

Protein Biomarkers in Asthma

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Abstract

Asthma is a chronic disabling respiratory disease that can be triggered by a variety of factors, including allergens, respiratory infections, psychological factors, occupational agents, exercise, atmospheric pollutants, and drugs. The asthma syndrome has been treated for decades according to a “one-fits-all” treatment strategy based on bronchodilators and steroids. With the availability of new forms of treatment targeting the different pathomechanisms of the asthma syndrome, such as anti-immunoglobulin E and cytokine-targeting therapies, the interest in biomarkers that can discriminate different forms of asthma according to their pathomechanisms has increased. This review attempts to provide an overview of protein biomarkers in asthma and how they might be used to discriminate different forms of asthma that may respond positively to sophisticated new targeted therapies.

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Introduction

The asthma syndrome affects more than 300 million people worldwide and it is severe burden for the affected patients and health care systems. The symptoms of asthma include breathlessness, cough, wheeze, and chest tightness, as well as chronic airway inflammation. Asthma and chronic obstructive pulmonary disease (COPD) sometimes have similar features and may co-occur in the same patient, which is designated as asthma-COPD overlapping syndrome (ACOS). Cigarette smoking is one of the most important causes of COPD, whereas several trigger factors and pathomechanisms are responsible for asthma. Figure 1 provides an overview of some of the most important trigger factors for asthma; immunoglobulin E (IgE)-associated allergy and respiratory infections are the most frequent and important ones, followed by pollutants and occupational agents that can induce bronchial hyperreactivity, as well as exercise, drugs, and psychological factors [1–3]. For decades the treatment of asthma has followed a “one-fits-all” strategy based on the local and/or systemic application of bronchodilators and medication (in most cases steroids and/or combinations of the two) aiming to reduce inflammation, [4]. With the emergence of new and more causative forms of treat-

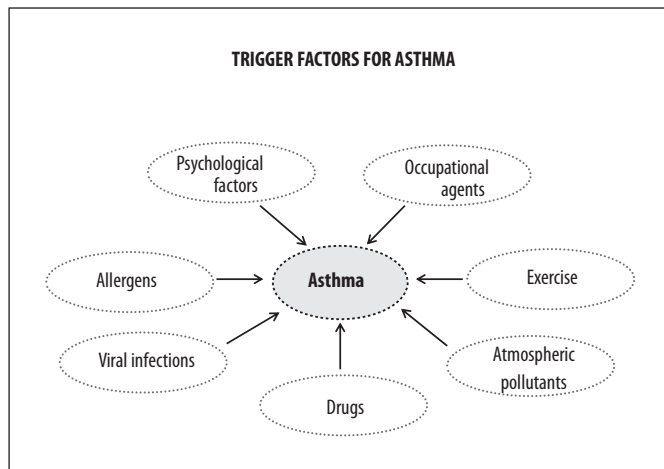


Fig. 1. Trigger factors for asthma.

ment for asthma, such as anti-IgE treatment and [5, 6] anti-cytokine therapies for allergic asthma, and in the case of anti-interleukin (IL)-5 for eosinophilic asthma [7–9], it has become apparent that treatment can be refined and focused on those groups of asthmatic patients who specifically benefit from the new targeted treatments, whereas those subjects who suffer from asthma caused by different pathomechanisms and therefore do not benefit from such targeted treatment, can be identified. Especially the introduction of cytokine targeting in asthma treatment has shown that only certain patient subgroups benefit. This has led to the classification of asthma into certain phenotypes and endotypes, respectively, which are characterized by the predominance of certain cell types such as eosinophils and neutrophils, according to the response to steroids, according to different courses of disease, and finally according to sophisticated biomarkers such as periostin [10]. Here we argue that it may be very useful to categorize the different forms of asthma according to the underlying pathomechanisms and trigger factors to enhance the precision of the prescription of highly refined forms of treatment and prevention. For example, patients suffering from allergic asthma may benefit not only from IgE-targeting strategies but also from allergen-specific immunotherapy, allergen avoidance, or other anti-allergic medications [11]. Likewise, it may be possible to treat asthma induced by respiratory viruses in the future by vaccination or treatment strategies specifically blocking viral infections [12–15]. We therefore suggest dedicating more attention to biomarkers and in particular protein biomarkers which

allow discrimination of different forms of asthma so that patients can benefit from more personalized forms of treatment.

Biomarkers: General Information

Biological markers, or biomarkers, are defined as biological or physiological traits that can be evaluated as indicators of normal or pathological processes or pharmacologic responses to a therapeutic approach. In biomedical studies, biomarkers are used not only as indicators of a disease and its features for the correct diagnosis but also as tools for determining the current state and predicting the outcomes of a disease and the predisposition to a disease and monitoring the efficiency of preventative measures and therapy.

Biomarkers are classified in different ways, utilizing a variety of parameters. Based on their characteristics, biomarkers can be divided into: imaging biomarkers (computerized axial tomography, positron emission tomography, magnetic resonance imaging) and molecular biomarkers (DNA, RNA, proteins, lipids, and small molecular metabolites) [16]. Cells with a specific phenotype inherent to the population can also be attributed to biomarkers.

Biomarkers can be measured in several biological fluids, such as: whole blood, plasma and serum; urine; sweat; sputum; nasal, bronchial, and lacrimal secretions; biopsy; and amniotic, cerebrospinal, peritoneal, pleural, and seminal fluids, and so-called “mucosal lining fluids” using, for example, naso- or bronchosorption devices [17, 18]. Measurements can be made either by biomedical imaging or in vitro by laboratory methods [16].

Among the nucleic acid biomarkers, specific mRNA, microRNA, and long non-coding RNA found in biological liquids and tissues also play an important role. The assessment of predisposition to allergic diseases is often also based on determination of DNA polymorphisms and patterns of DNA methylation [19]. Leukotrienes can serve as lipid biomarkers. The well-known low-molecular metabolites malondialdehyde and nitric oxide are also widely used as biomarkers [20]. Additionally, soluble proteins make up a significant fraction of biomarkers for both normal and pathological states.

Frequently, quantitative analysis of a biomarker is beneficial for disease monitoring and the response to therapy as it allows monitoring of any changes in biomarker levels (or cell counts) at different stages of disease or treatment.

Protein Biomarkers and Their Role in Asthma

Proteins are one of the most informative types of biological markers. For many diseases, numerous protein biomarkers have been discovered and explored with applications in clinical diagnosis, disease progression prediction, and personalized treatment. Since these markers are often isolated and detected by noninvasive means (from serum, plasma, urine, etc.), there is often no need for expensive interventions to obtain those samples [21].

Proteins are differentially expressed and processed during various disease states, thereby providing multifaceted information about the particular disorder. Differential protein processing, like changes in protein folding or glycosylation, can deviate from normal posttranslational modifications during disease. For example, shedding of membrane differentiation molecules from immune cell membranes is a common posttranslational protein modification in normal conditions and inflammation. As a result, the level of soluble differentiation molecules and histocompatibility molecules is elevated in biological fluids [22–24]. With regard to allergies, the most known soluble protein biomarkers are IgE and IgG antibodies specific for the disease-causing allergens, cytokines, enzymes, and other proteins, such as soluble differentiation molecules of the immune cells, i.e., membrane protein markers.

