REVIEW ARTICLE

Serum biomarkers for allergy in children

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Abstract

A large number of studies investigating various biomarkers for allergy have been published over the past decades. The aim of this review was to evaluate these biomarkers on their diagnostic and/or predictive value. To this date, no single or specific biomarker for allergy has been identified. As allergy is not one disease, but a collection of a number of allergic conditions, it is more plausible a combination of clinical history, clinical readouts, and diagnostic markers will be needed.

Atopic individuals may or may not have symptoms of allergy, but are genetically predisposed to develop one or more allergic diseases (i.e., allergic rhinitis, hay fever, asthma, atopic dermatitis, and food allergies) and have a strong familial basis. These individuals may produce IgE antibodies and develop an allergy against otherwise harmless environmental substances. Effective management of allergic diseases relies on the ability to make an accurate diagnosis. The diagnosis of allergic disorders is most often made on clinical history, supported by the results of skin tests and diagnostic markers, for example, immunoglobulin E (IgE). Up to now, the best way of diagnosing food allergy is a double-blind placebo-controlled food challenge. Although skin testing has advantages of relative sensitivity and specificity, rapid results, and good tolerability, it is subject to some operator, observer, and interpretation variability. IgE is also known to be elevated in non-allergic conditions, and many allergic patients have IgE levels within the normal range. Very recently, Schoos et al. (1) discovered that there is a substantial disagreement between skin prick test (SPT) and specific IgE for diagnosing allergic sensitization in young children, which increases with age for food sensitization. Therefore, research efforts are focused on improving diagnostic tests and on discovery of other allergic biomarkers that have better predictive, diagnostic, or prognostic value. A systemic database search was conducted using PubMed and Google Scholar for studies of serum biomarkers in atopic dermatitis (AD), asthma and allergic rhinitis, eosinophilic esophagitis (EoE), and food allergy in children from 0 to 18 years of age.

Immunoglobulin isotypes, subclasses, and free light chains

IgE is known for its involvement in type I hypersensitivity reactions; allergen-specific IgE binds to the receptor FcεR on mast cells and basophils. Re-exposure to the same allergen results in cross-linking of cell-bound IgE, which evokes a cascade of reactions. Longitudinal studies have shown that levels of IgE increase with age from birth, regardless of atopic status, but the increase in atopic children is faster and they continue to have high levels in adulthood (2). The observation of high levels of total IgE in allergic individuals compared to healthy controls lead to determining a cutoff level of total IgE for diagnosis of allergy. It was observed that the probability is very high in predicting allergy when the total IgE level is above 200 kU/l (3). In AD patients, the total IgE was significantly higher than that of control group (4). Nevertheless, a high total IgE level alone is of limited value as a marker of allergy as it does not give any clue to sensitizing allergens in an individual. Hence, attention was paid more on specific IgE as a biomarker (5). The presence of specific IgE in serum indicates that the individual has been exposed to the allergen earlier (sensitized) and specific IgE against a particular allergen above a level of 0.35 kU/l is considered positive for that allergen (6). It should be noted that a positive result does not always correlate well with the clinical features of a person. People with a positive test for a specific allergen may not necessarily exhibit symptoms when exposed to that allergen. Although a positive test for aeroallergens generally correlates well with the clinical expression, much controversy exists for food allergens (5). Specific

IgE can be used as a sensitization or predictive marker. Hen's egg-specific IgE at the age of 12 months may be a valuable marker for subsequent allergic sensitization to allergens that cause asthma, allergic rhinitis, and atopic dermatitis (7). In children who were hospitalized for wheezing at <2 years of age, it was found that specific IgE of ≥0.35 kU/l to wheat, egg white, or inhalant allergens is predictive of later childhood asthma (8). Molecular-based allergy diagnostics have recently become available in the clinical practice and offer new opportunities for improved characterization of the sensitization profile by identifying potentially dangerous proteins and suggesting a more precise prognosis and improvements in the management of allergy (9). Using molecular-based allergy diagnostics, prediction of peanut allergy in pediatric patients was much improved showing Ara h 2 and Ara h 6 were the best predictors of peanut allergy (10). Tosca et al. (11) studied egg allergic children and concluded that molecular-based allergy diagnostic should be performed in children with hen's egg allergy, especially with anaphylaxis, for better defining the sensitization profile and improving the prognosis and management of hen's egg allergic children. An investigation of the added value of measuring IgE to wheat components in a group of children with a diagnosed wheat allergy even revealed that levels of IgE to wheat gluten-derived components correlated well with wheat challenge outcome and severity and that many children diagnosed as wheat allergic had outgrown their allergy and were unnecessarily on a wheat-free diet (12).

