutic strategies and minimizing unnecessary side effects.

Effective collaboration among clinicians, researchers, and industry partners is essential to further unravel the complexities of severe asthma and translate these findings into practical clinical applications that improve patient care. It is important to note that our study's primary limitation stems from its exclusive focus on the PubMed platform. Additionally, despite our emphasis on pediatric studies, we included articles primarily addressing asthma in adults due to limited availability.

Biomarkers associated with severe asthma, such as eosinophils, FeNO, and various inflammatory cytokines, provide valuable insights into the specific inflammatory pathways involved. These biomarkers not only aid in diagnosing severe asthma but also guide clinicians in selecting targeted therapies, such as monoclonal antibodies, to effectively modulate the inflammatory response. The advancing field of biomarker research in severe asthma also encompasses genomics and proteomics, providing a deeper understanding of the genetic and protein-level variations contributing to the disease. This knowledge could lead to the identification of novel therapeutic targets and the customization of treatment plans based on an individual's unique molecular profile. We believe that exploring biomarkers in managing severe asthma represents a pioneering approach in precision medicine. As our understanding of these biomarkers deepens, so does the potential to revolutionize the care of individuals with severe asthma, offering targeted and effective interventions that improve overall outcomes and quality of life.

Abbreviations

IL-4, interleukin-4; IL-5, interleukin-5; IL-13, interleukin-13; IgE, Immunoglobulin E; FEV1, forced expiratory volume in one second; Th2, T2-helper; ILC-2, Group 2 innate lymphoid cells; ECP, eosinophil cationic protein; FeNO, fractional exhaled nitric oxide; COPD, chronic obstructive pulmonary disease; CLC, Charcot-Leyden Crystal Galectin; *EMR4P*, EGF-like module-containing mucin-like hormone receptor-like 4 pseudogene; *IL-5RA*, interleukin-5 receptor alpha; *FRRS1*, Fibroblast Reducing Substance 1; *HRH4*, Histamine Receptor H4; *SLC29A1*, Solute Carrier Family 29 Member 1; *SIGLEC8*, Sialic Acid-binding Ig-like Lectin 8; *IL1RL1*, interleukin 1 Receptor-like 1; SNPs, single nucleotide polymorphisms; *ADRB2*, beta-2 adrenergic receptor gene; *ORMDL3*, *ORMDL* sphingolipid biosynthesis regulator 3; *FLG*, filaggrin gene; *HLA-G*, human leukocyte antigen-G; CHI3L1 or YKL-40, chitinase-3-like protein 1; TSLP, thymic stromal lymphopoietin; *CDHR3*, cadherin-related family member 3; MMP10, matrix metalloproteinase-10; CCL4, C-C motif chemokine ligand 4.

Availability of Data and Materials

Not applicable.

Author Contributions

CI, AK, GD, CLB, EDA and SF contributed to the concept and designed the research study and wrote the paper. AK, CLB, EDA and SF performed the research. FD, GC and MMdG designed the work and revised the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. Giorgio Ciprandi is serving as one of the Editorial Board members of this journal. We declare that Giorgio Ciprandi had no involvement in the peer review of this article and has no access to information regarding its peer review.

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