Molecular cloning technologies developed during the last decades have led to the real revolution in biomedical research and clinical diagnostics of many diseases including allergies. It is now possible to determine the 3-dimensional structure and molecular characteristics of disease-causing allergens or specific IgE towards them, create recombinant molecules based on the sequences of allergens, and use these recombinant allergens for highly refined allergy diagnosis (i.e., molecular allergy diagnosis and component-resolved diagnosis) [25–28]. Such revolutionary approaches brought the molecular level of allergology to routine clinical practice, allowing doctors to understand allergies at a molecular level [29]. Moreover, it has been found that the natural immune response towards rhinovirus, the most common asthma-triggering respiratory virus, is directed against an N-terminal peptide of the VP1 coat protein [30]. It has therefore become possible to detect rhinovirus infections by measuring specific antibody responses against this VP1 peptide [31–33].

In this review, we concentrate on different protein biomarkers, which can be used for prediction, prevention, diagnostic, therapeutic, and monitoring purposes in asthma.

Biomarkers in Phenotyping and Endotyping of Asthma

Asthma is a heterogeneous chronic inflammatory disease of the lungs. The heterogeneity of asthma is the consequence of multiple factors involved in the development of this disorder. In general, the diagnostic approaches for differentiation between IgE- and non-IgE-mediated allergic diseases and asthma were presented in a review by Incorvaia et al. [34]. The following tests were considered for respiratory allergy in routine practice: the skin prick test, in vitro measurement of sIgE and nasal IgE, and nasal provocation and atopy patch tests [34].

The past two decades of active investigations have increased our understanding of the pathophysiological nature of asthma, leading to classification of the disease into groups called phenotypes and endotypes [35, 36].

The eosinophilic phenotype represents the major features of classic atopic asthma, including responsiveness to corticosteroids, with the exception of a small subgroup of severe asthma where inhaled corticosteroids are not so effective even in the presence of eosinophils. This asthma phenotype probably reflects ongoing adaptive immunity while responding to an allergen. Monitoring of eosinophilic asthma using such biomarkers as blood eosinophils, serum IgE, exhaled nitric oxide, and serum periostin is available in clinical practice [37].

Eosinophilic inflammation is the most focused phenotype because many novel treatments target the Th2 pathway. Measurement of fractional exhaled nitric oxide (FeNO) in eosinophilic airway inflammation is able to show a significant correlation with sputum eosinophilia and asthma severity instead of sputum eosinophil count. The results of the latter method could be distorted because of the influence of corticosteroid treatment, and for this reason FeNO may be a more advantageous and informative approach. Therapy algorithms based on the measurement of FeNO allow cost-effective maintenance of asthma control/quality of life. However, according to some studies, the discordance between FeNO levels and treatment response or adjustment is notable. For this reason, it may be better to use several types of biomarkers for therapy monitoring. For Th2-targeted therapy (such as lebrikizumab or omalizumab) of eosinophilic airway inflammation, serum periostin is an informative marker although more replication studies along with FeNO are needed. YKL-40, periostin, OPN, and metabolites such as choline, arginine, acetone, and protectin D1 are known to be connected to asthma severity and airflow limitation [38]. In contrast to the eosinophilic phenotype, the neu-

trophilic phenotype of asthma is characterized by the presence of neutrophils and an inadequate response to corticosteroid treatment even in mild asthma. This phenotype reflects the activation of the innate immune system in response to pollution and infection. To date, biomarkers of neutrophilic asthma and airway remodeling for routine laboratory analyses are unknown [37] and no neutrophilic phenotype-dependent effective treatment has been described [39].

The paucigranulocytic phenotype of asthma is thought to be non-inflammatory and caused by smooth muscle dysfunction [37].

Unlike phenotypes, patients with the same asthma endotype are characterized by the same pathophysiological processes leading to disease development, progression, and presentation. Endotypes are based on multiple disease-related factors including the clinical nature of the disease, genetic susceptibility, environmental risk factors, and the age at disease onset [35]. Currently, emerging endotype-driven strategies in asthma are focused on solving individualized clinical problems of disease outcomes.

In their work, Liang et al. [40] used circulating biomarkers to discriminate asthma subtypes and determine whether a systemic inflammatory endotype of asthma could be identified. To do this, the expression profiles of 20 serum cytokines were assessed by anti-human cytokine antibodies with subsequent hierarchical clustering analysis based on principal component analysis-transformed data. The completed analysis led to the identification of the endotype, which is characterized by systemic inflammation and severe symptoms. The levels of systemic inflammation markers leptin and vascular endothelial growth factor were elevated, while the levels of anti-inflammatory soluble receptor for advanced glycation end products were reduced. Such changes in vascular endothelial growth factor, leptin, and soluble receptor for advanced glycation end products may contribute to the systemic inflammation of the asthma endotype identified in the study [40]. Molecular biology methodologies are also helpful in asthma phenotyping. For example, transcriptomics is a quite new, yet promising, method for asthma studies. Using microarrays, Raedler et al. [41] determined different immune signatures of children with allergic and nonallergic asthma. Despite similarly high numbers of Treg cells, the secretion of IL-5, IL-13, and IFN- γ was significantly suppressed in non-allergic asthma patients but not in allergic asthma. Patients with allergic asthma were characterized by a decrease in important regulators of innate immunity – gene expression of chloride intracellular channel 4 and tuber-

ous sclerosis 1. The immune signature for non-allergic asthmatic patients had increased levels of pro-inflammatory IL-1 β and IL-17-shifted immunity and insufficient suppression of IL-5, IL-13, and IFN- γ . Increased gene expression levels for anti-inflammatory IL-37, proline-serine-threonine phosphatase-interacting protein 2 (PSTPIP2), and the neutrophil-associated genes CD93, triggering receptor expressed on myeloid cells 1 (TREM1), and regulator of G-protein signaling 13 (RGS13) were observed in non-allergic asthma children [41]. The authors proposed using the data for biomarker chip development and for better stratification of children suffering from different asthma phenotypes.

As mentioned previously, asthma is caused by several triggers, most commonly by IgE-associated allergy and respiratory virus infection. New forms of molecular biology tests, based on molecular cloning, have been proposed for determination of diseases caused by allergen molecules and respiratory virus infections in routine practice, which allow distinguishing and separating those groups of patients. For example, for identifying causative rhinovirus groups, measurement of antibodies towards N-terminal fragments of the human rhinovirus coat protein VP1 can be useful [31, 32]. Specific IgE towards single allergen molecules or complex IgE and IgG reactivity profiles can be biomarkers and even predictors of allergic asthma. This approach is mentioned in a separate section within our review.

It is necessary to underline that when using biomarkers for the detection of asthma phenotypes and endotypes one should be aware about the accuracy of different types of markers. Westerhof et al. [42] analyzed the accuracy of biomarkers for sputum eosinophilia ($\geq 3\%$) in different phenotypes of adult asthma. In that study, the levels of eosinophils in blood and sputum, FeNO, and total IgE were investigated. Their analysis showed comparable diagnostic accuracies for blood eosinophils and FeNO in adult patients with asthma, irrespectively of the asthma phenotype, such as severe, nonatopic, obese, and smoking-related asthma [42]. However, these markers had a superior accuracy compared to total IgE. According to the study performed by Wagener et al. [43], serum periostin could be a more accurate biomarker for eosinophilic inflammation as blood eosinophils and FeNO showed contradictory results in predicting eosinophilic airway inflammation [43]. Alternatively, in patients with mild to severe asthma, blood eosinophils seemed to have the highest accuracy in the identification of sputum eosinophilia [43]. This information should be taken into account when diagnosing several asthma phenotypes.

Protein Asthma Biomarkers according to Their Roles in Asthma Pathogenesis

Currently, various protein biomarkers are used to provide insight into the underlying biology in human studies of patients with asthma (Table 1). In addition to well-investigated molecules such as cytokines, several other proteins participating in inflammatory cellular pathways have emerged as possible biomarkers of asthma.