Besides IgE, other immunoglobulin isotypes may play a role in allergic reactions. Immunoglobulin isotypes (IgG, IgA, IgM, IgD, and IgE) were investigated in serum of children with mild to moderately severe asthma. Serum levels of IgG, IgD, and IgE were elevated significantly in patients with asthma compared to controls, while IgA and IgM levels were normal (13). Humoral responses to food antigens may reflect the tendency of a child's immune system to develop tolerance to antigens. In an allergy prevention trial in high-risk children, it was found that atopic children had higher ovalbumin (OVA)-IgA, OVA-IgG, OVA-IgG1, and egg-IgE but lower OVA-IgG4/egg-IgE ratios than non-atopic children. Therefore, allergy was associated with more intense IgA and IgG responses to OVA. Furthermore, OVA-specific IgA and IgG antibodies may help in assessing the risk for atopy (14).

Serum and secretory IgA concentrations have been suggested to be inversely associated with allergic symptoms in children. A cohort of full-term newborns was prospectively followed up from birth to age 20 years with measurement of serum total IgA at ages two and six months and re-assessed for the occurrence of allergic symptoms, with skin prick testing and measurement of serum IgE. Children with respiratory allergic symptoms and sensitization had a higher serum IgA concentration at age two months than non-atopic subjects. Increased serum IgA concentration at age two months was associated with the development of subsequent allergic symptoms and sensitization in childhood and adolescence (15).

IgD may also have some role in allergic reactions. IgD was found to bind to basophils and mast cells and activate these cells to produce antimicrobial factors to participate in respiratory immune defense in humans. Total IgD and IgE were investigated in atopic subjects, showing that mean serum IgD and IgE were significantly higher in atopic subjects than that in normal subjects. However, total serum IgD was not significantly correlated with total serum IgE, indicating that immunoregulatory control of the basal levels of the two isotypes is not linked (16). Serum IgD and IgE levels were also measured in children with atopic asthma to determine the relationship with clinical status. A significant increase in IgD levels was observed in children at first signs of asthma, and the following normalization after 18 months may represent a non-specific response or an attempt of the organism to block asthma, therefore favoring immunologic tolerance (17). Both studies were conducted >15 years ago, and recent data on IgD are therefore lacking.

Allergen-specific IgE antibodies are implicated in allergic diseases, while allergen-specific IgG antibodies have been proposed to prevent allergic reactions. In particular, IgG4 has been hypothesized to act as blocking antibody, capable of preventing IgE-mediated effector cell triggering. Serum IgE and serum IgG4 were evaluated in a group of asthmatic children that were skin prick test positive to at least two allergens. A significant decrease in serum-specific IgE to house dust mite and pollen allergens was observed; however, no significant variations were shown by IgG4 and IgG4/IgE ratio. The positive correlation between specific IgE and specific IgG4 throughout the entire study suggests a relationship between these classes of immunoglobulins (18). Another study investigated whether the immune response (IgG and IgG4) to peanut differs in IgE-sensitized and non-sensitized young children who were followed from birth to 5 years of age. Here was found that peanut-specific IgG or IgG4 levels were elevated in peanut-sensitized children especially those avoiding peanuts. In this study, IgG and IgG4 do not seem to indicate tolerance or protection from sensitization (19). It is known that egg sensitization, particularly persistent sensitization, is a risk factor for later asthma. However, little is known about accompanying IgG and subclass responses and how they might relate to asthmatic outcome. OVA-IgG and subclass responses were assessed throughout the first 5 years of life in relation to duration of egg sensitization and later asthma. The kinetics of OVA-IgG and OVA-IgG1 responses, but not OVA-IgG4, differed between egg sensitized and non-egg sensitized children. Only persistently sensitized children had a rise in OVA-IgG1 concentration through the first year of life, and at 1 year of age, they had significantly higher OVA-IgG and OVA-IgG1 than either transiently sensitized or non-egg sensitized children. High OVA-IgG1 was associated with later asthma. OVA-IgG and subclass responses relate to the duration of egg sensitization. Measurement of OVA-IgG1 concentration in infancy might offer a useful adjunct to identify those at an increased risk of asthma (20).