Cytokines

Cytokines have long been explored and studied as potential biomarkers of the disease. The complex biology of the asthma syndrome has been linked to a number of IL and chemokines as potential disease biomarkers. However, cytokine profiles may be also altered in other diseases and hence might not be specific for asthma.

Interleukins

Several IL are tightly associated with processes involved in the development of asthma. In particular, IL-4 is considered to be the predominant cytokine of the Th2 pathway in allergic airway inflammation. Briefly, in asthma pathogenesis, IL-4 induces differentiation of Th2 lymphocytes from naive T cells leading to Th2 cytokine release; IL-4 induces the IgE isotype switch and mucus secretion; IL-4 then induces the adhesion molecules on endothelial cells leading to the promotion of fibrosis and thrombosis and primes the vessel wall for eosinophil extravasation [44]. IL-4 has been reported as a trigger for barrier dysfunction in airway epithelial cells, but another study found that only Th1 cytokines affect epithelial barrier function [99]. Allergen challenge studies demonstrated that such induction is a generalized phenomenon for both the upper and the lower airways. IL-4 can be produced by T cells, basophils, eosinophils, mast cells, and recently discovered innate lymphoid cells (ILC) 2 cells.

IL-5 facilitates trafficking and maintenance of eosinophils within the lung tissue by directly recruiting eosinophils from the circulation and inducing eosinophil differentiation from the bone marrow [50]. IL-13 plays an indispensable role in mounting bronchial hyperreactivity and goblet cell hyperplasia, caused by an elevated expression of the mucin proteins MUC5AC and MUC5B, ultimately leading to mucus overproduction and airway obstruction [51]. Izuhara et al. [45] found that IL-4 and IL-13 induce periostin expression. Data collected from patients with asthma have shown that IL-4, IL-5, and IL-13 have the potential to serve as an allergic asthma biomarker profile [21]. It is especially important for the pre-

scription of anti-cytokine therapies targeting IL-4, IL-5, and IL-13 in asthma patients.

IL-25 activates innate lymphoid cells. In response to airway injury by rhinovirus infection or allergen stimulation, epithelial cells can produce IL-25, which in turn can act on nearby innate lymphoid cells [64]. IL-25 induces airway remodeling due to goblet cell hyperplasia, subepithelial collagen deposition, and angiogenesis [53]. The level of IL-25 is increased in acute asthma attacks.

IL-33 is important in the innate and adaptive immune response in the mucosal organs for chronic airway inflammation and tissue remodeling [54]. In innate immunity, IL-33 activates eosinophils, basophils, mast cells, and ILC2. For adaptive immune responses, IL-33 influences dendritic cells to promote the development of Th2, Tfh, and Treg cells. On the cytokine level, IL-33 induces IL-8 and IL-17F expression and participates in rhinovirus-induced CXCL10 production. Levels of IL-33 seem to correlate with asthma severity. IL-33 can be detected in serum, induced sputum, bronchoalveolar lavage (BAL) fluids, and epithelial cells of airways. IL-25 and IL-33 may be utilized as surrogate biomarkers of atopic and non-atopic asthma [21].

IL-9 promotes IL-4-driven antibody-inducing goblet cell metaplasia. IL-9 functions in multiple potential asthmatic roles, from promoting mast cell growth and development to initiating IL-4 expression and subsequent B-cell production of allergic antibodies. IL-9 has also been evaluated for its use as an asthma biomarker given its heightened expression in the lungs of asthmatic patients [61].

IL-15 is known as a T cell growth factor important for the development of Th17 cells. Its role in allergy is still being investigated. According to the analysis of cytokine panels, IL-15 is hypothesized to play a key role in the development of atopy. In several studies, IL-15 has been demonstrated to prevent the development of allergic inflammation by reactivating antigen-specific CD8⁺ cells [64, 65].

IL-20 regulates hematopoiesis, proliferation, and differentiation of keratinocytes and epidermal cells during inflammation. A positive correlation between serum levels of IL-20 and the Th2 cytokines (IL-4, IL-5, and IL-13) was found in asthma patients. Wu et al. [66] proposed IL-20 as potential marker of asthma severity, as IL-20 increases in direct correlation with the severity of asthma.

IL-1 α is strongly associated with acute and chronic inflammation. IL-1 α and IL-33 are similar in that their precursor forms can bind to their respective receptors and trigger signal transduction [55]. IL-33 is a novel IL-1 fam-

Table 1. Protein biomarkers for asthma

Biomarker	Producers in asthma	Function in asthma pathogenesis	Associations/clinical implications	Biological fluids/sample	References
Cytokines					
IL					
IL-4	Basophils, eosinophils, mast cells, ILC2 cells, CD4+ Th2 cells	Activation of GARA3, promotion of fibrosis and thrombosis, induction of periostin expression	Increase in asthma/ diagnostic and target treatment, monitoring of the clinical efficacy of SIT	Serum, allergen-activated PBMC, sputum, BAL fluid; tissue	21, 39, 44–49
IL-5	CD4+ Th2 cells	Eosinophil activation, increased generation; B cells: proliferation; trafficking and maintenance of eosinophils; airway remodeling	Increase in asthma/ monitoring of the clinical efficacy of SIT	Serum, allergen-activated PBMC, local nasal fluid, sputum, BAL fluid	21, 39, 46, 47, 49, 50
IL-13	CD4+ Th2 cells, mast cells, NKT cells	B cells: isotype switching to IgE; epithelial cells: increased mucus production; fibroblasts: increased collagen synthesis; macrophages: alternative activation, induction of periostin expression	Mounting bronchial hyperreactivity, mucus overproduction, airway obstruction/diagnostic and target treatment, monitoring of the clinical efficacy of SIT	Serum, allergen-activated PBMC, local nasal fluid, sputum, BAL fluid	21, 39, 45–47, 49, 51
IL-25	T cells, mast cells, eosinophils, macrophages, mucosal epithelial cells	Activation of ILC, induction of TSLP, IL-4, IL-5, I and L-9, contribution to abnormal angiogenesis, airway remodeling	Associations with allergic asthma	Serum or plasma, sputum, BAL fluid	21, 52, 53
IL-33	Epithelial cells	Activation of ILC, activating intracellular molecules in the NF- κ B and MAP kinase signaling pathways that drive production of type 2 cytokines (e.g., IL-5 and IL-13) from polarized Th2 cells, mast cells, eosinophils, and basophils	Associations with asthma severity, negative correlation with lung function	Serum or plasma, sputum, BAL fluid	21, 39, 49, 54–60
IL-9	Th2 cells, Th9 cells, mast cells, eosinophils	Promotion of mast cell growth, reduction of the production of IFN- γ , development of IL-4 expression, synergistic effect on IL-4-induced IgE production by B cells	Diagnosis, monitoring of the clinical efficacy of SIT	Serum, local nasal fluid	21, 39, 46, 60–63
IL-15	Mononuclear phagocytes	T-cell growth factor for Th17 cells, survival and proliferation of memory T cells, development of atopy	Preventive	Serum or plasma	64, 65
IL-20	CD4+ Th2 cells, monocytes, NKT cells	Enhancement of the proliferation of multipotent hematopoietic progenitors	Increase in asthma, associations with asthma severity	Serum	49, 66
IL-1 β	Macrophages, dendritic cells, fibroblasts, endothelial cells	Acute-phase reactants, activation of cytokine secretion, decreased inflammatory responses	Increase in nonallergic asthma in children	Stimulated PBMC	49, 60, 67
IL-17	Th17 helper cells, cytotoxic T cells, $\gamma\delta$ T cells	Promotion of neutrophil recruitment	Nonallergic asthma in children	Stimulated PBMC	49, 63, 67, 68
IFN- γ	Th1 cells, CD8+ T cells, NK cells, ILC	Classical activation of macrophages, B cells, isotype switching to IgG, Th1 differentiation, increased expression of class I and class II HLA molecules, increased antigen processing and presentation to T cells	Steroid-resistant asthma patients/prognosis of patients resistant to treatment	Stimulated CD8+	39, 46, 49, 63, 68–70

Table 1 (continued)

Biomarker	Producers in asthma	Function in asthma pathogenesis	Associations/clinical implications	Biological fluids/sample	References
Chemokines					
Eotaxins CCL11 CCL24 CCL26	T cells, fibroblasts, endothelial cells, alveolar macrophages	Mobilization of eosinophils into tissues with release of biologically active lipids as paracrine mediators of inflammation and release of pre-formed cationic granules and cytokines	Increase in asthma, high level during asthma exacerbation, negative correlation with lung function/diagnosis and prognosis, monitoring of the clinical efficacy of SIT	Sputum, tissue, serum, BAL fluid	21, 46, 49, 71–73
CCL17 (TARC)	Dendritic and epithelial cells	Potent chemoattractant for CCR4 expressing Th2 cells	Levels significantly decline following steroidal treatment/indicator of steroid treatment efficacy	Sputum, serum	21, 74
CCL5 (RANTES)	Mononuclear cells	RANTES promotes the migration and activation of the inflammatory cells, including monocytes and eosinophils, and is involved in allergic inflammation	Detection and monitoring of inflammation in patients with asthma	Serum, inhaled breath condensate	21, 48, 75–77
MCP-4/ CCL13	Epithelium and endothelial cells	Attractant of eosinophils, monocytes, and basophils, promotion of histamine release from IL-3-primed basophils, activation of eosinophil respiratory burst	Prediction of susceptibility to asthma, severity of asthma exacerbation	Sputum, BAL fluid, serum	21, 78
TSLP	Mainly by nonhematopoietic cells: fibroblasts, epithelial cells, and different types of stromal cells	Stimulator of naive CD4 cell proliferation priming to Th2 phenotype activation of mast cells	Asthma exacerbations	Sputum, BAL fluid, serum	21, 39, 79–81
CXCL10 (IFN- γ -induced protein 10; IP-10)	Endothelial cells, CD4+, CD8+, NK, and NKT cells, dependent on IFN- γ	Chemoattraction for monocytes/macrophages, T cells, and dendritic cells, promotion of T-cell adhesion to endothelial cells	Asthma exacerbations, discrimination of acute exacerbations in patients with virus-induced asthma	Biological fluids, tissue	38, 48, 76, 82
Periostin	Cells from the mesenchymal lineage	Eosinophilic inflammation, regulation of subepithelial fibrosis and mucus production	Biomarker for type 2 inflammation, distinguishing of patients who benefit from anti-IL-13 and anti-IgE-treatment, prognosis for risk of asthma exacerbations, prognosis for lung function decline	Serum	38, 43, 45, 49, 84, 85
Chitotriosidase	Mature monocyte-derived macrophages; lung macrophages, neutrophils, lung alveolar epithelial cells	Chronic inflammation; cytolytic enzyme activity: hydrolytic and transglycosylation activity	Non-2-type biomarker	Serum	86
ECP	Eosinophils	Toxic to airway epithelium	Increase in asthma, negative correlation with lung function, decrease under steroid treatment/severity of asthma, treatment monitoring	Serum	49, 87

Table 1 (continued)

Biomarker	Producers in asthma	Function in asthma pathogenesis	Associations/clinical implications	Biological fluids/sample	References
Soluble antigens					
sCD14	Epithelial, endothelial, smooth-muscle cells	Development and manifestation of atopy	Exacerbation of asthma in children/predictor of infant wheezing and prolonged cough	Serum, cord blood	88–92
sHLA-G	Airway epithelium	Nonclassical major histocompatibility complex, overexpression or lack of suppression of HLA-G contributes to asthma development	Increasing in atopic asthma	BAL fluid, plasma	93–98

IL, interleukin; RANTES, regulated on activation, normal T-cell expressed and secreted; SIT, specific immunotherapy; ILC, innate lymphoid cells; MCP, monocyte chemoattractant protein; PBMC, peripheral blood mononuclear cells; TSLP, thymic stromal lymphopoietin; TARC, thymus and activation-regulated chemokine; sHLA-G, human leukocyte antigen-G; ECP, eosinophil cationic protein; BAL, bronchoalveolar lavage.

ily cytokine, activating eosinophil functions targeting the production and release of pro-inflammatory mediators, such as superoxide anion, a granule protein, and IL-8 [56]. Furthermore, IL-33 supports the survival of mast cells and Th2 cells, involved in eosinophilic inflammation and induces cytokine production by those cells in mucosal tissues [57]. Together with activated STAT5, IL-33 promotes the production of IL-13 and IL-5, but not of IL-4 or IL-2 [58]. In addition to binding to their cell surface receptors, IL-1 α and IL-33, the intracellular precursor forms of IL-1 α and IL-33 also translocate to the nucleus and influence transcription [59].

IFN- γ is an important cytokine for innate and adaptive immunity against viral, some bacterial, and protozoal infections. Specifically, IFN- γ is produced predominantly by natural killer (NK) and NK T cells as part of the innate immune response and by CD4 Th1 and CD8 cytotoxic T lymphocyte effector T cells as part of adaptive immunity [69]. IFN- γ is also produced by non-cytotoxic ILC, a family of immune cells first discovered a few years ago [70]. IFN- γ is the primary cytokine that defines Th1 cells. IFN- γ turns Th0 cells into Th1 cells, suppressing Th2 cell differentiation.

Th2 cells are dominant in mild/moderate asthma, and patients with mild/moderate asthma respond to low doses of inhaled corticosteroids. In contrast, certain patients with severe asthma are not responsive even to high doses of systemic corticosteroids and have IFN- γ (Th1) immune responses with reduced Th2 and IL-17 responses [68].

Given this data, theoretically, cytokines can be used as markers for disease prognosis; defining cytokines for im-

mune cells and direction of response include IFN- γ for Th1 cells, IL-4 for Th2 cells, and IL-17 for Th17 cells.

Chemokines

Eotaxins

Eosinophilia in lung tissue is driven partially by the recruitment of eosinophils to the lung mucosa and interstitium due to airway-produced IL-5, as well as production of eotaxin (CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26). Eotaxins are chemokines with a high specificity for eosinophil populations given this cell type's preferential expression of eotaxin receptors, CCR2, CCR3, and CCR5 [71]. An increased expression of eotaxin at sites of allergic inflammation has been observed in both atopic asthmatics and nonatopic asthmatics and can also be found in increased levels in patient sputum [72, 73]. Thus, eotaxins can be considered as potential biomarkers for diagnosis and prognosis of asthmatic disease featured with eosinophilia.

Monocyte Chemoattractant Protein-4

Allergen-induced expression of monocyte chemoattractant protein-4 (MCP-4/CCL-13) within asthmatic airways has also been suggested as a potential biomarker. It signals through receptors CCR2, CCR3, and CCR5 and is a potent chemoattractant for asthma-related cell populations including eosinophils, monocytes, and basophils. MCP-4 signaling promotes histamine release from IL-3-primed basophils. In addition, MCP-4 is an activator of the respiratory burst in eosinophils. When allergic inflammation occurs, MCP-4 levels are significantly elevated in asthmatic sputum, BAL fluid, and respiratory epi-

thelium and within the cells that line the airways. Following an allergen-induced challenge, TNF- α and IL-1 β trigger the release of MCP-4 from the epithelium and endothelial cells that are inflamed, which in turn facilitates the recruitment of other types of asthma-inducing inflammatory cells and the cytokines they produce [78]. Blood levels of MCP-4 can predict the susceptibility to asthma, the severity of asthma exacerbations, and therefore potentially the efficacy of asthma-control therapy.