Immunoglobulin molecules are composed of heavy and light chains that form whole intact Ig molecules in activated plasma cells secreting antibodies. Heavy and light chains are assembled in the endoplasmic reticulum, following a complex and highly orchestrated process of B-cell development and immunoglobulin synthesis. During these processes, light chains (both kappa and lambda Ig-fLC) are always produced in slight excess over heavy chains. Although immunoglobulin free light chains

(Ig-fLCs) are considered to be spillover products, many studies showed that an increased concentration of Ig-fLC can be seen in a number of autoimmune/inflammatory diseases (21–23). In addition, Ig-fLC might play a role in allergic responses. In children with severe AD, an increase in Ig-fLC reflected disease was found without association with total IgE levels. Therefore, Ig-fLC might represent an additional diagnostic marker independent of total IgE levels (24). In another study, increased Ig-fLC levels were associated in infants with AD (25). Recently, we analyzed allergy markers in 91 children (62 boys and 29 girls) with EoE and a control group of 45 age-matched children who had non-EoE gastrointestinal allergic symptoms. Here, serum Ig-fLC appeared higher in females than in males during EoE compared to the control group (26). An overview of the immunoglobulins is depicted in Table S1.

Cytokines

Anti-inflammatory cytokines

In recent years, accumulating data in humans have identified Th2 cytokines (IL-4, IL-5, IL-10, and IL-13) as major contributors to allergy and asthma and have shown to be elevated in allergic patients (27, 28). IL-4 is a pleiotropic cytokine produced by activated T cells, mast cells, and basophils. In allergic children, serum levels of IL-4 were significantly elevated, particularly at age 13–24 months. The serum levels of IL-4 did not differ in children with different clinical manifestations of allergy, such as bronchial asthma and AD (29), although one study in asthmatic children suggests that serum level of IL-4 may be elevated in concert with decreased level of IFN-γ in asthma (30). IL-5 is produced by a number of cell types and is responsible for the maturation and release of eosinophils in the bone marrow. In humans, IL-5 is a very selective cytokine as a result of the restricted expression of the IL-5 receptor on eosinophils and basophils. Eosinophils are a prominent feature in the pulmonary inflammation that is associated with allergic airway diseases (31). IL-5 has been studied in children with EoE, but no elevated concentrations were detected (32). IL-10 is a cytokine with pleiotropic effects in immune regulation and inflammation. It downregulates the expression of Th1 cytokines, MHC class II antigens, and costimulatory molecules on macrophages. It also enhances B-cell survival, proliferation, and antibody production. IL-10 can block NF-κB activity and is involved in the regulation of the JAK–STAT signaling pathway. It was seen that IL-10 levels in the asthmatic children were significantly lower than those of the normal controls (33). IL-13 directs many of the important features of airway inflammation and remodeling in patients with allergic asthma. Several promising therapies for asthma that target the IL-13/IL-4/signal transducer and activator of transcription 6 pathway are in development, including anti-IL-13 mAbs and IL-4 receptor antagonists (34).

Proinflammatory cytokines

IL-16 is a pleiotropic cytokine that functions as a chemoattractant and as a modulator of T-cell activation. Serum levels of IL-16 of AD patients were significantly higher than those of controls and declined significantly after treatment with clinical improvement. A positive correlation was found between SCORAD and IL-16 in the acute exacerbation phase (35). IL-17 is a major cytokine player in T-cell-mediated leukocyte-associated inflammation. Asthmatic children under the age of 5 years were divided into mild, moderate, or severe asthma. Serum IL-17 concentrations were significantly higher in patients with severe asthma than in the other two groups of children with mild and moderate disease (36). IL-18 is a cytokine that belongs to the IL-1 superfamily and is able to induce severe inflammatory reactions, which suggests a role in inflammatory disorders. In a study in patients with AD, it was found that the mean serum level of IL-18 in the AD group was significantly higher than that of controls. IL-18 was also significantly higher in the sera of the patients with severe AD than in those with milder disease, with a correlation to IgE levels and SCORAD (37, 38). IL-18 was also studied in a group of asthmatic children, and lower levels of serum IL-18 were found in a group of asthmatic children especially during exacerbation (39). IL-21 is a member of IL-2 family cytokine has potent regulatory effects on cells of the immune system, including natural killer (NK) cells and cytotoxic T cells. A significantly decreased level of IL-21 was observed in children suffering with severe AD compared with controls (4). IL-31 is a novel Th-cell-derived cytokine that plays an important role in human T-cell-mediated skin diseases. Serum IL-31 levels were significantly higher whether during AD flare or quiescence than those in controls. IL-31 levels were significantly higher in the high disease severity group compared with the moderate or low severity group. Moreover, serum IL-31 levels correlated positively with SCORAD (40, 41). IL-33 is a member of the IL-1 family that potently drives production of Th2-associated cytokines. IL-33 was investigated in only one study in asthmatic children, and IL-33 was found to be significantly higher in the patient group compared to the control (42). Thymic stromal lymphopoietin (TSLP) is an epithelial cell-derived cytokine that can potently activate immature CD11c+ myeloid dendritic cells, which subsequently prime CD4 + T cells to produce allergy-promoting cytokines such as IL-4, IL-5, IL-13, and TNF-α, but downregulate IL-10 and interferon (IFN)-γ. Polymorphisms in the gene encoding the cytokine TSLP are associated with the development of multiple allergic disorders in humans, suggesting that TSLP is a critical regulator of allergic diseases (43). In children with AD, it was found that serum TSLP levels were significantly higher than normal controls but there were no differences in children with atopic and non-atopic eczema. Besides, serum TSLP levels in children with AD were not significantly correlated with disease severity, blood eosinophil counts, and serum total IgE levels. This suggests that TSLP may play a contributory role in the pathogenesis of AD regardless of the presence of atopy (44). B-cell-activating factor (BAFF) is a cytokine that belongs to the TNF superfamily, best known for its role in the survival and maturation of B cells. Serum BAFF level in children with atopic eczema was significantly higher than in non-atopic eczema children or healthy controls. Serum BAFF level was significantly correlated with total serum IgE level and total eosinophil count. It was also positively correlated with serum