Thymic stromal lymphopoietin (TSLP) is another potent cytokine, which is released by inflamed epithelium and has links with asthma exacerbation. This cytokine is required for a normal CD4 T cell response – TSLP acts directly on naive, but not memory, CD4 T cells to stimulate their proliferation in response to antigens [79]. However, the interaction of TSLP with dendritic cell populations favors the polarization of primed CD4 T cells into the Th2 phenotype. TSLP also activates mast cells which in turn can produce cytokines that increase the preferential progression of Th2 polarization. TSLP induces key changes in dendritic cells such that, during antigen ligation to cognate T cells, DC also are promoted to express the chemokine ligands 17 (CCL17) and CCL22, as well as inducing surface expression of the OX40 ligand [80]. TSLP may also influence the regulatory T-cell compartment to affect allergic airway disease, as TSLP inhibits IL-10-mediated Treg function and the formation of inducible Tregs to exogenous antigen [81].

CCL-17 (thymus and activation regulated chemokine) induces chemotaxis in T cells via the CCR4 receptor. CCL-17 is produced by dendritic and epithelial cells and released upon contact with an allergen. CCL-17 is a potent chemoattractant for CCR4-expressing Th2 cells which then feeds forward into the allergen-derived inflammatory response. CCL-17 levels are significantly elevated in asthmatic patient's sputum and in serum samples from children suffering from asthma. CCL-17 can serve as an indicator of treatment efficacy. It has been shown that its levels significantly decline following steroid treatment [74].

CCL-5 (regulated on activation, normal T-cell expressed and secreted; RANTES) promotes the migration and activation of inflammatory cells, including eosinophils, and is involved in allergic inflammation. In addition, increased serum RANTES levels in children with atopic asthma in comparison with healthy children have been observed [76]. Moreover, children with unstable asthma have significantly higher RANTES levels in inhaled breath condensate. The use of RANTES has been proposed for detecting and monitoring inflammation in patients with asthma [77].

CXCL10

The secretion of IFN- γ -induced protein 10 (IP-10) by CD4+, CD8+, NK, and NKT cells is dependent on IFN- γ . IP-10 is a secreted protein and can be measured in biological fluids and tissue samples. While IP-10 and other inflammatory cytokines are often used as predictors of breast, ovarian, lung, prostate, and colorectal cancer development and progression, they can be adapted as markers of asthma inflammation. IP-10 is a predominant chemokine measured during human allergic pulmonary late-phase reactions. A significant increase in serum levels of IP-10 during an asthma attack in children [76] and in patients with occupational asthma [82] in contrast to healthy controls suggests that IP-10 is a serological marker of exacerbation. Moreover, IP-10 values can be useful for discrimination of acute exacerbations in patients with virus-induced asthma, as serum IP-10 levels were significantly higher in non-sensitized patients [83].

sCD14. CD14 is a co-receptor of glycoprotein nature (soluble or membrane bound) for the interactions between lipopolysaccharide (LPS) and Toll-like receptor 4 with a subsequent production of IL-6, IL-8, IL-12, and TNF- α . Soluble CD14 (sCD14) activated by LPS is highly required for epithelial, endothelial, and smooth muscle cells because of the absence of membrane-bound CD14 in these cell types. sCD14 is reported to have both pro- and anti-inflammatory effects by mediating LPS transfer to plasma lipoproteins with subsequent neutralization of its activity and taking LPS from membrane-bound CD14 [88]. Studies have found that both adults and children with acute forms of asthma and other allergic diseases present with increased levels of sCD14. Additionally it has been demonstrated that, in patients with asthma, levels of sCD14 continued to be elevated 18 h segmental allergen provocation. Those with atopic dermatitis, however, exhibited greater sCD14 levels within the acute stage, which was noted to be correlated with improvement in skin intensity score [89–91]. A Taiwanese study recorded sCD14 levels at birth and collected questionnaires from the children's mothers asking about parental medical and allergic histories. These questionnaires were then repeated at 1, 6, 12, and 18 months [92]. At the 1-year mark, wheeze was prevalent in 14% of the participants, and sCD14 levels in these children were significantly higher than in the 177 children with no reported wheeze. Factors significantly predictive for wheeze at 1 year included sCD14 levels, duration of day care attendance, and parental smoking, with each pack per day of cigarettes associated with a 2-fold risk. Also, prolonged cough was reported in 7% of the children at 1 year, and these chil-

dren were found to have significantly higher sCD14 levels in their cord blood. After statistical analysis, cord blood sCD14 was found to be the single independent predictive biomarker for wheeze and prolonged cough by the age of 1 year, indicating that cord blood sCD14 could be a vital predictive biomarker.

Soluble human leukocyte antigen-G (sHLA-G) is a non-classical major histocompatibility complex with an important tolerogenic function during pregnancy and cancer development [93]. Previously, soluble forms of HLA-G were tested as prognostic factors in maternal plasma in early and late pregnancy [94] and in BAL of lung cancer patients [95], and measured as immunomodulatory factors in multiple sclerosis [96], psoriasis, and other inflammation diseases. To support the role of sHLA-G in asthma pathogenesis, White et al. [97] measured sHLA-G levels in BAL of patients with mild persistent asthma. They found increased levels of sHLA-G in asthmatics compared to controls and supposed that overexpression or lack of suppression of HLA-G contributes to asthma development. Plasma sHLA-G levels were also significantly higher in atopic asthmatic children than in healthy ones; however, no significant association was observed between plasma sHLA-G, total IgE, and allergen-specific IgE levels [98].

Periostin

Serum periostin was proposed to be among the novel markers for eosinophilic inflammation [43]. This matricellular protein encoded by the POSTN gene interacts with multiple signaling cascades, thus modulating the expression of downstream genes, regulating cellular interactions within the extracellular matrix. Periostin is involved in the development of many respiratory diseases through its secretion after epithelial injury and subsequent involvement in airway remodeling and other aspects of pulmonary pathophysiology. In asthma, periostin can be used as biomarker of type 2 inflammation. Expression of the POSTN gene in bronchial epithelial cells is upregulated by IL-3 and IL-4. Serum periostin aids in distinguishing patients who demonstrate an increased clinical benefit from treatment with anti-IL-13 (lebrikizumab and tralokinumab) and anti-IgE (omalizumab). Moreover, it may be used for the prognosis of an increased risk of asthma exacerbations and progressive lung function decline. Periostin may serve as a systemic biomarker of eosinophilic airway inflammation as it may regulate subepithelial fibrosis and mucus production in asthma. Additionally, periostin expression is elevated in the lungs of patients with idiopathic pulmonary fibrosis,

and thus its serum levels can serve as predictors of clinical progression. In conclusion, periostin may contribute to the development of multiple respiratory diseases through a variety of pathological processes, and levels of periostin in peripheral blood may be used as a biomarker for treatment response and disease progression [38, 84, 85].

Serum chitotriosidase and YKL40 (human cartilage glycoprotein 39) can also serve as biomarkers of asthma due to their significantly elevated levels when compared to healthy controls, despite the strong influences of genotype and age. Correlations between lung function and expression of chitotriosidase and YKL40 have been demonstrated; levels of circulating chitinase were not affected by acute exacerbations, allergen-induced airway obstruction, or corticosteroid treatment. It can be concluded that chitotriosidase and YKL40 are relatively steroid-insensitive substances and could be used as non-T helper cell type 2 biomarkers that have a distinct relation to chronic inflammatory disease processes [86].

Combination of Biomarkers in Asthma and Protein Profiling

Using protein multiplex arrays, including detection of the most important cytokines, chemokines, and growth factors, it was found that several proteins could serve as potential disease biomarkers in combination and can be measured simultaneously.

For example, simultaneously measuring of IL-4, IL-5, and IL-13 levels with eosinophils in serum and tissue can determine asthma endotype subsets [47]. Serum fibroblast growth factor, hepatocyte growth factor, and SCGF β characterize the moderate and severe asthma groups [100], and in combination with IL-18 they are positively correlated with poor asthmatic control and negatively associated with quality of life scores. A strong correlation between RANTES (CCL5) levels and FEV₁, and between expression levels of TNF- α and TGF- β ₁ and bronchial hyperreactivity, has also been shown [101]. Elevated cytokine levels IL-1b and macrophage inflammatory protein 3a/CCL20 in sputum samples are associated with asthma severity [102]. Multiplex arrays can be also used to monitor the cytokine profiles of asthmatic patients undergoing corticosteroid treatment [48]. Patients treated with corticosteroids showed decreased levels of CXCL10 and increased levels of IL-4 and RANTES compared to untreated controls, and these changes were correlated with improved FEV₁ and over-

all airway obstruction. These findings strongly suggest that IL-4 and RANTES levels in exhaled breath condensate could not only be applied as predictive biomarkers for the success of steroidal therapies but also mark cytokines as therapeutic targets for disease intervention and monitoring.

In conclusion, the protein biomarkers can be classified into non-invasive sample types including serum, sputum, exhaled breath condensates, and BAL fluids. This makes them appealing for routine practice. Due to the heterogeneity and multifactorial nature of asthma, there seems to be no unique or specific protein that can individually serve as an asthma marker. One may therefore consider studying the usefulness of combinations of the above-mentioned factors for the discrimination of different forms of asthma and eventually select certain biomarkers or biomarker combinations for individual patients in a personalized manner.

IgE Specific for Certain Allergen Molecules or Combinations of Molecules as Biomarkers and Predictors of Allergic Asthma

Determination of predictive biomarkers for the atopic asthma endotype is a requirement nowadays. According to current clinical recommendations and guidelines, it is important to identify the asthma-associated sensitization patterns and minimally invasive asthma biomarkers [103]. The basis for this suggestion are recent investigations demonstrating that IgE specific for different allergen molecules and protein families are associated with different symptoms and severity of allergic diseases [104]. According to GINA 2017, among the factors that can trigger or worsen asthma symptoms, domestic and occupational allergens, such as cockroach, house dust mite and pollen, are particularly important [105].

From epidemiological investigations and birth cohort studies, we know that the development of asthma is preceded by early sensitization followed by clinical symptoms [106–107], often rhinitis, during childhood and may be predicted by measuring IgE reactivity to few allergen molecules [108]. As asthma has early origins and there are differences in its pre-/early- school forms [109], it is crucial to identify biomarkers of IgE sensitization before the clinical manifestation. This can help prevent molecular spreading, disease manifestation, and/or asthma progression.

The last 25 years have been revolutionary in allergy diagnostics, as molecular diagnostic tests have been de-

veloped. This has been possible through knowledge regarding the structures of disease-causing allergens [33]. Today it is possible to determine specific IgE towards multiple recombinant or purified natural allergens [28]. Thereby we are now at a new (molecular) level of determination of the genuine trigger of allergic reactions and studying the mechanisms of allergic inflammation development [29].

Important Asthma-Associated Allergen Molecules Recognized by IgE

Perennial allergens, more often than pollen, are associated with the severity of bronchial hyper-responsiveness, with a large impact on asthma severity and prognosis [110].

More than 80% of asthmatic children are sensitized to at least one indoor allergen [107], and IgE sensitization to house dust mite allergens, pests (cockroach and rodents), pets (dogs and cats), and mold is strongly associated with asthma development [106, 107, 111–113]. Given these facts, measurement of IgE responses to perennial allergens is a major focus for asthma diagnosis and prediction (Table 2a).

Mite Allergens and Asthma

Sensitization to house dust mites predicted the persistence of wheezing and relapse in childhood asthma when 1,139 children from New Zealand were followed up from the ages of 9–21 years [106]. It was found that high levels of IgE to house dust mite allergen extract increased the risk of acute wheezing in children with asthma [114].

Resch et al. [115] demonstrated that asthmatic children with a house dust mite allergy are characterized by a broad IgE recognition pattern towards house dust mite (*Dermatophagoides pteronyssinus*) allergens, and by significantly higher IgE levels to Der p 1, Der p 2, Der p 5, and Der p 23, than non-asthmatic children. Moreover, the sum of IgE levels to different house dust mite allergens was also higher in children with asthma [115].

Thanks to longitudinal studies, we now understand the evolution of sensitization and prediction IgE levels towards different house dust mite allergens. In healthy preschool children, early IgE sensitization to Der p 1 or Der p 23 is strongly associated with asthma at school age [116]. According to a latter analysis, Der p 1 (cysteine protease) and the new major house dust mite allergen Der p 23 (peritrophin-like protein) [117, 118] may be proposed as predictive markers for asthma development.

Among 30 known allergenic determinants from *Blomia tropicalis*, Blo t 1, Blo t 5, Blo t 10, and Blo t 21 are species-

Table 2. Important marker indoor and outdoor allergens of IgE sensitization in asthma**a** Perennial allergens

Source	Allergen molecule	Protein family	Molecular weight, kDa	References
House dust mite <i>Dermatophagoides pteronyssinus</i>	Der p 1 Der p 23	cysteine protease peritrophin-like protein	25 10.3	114–116 117, 118
<i>Blomia tropicalis</i>	Blo t 5	group 5 mite allergen	14	120
Cockroach <i>Periplaneta americana</i>	Per a 2	aspartic protease-like	42	121
<i>Blattella germanica</i>	Bla g 1 Bla g 2	transmembrane protein aspartic protease	46 36	122 122
Pets and rodents Cat	Fel d 1	uteroglobin	14	111
Dog	Can f 1	lipocalin	23–25	111
Mouse	Mus m 1	lipocalin	19	123
Mold <i>Alternaria alternata</i>	Alt a 1	unknown	30	124
<i>Aspergillus fumigatus</i>	Asp f 1	mitogillin	18	125

b Pollen allergens

Source	Allergen molecule of genuine sensitization	Biochemical name*	Molecular weight, kDa	References
Weed pollen Ragweed (<i>Ambrosia artemisiifolia</i>)	Amb a 1	pectate lyase	38	143
Mugwort (<i>Artemisia vulgaris</i>)	Art v 1 Art v 3	defensin-like protein nonspecific lipid transfer protein type 1	13–16 12	143 143
Wall pellitory (<i>Parietaria judaica</i>)	Par j 1 Par j 2	phospholipid transfer protein phospholipid transfer protein	15 10–14	143 143
Goosefoot (<i>Chenopodium album</i>)	Che a 1	trypsin inhibitor Ole e 1-like protein	17	143
Saltwort (<i>Salsola kali</i>)	Sal k 1 Sal k 5	pectin methylesterase Ole e 1-like protein	43 18.2	143 143
Grass pollen Timothy grass (<i>Phleum pratense</i>)	Phl p 1 Phl p 5	β-expansin ribonuclease	27 32	144 144
Tree pollen Birch (<i>Betula verrucosa</i>) (<i>Betula pendula</i>)	Bet v 1	pathogenesis-related protein, PR-10, Bet v 1 family member	17	145
Cypress (<i>Cupressus arizonica</i>)	Cup a 1	pectate lyase	43	145
Japanese cedar (<i>Cryptomeria japonica</i>)	Cry j 1	pectate lyase	41–45	145
Olive tree (<i>Olea europaea</i>)	Ole e 1 Ole e 7 Ole e 9	trypsin inhibitor nonspecific lipid transfer protein 1 3-β glucanase	16 9–10 46	145 142 142
Plane tree (<i>Platanus acerifolia</i>)	Pla a 1 Pla a 2	putative invertase inhibitor polygalacturonase	18 43	145 145