BAFF and egg-specific IgE level in atopic eczema. This indicates that BAFF level is high in atopic eczema and might be a useful marker for atopic eczema (45). An overview of the cytokines is depicted in Table S2.

Chemokines

Chemokines are small, secreted molecules that regulate leukocyte trafficking. The human chemokine system currently includes more than 50 chemokines and 18 chemokine receptors. Based on the position of the first two of the four conserved cysteine residues, chemokines are divided into four subfamilies: CXC, CC, C, and CX3C (46). Chemokines can be divided into two categories in terms of their physiologic features, inflammatory and homeostatic. Inflammatory chemokines are expressed in inflamed tissues on stimulation by proinflammatory cytokines. These chemokines are specialized for the recruitment of effector cells, including monocytes, granulocytes, and effector T cells. Increased levels of various chemokines and chemokine receptors have been found in tissue samples collected from patients with allergic diseases, but the role of individual chemokines and chemokine receptors in the pathogenesis of allergic inflammation is not always known (47). There are 13 inflammatory chemokines known; in this review, only the chemokines with strong indications of their involvement in allergic diseases in children are described.

Thymus and activation-regulated chemokine (TARC/CCL17) is produced by dendritic cells, endothelial cells, keratinocytes, and fibroblasts. TARC is designated a Th2 type chemokine as it binds to CC chemokine receptor 4 (CCR4). TARC is known to be especially involved in skin-related allergies, and increased levels are found in children with AD compared to controls. Moreover, TARC levels seem to be specifically correlated to disease activity (48–54). Little is known about TARC in other allergies like EoE, food allergy, or asthma. In our own study of serum TSLP, TARC, and Ig-fLC, we found that TARC levels did not play a role in EoE; however, TARC levels were significantly higher in children with gastrointestinal allergies other than EoE (26). In the literature, three cases with high TARC levels of gastrointestinal food allergies in neonates and infants were described (55). These findings could indicate that serum TARC may be related to the part of gastrointestinal food allergies in infancy, but further research will be needed. Therefore, TARC may be a useful laboratory marker for the diagnosis of AD, especially cases which are moderate to severe, and for the evaluation of disease activity of AD.

Cutaneous T-cell-attracting chemokine (CTACK/CCL27) is a member of the CC chemokine family and a functional ligand for CCR10. It is selectively expressed in skin and attracts CCR10-expressing skin-homing memory T cells. The epidermal keratinocyte is a main source of CTACK, suggesting the involvement of various inflammatory skin diseases. In many studies was found that serum CTACK levels in patients with AD were significantly higher than those in healthy control subjects (52–54, 56) and CTACK levels in patients with AD significantly correlated with severity, soluble IL-2 receptor, soluble E-selectin, TARC, and macrophage-derived chemokine (MDC) levels (57). Serum CTACK concentration appears to be a skin-specific objective marker that correlates with various clinical and laboratory parameters of AD. No involvement of CTACK in other allergic disorders was yet reported.