* According to the WHO/IUIS Allergen Nomenclature Subcommittee (August 2017).

specific molecules. There is a difference in sensitization patterns to *B. tropicalis* as it relates to allergic disease development and progression. Patients with asthma often have sensitization to Blo t 5, Blo t 6, and Blo t 11 in tropical regions. It was demonstrated that sensitization to Blo t 5 was 3 times higher in patients with asthma symptoms than in patients with eczema. Moreover, 96.7% of asthmatic patients in Singapore were sensitized to Blo t 5 instead of to *D. pteronyssinus* molecules [119]. It is necessary to emphasize that the Blo t 5 molecule has a low cross-reactivity with the Der p 5 allergen and can be suggested as asthma biomarker in tropical regions [120]. The same study showed that true co-sensitization to *D. pteronyssinus* and *Blomia* allergens resulted in a higher risk of asthma.

Cockroach Allergens and Asthma

Investigation of the association between cockroach allergy and asthma has a 50-year worldwide history. It was reported that 55% of children and young adults with allergic respiratory symptoms had a positive skin test reaction to cockroach allergens in Brazil [126]. Children with asthma who were sensitized and exposed to high levels of cockroach allergens had increased asthma morbidity [127].

There is no clinically specific symptom of cockroach asthma, but a longer duration and a higher proportion of steroid dependency are markers for disease severity. North American children with Bla g 1 or Bla g 2 exposures higher than 2 U/g of dust in the living room or kitchen have a risk of asthma development of 35% [127].

Currently, 12 groups of allergen molecules of the most common cockroach species are listed in the allergen database of the WHO/IUIS Allergen Nomenclature Subcommittee: Bla g 1–9 and Bla g 11 from *Blattella germanica*, and Per a 1–3, Per a 6–7, and Per a 9 to Per a 12 from *Periplaneta americana* (www.allergen.org). Although the IgE reactivity profiles are heterogeneous, the Bla g 1 (mid-gut microvilli protein homolog), Bla g 2 (inactive aspartic protease), Bla g 4 (lipocalin), and Bla g 5 (glutathione S-transferase) allergen mixture covered 95% of cockroach allergies in the USA [128].

Bla g 7 and Per a 7 (tropomyosins) can be important for patients in other specific regions. For example, within the Brazilian population, Pen a 7 is a dominant allergen in cockroach allergy, and 42% of patients with asthma and rhinitis had a positive response to rPen a 7 [122].

A marker for severity of disease among cockroach-allergic patients in Taiwan was IgE to Per a 2 (aspartic protease-like), which is more frequent among the group of patients with persistent asthma [121].

Rodent Allergens and Asthma

Previously, rodent allergy was within the scope of occupational medicine; however, today, allergens of rodents, and in particular those from mice, are important in home, school, and day care center environments [107, 129]. Mouse allergen molecules might already be present at schools and are not passively transferred from homes [130]. Mus m 1 (lipocalin/urinary prealbumin) is the major allergen of *Mus musculus* and can be found in urine, hair follicles, and dander of mice.

Exposure to mouse allergens and sensitization to Mus m 1 were significantly associated with asthma and asthma-related death in children [123].

Pet Allergens and Asthma

According to recent investigations in the USA, asthma attacks in patients with sensitization to cat and dog allergens are associated with exposure to high levels of the allergens and have increased to 1 million for dog-sensitized and more than 500,000 for cat-sensitized patients [131] within this population. In Europe, having a cat allergy is a major risk factor for the developing asthma and rhinitis.

The WHO/IUIS Allergen Nomenclature Database includes 8 groups of allergen molecules of *Felis domesticus* (cat) and 7 groups of *Canis familiaris*.

Scrutiny of the newborn cohort BAMSE (Stockholm, Sweden) and further follow-up until the age of 16 years, performed as a cross-sectional and longitudinal population-based study, traced an increase in sensitization against cats and dogs with age and revealed allergen molecules as predictors of respiratory allergy [111].

Fel d 1 (uteroglobin) is the major and most dominant sensitizing cat allergen during early childhood. Sensitization to Fel d 1 is a prognostic marker of cat allergy, whereas sensitization to Can f 1 is an important predictor of dog allergy [111].

In northern Sweden, 3 pet allergic molecules were found to have the strongest relationship to asthma: Can f 1, Can f 5, and Fel d 1 [111].

Despite the fact that a large proportion of children are sensitized against Can f 5 in comparison to other dog allergens, dog allergy symptoms developed only in 1 child out of 10. IgE reactivity towards Fel d 1 and Can f 1 at the age of 4 and 8 years was associated with allergy symptoms at the age of 16 years [111].

Mold Allergens and Asthma

Clinical asthma among schoolchildren had a strong association with exposure to mold in classrooms in Taiwan. The symptoms improved during the vacation pe-

riod [132]. Using a meta-analysis, Sharpe et al. [133] summarized that an increase in asthma exacerbation in children around the world correlated with exposure levels of *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* species.

Alternaria alternata

In 2006, Salo et al. [112] demonstrated a correlation between exposure to *Alternaria alternata* in US homes and clinical symptoms of asthma. Sensitization to *Alternaria* species is also associated with life-threatening asthma exacerbations [134] and morbidity among asthmatic patients [135].

The asthma attack-associated molecule seems to be the major allergen from *A. alternata*, i.e. Alt a 1, and IgE to Alt a 1 may be a risk factor for asthma development and progression in children [124, 136].

Aspergillus fumigatus

The WHO/IUIS Allergen Nomenclature Database includes 23 groups of allergen molecules of *Aspergillus fumigatus*. From this list, Asp f 1 (Mitogillin family) is a risk allergen for asthma [125], induced by *A. fumigatus*, while Asp f 2 (ASPND1 family) [137], Asp f 4, and Asp f 6 (manganese superoxide dismutase) are specific markers of bronchopulmonary aspergillosis.

Outdoor Allergens and Pediatric Asthma

The associations between outdoor environment and pediatric asthma in North America were analyzed in a review of 33 papers published in 2010–2015. Strong correlations between tree pollen (even at low levels) and pediatric asthma were described, especially during the spring season [138].

Correlation of asthma with sensitization to pollens had been found in Helsinki, wherein adult asthma patients had IgE reactivity towards various pollen allergens [139]. In general, in the Northern part of the world, the common pollen sensitizer sources for patients with seasonal asthma are birch, timothy grass, mugwort, and ragweed pollen.

Salsola kali pollen is an asthma-important source in semidesert areas of Central Asia, Saudi Arabia, Kuwait, Iran, and western parts of the United States, where Salkwort represents a major allergen source [140].

Parietaria judaica, *Oleaceae*, and *Cupressaceae* species are risk allergen sources for the development of asthma in Mediterranean countries. In Italy, for example, 52% of monosensitized *Parietaria* patients suffer from bronchial asthma associated with rhinoconjunctivitis [141]. Olive

pollen can also trigger asthma during pollination time [142].

Chenopodium pollen has been reported to induce asthma in Africa, Australia, Asia, North America, and Europe.

The following pollen allergen molecules are interesting as IgE biomarkers for the atopic asthma endotype but in special regions (Table 2b).

Weed pollen

Art v 1 (defensin-like protein) is a specific marker allergen for clinically manifested mugwort pollen allergy in Central Europe [143]. In the Mediterranean area Art v 3 (LTP) can trigger respiratory symptoms in sensitized patients [143].

Amb a 1 (peptate lyase) has important role as a primary sensitizer and trigger for ragweed pollen allergic rhinitis, exacerbation of asthma, and allergic conjunctivitis [143].

Par j 1/2 (nsLTP) are major allergens of wall pellitory pollen and they are clinically useful for identifying the primary *Parietaria* pollen sensitization [143].

Che a 1 (trypsin inhibitor) from goosefoot is also noted.

Sal k 1 (pectin methylesterase) can be considered as a specific marker for *S. kali* pollen sensitization [143].

Grass Pollen [144]

A longitudinal study showed the evolution of sensitization development toward grass pollen molecules in German children from the ages of 3 to 16 years. Some children kept their sensitization profiles, whereas in some others IgE antibodies appeared first to Phl p 1 and then to Phl p 4 and Phl p 5, followed by Phl p 2, Phl p 6, and Phl p 11. IgE to Phl p 7 (polcalcin) and Phl p 12 (Profilin) had been considered as a marker of disease severity and prolongation in this study, but only few subjects were sensitized against these components [146]. It should be also noted that early sensitization against a given allergen and frequency of IgE recognition are not related to the allergenic potency of a given allergen. For example, Phl p 4 is a major grass pollen allergen according to the IgE recognition frequency but induces only weak if any allergic reactions [147]. In fact, group 5 allergens seem to be the most potent allergens in inducing asthma attacks because of their abundance and unique ability to trigger strong mast cell and basophil activation [148–150].

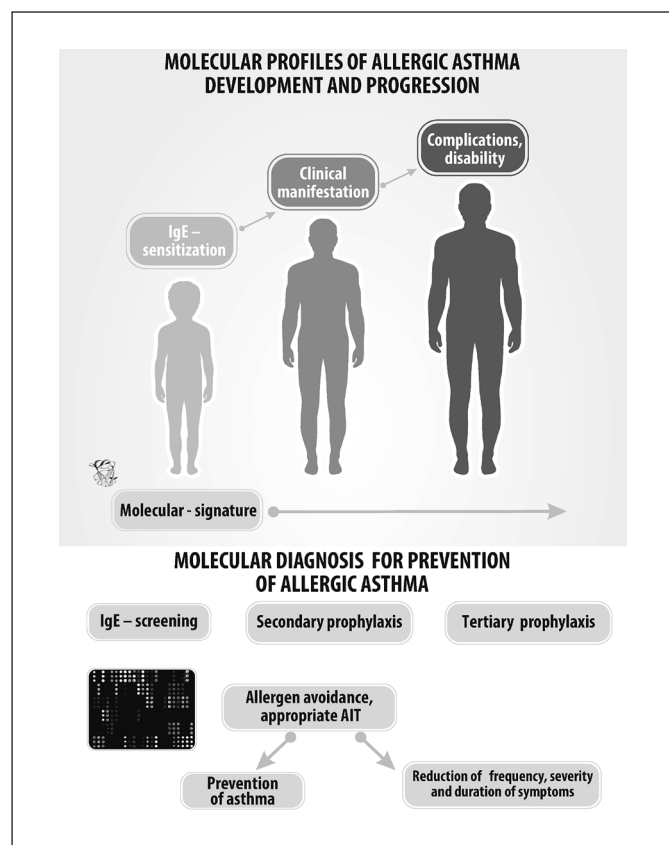


Fig. 2. Importance of molecular diagnosis for monitoring of the development of allergic asthma and for its prevention.

Tree Pollen

Bet v 1 (PR-10 protein) is marker for sensitivity toward tree pollen of the Fagales family. Considering IgE level toward the major birch pollen allergen Bet v 1 in early childhood, it is possible to assume that the clinical manifestation of birch pollen is associated with allergic diseases at the age of 16 years in 54% of those children.

Cup a 1 or Cry j 1 (pectate lyases) from cypress and Japanese cedar have a high homology. Allergy to *Cupressaceae* species has become a real problem in Mediterranean countries in the last 30 years. An association of asthma with conifer pollen was reported in areas with a high cypress exposure, where 63% of patients with conifer sensitization had asthma [151]. An association between clinical symptoms and IgE to Cup a 1 was also reported. Ole e 1 (Ole e-1 family) is marker allergen of sensitization to the *Oleaceae* family (mostly olive and ash). A statistically significant association between severe asthma and IgE Ole e 10 (a major allergen with homology to the C-terminal domain of Ole e 9) was found in Spain [152]. At the

same time, patients with olive pollen allergy and plant-derived food anaphylaxis reacted mostly to Ole e 7. Pollen-latex-fruit syndrome had been found to be in association with IgE to the Ole e 9 allergen molecule. Based on that, IgE to concrete olive pollen allergen can be a marker of different olive-allergic patients' phenotypes.

Nowadays, different techniques of allergy molecular diagnostics are available on the market. Immuno Solid-phase Allergen Chip (ISAC) technology is the most complete platform for simultaneous measurement of specific IgE against 112 allergenic molecules in a single test using a small amount of patient serum, which is very important for children [26].

Determination of a personal allergen molecular profile of sensitization in childhood can predict the development of allergic asthma and clinical outcomes. This approach can help to prescribe an adequate secondary and tertiary prophylaxis of asthma in every individual and more accurately choose personalized treatment strategies (Fig. 1). Similar tests are now becoming available also for rhinovirus-induced asthma, which is very important in childhood [31, 32].

Based on the determination of asthma-related IgE sensitizations to certain allergens, it becomes also possible to influence the sensitization profile of the whole population by controlled anthropogenic transformation of the environment for the subsequent primary prevention of allergic diseases [140]. Safe, convenient, and effective allergy vaccines are currently under clinical evaluation and show a high potential for treatment and eventual prophylactic vaccination in allergic asthma [153–155].

In conclusion, there are many biomarkers under consideration for the stratification of patients suffering from asthma. However, cellular biomarkers, cytokines, and other proteins which can be measured as surrogates for inflammation may be elevated or altered also in other pathological conditions and are not highly specific for the individual forms of asthma.

Allergic asthma can be detected best and in the most comprehensible way by measuring specific IgE in multiplex tests such as allergen microarrays, which allow assessment, in a single determination, of IgE specificities against the most relevant respiratory allergens [27]. Allergen chips are even more sensitive in detecting allergic sensitization than conventional tests based on allergen extract-based skin testing or measurement of IgE reactivity to allergen extracts [156]. Importantly, allergen microarrays allow determination of sensitization to respiratory allergens and the culprit allergen source quickly and hence are ideal first-line tests for the diagnosis of allergic

asthma. Similar antibody-based tests are currently being developed to identify respiratory viruses as culprit agents for asthma so that it will be soon possible to firmly differentiate between allergic and virus-induced forms of asthma [31, 32]. Another important result from recently conducted birth cohort studies is that it seems to be possible to predict the development of allergic respiratory diseases according to “clinically silent” IgE reactivity profiles measured early in the life of children (Fig. 2), which opens possibilities for the prevention of asthma before the onset of symptoms [108, 157, 158]. Since respiratory allergy and asthma severely affect the life of children, one may consider to initiating preventive medical examinations (i.e., “allergy passport”) based on determination of IgE reactivity profiles early in childhood to identify children who may benefit from preventive measures (Fig. 2).

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