Eotaxins are a CC chemokine subfamily of monocyte chemotactic proteins, responsible for the recruitment of eosinophils. In humans, three eotaxins are known: eotaxin-1 (CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26). Elevated levels of only serum eotaxin-1 were found in children suffering from food allergy (58).

Mucosa-associated epithelial chemokine (MEC/CCL28) is considered to regulate the chemotaxis of cells that express the CCR3 and CCR10. MEC is expressed by columnar epithelial cells in the gut, lung, breast, and the salivary glands and drives the mucosal homing of T and B cells that express CCR10, and the migration of eosinophils expressing CCR3. MEC is highly upregulated in inflammatory skin diseases such as AD. Serum MEC levels in AD, whether during flare or quiescence, were significantly higher than those in healthy children. Serum MEC levels were not correlated with the serum total IgE values in AD. MEC might contribute to the pathogenesis of AD, probably through the selective migration and infiltration of effector/memory Th2 cells in the skin. MEC may also represent an objective prognostic marker for disease severity (59).

Macrophage-derived chemokine (MDC/CCL22) is a CC chemokine and is a selective chemoattractant for CCR4-expressing cells, in addition to TARC. In two studies in children with AD, several chemokines were analyzed. Only MDC and TARC serum levels correlated with SCORAD and may be a useful inflammatory marker for assessing severity of AD in infants and young children (60, 61). Both studies date from 2002/2003, and recent data of MDC in allergic children are lacking. An overview of the chemokines is depicted in Table S2.

Markers of mast cell degranulation

Mast cells are the primary effector cells of immediate hypersensitivity reactions in humans. Upon mast cell activation, both preformed and newly synthesized mediators are secreted. Preformed mediators are histamine, heparin, chondroitin sulfate E, and several proteases such as tryptase (α-protryptase and ß tryptase), chymase, mast cell carboxypeptidase, and cathepsin G. Newly preformed mediators include leukotriene C_4, prostaglandin D_2, and cytokines (tumor necrosis factor-α, IL-4, 5, 6, and 13). The release of all these mediators can be used as clinical markers for mast cell activation, where ß-tryptase levels seem to be the most specific measure of mast cell activation in biologic samples (62). An overview of the mast cell degranulation markers is depicted in Table S3.

Markers of eosinophil activation

In addition to mast cells, eosinophils are also important effector cells in human allergic diseases; they play a significant role in promoting allergic inflammation through the release of

proinflammatory mediators, and it is known that circulating eosinophils are elevated in patients with asthma (63). Eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) are cytotoxic agents secreted by activated eosinophils. Pucci et al. (64) found that serum ECP levels in infants and young children with AD correlate with disease severity, while other studies did find elevated levels, but no correlation to disease severity (65, 66). Serum ECP was also studied in asthma and allergic rhinitis. Patients with asthma showed higher ECP levels than healthy children and partially controlled asthmatics had significantly higher levels of ECP than controlled asthmatics, whereas controlled asthma showed no differences in ECP versus healthy children. Therefore, ECP may be helpful in the assessment of asthma control (67). Like serum ECP in AD, also in asthma, decreased ECP levels were seen after treatment (68), suggesting ECP is a good marker for monitoring effectiveness of treatment. In allergic rhinitis, severity of nasal obstruction was positively correlated with the duration of rhinitis and the levels of serum ECP in childhood persistent allergic rhinitis, indicating that disease severity might be related to chronic inflammatory process (69). Like ECP, EDN is secreted by activated eosinophils during allergic and inflammatory processes. It is suggested that EDN is more useful than ECP in evaluating disease severity. This may partially due to the recoverability of EDN (not sticky, 100% recovery rate), as ECP is a sticky and more highly charged protein. In terms of clinical utility, EDN level is a more accurate biomarker than ECP when analyzing the underlying pathophysiology of asthma. As a monitoring tool, EDN has shown good results in children with asthma as well as other allergic diseases. In children too young to fully participate in lung function tests, EDN levels may be useful as an alternative measurement of eosinophilic inflammation. Therefore, EDN may be a biomarker for the diagnosis, treatment, and monitoring of asthma/allergic disease (70). Lee et al. (66) studied, together with ECP, EDN as a marker for AD. In this study, unlike ECP, EDN did correlate with disease severity and a decrease of EDN after treatment was seen. Also in EoE, serum EDN levels were significantly higher in subjects with EoE than in controls, although more studies are needed to assess serum EDN in establishing EoE diagnosis, assessing response to therapy, and/or monitoring for relapse or quiescence (32). An overview of the eosinophil activation markers is depicted in Table S3.

Soluble immune receptors

Soluble immune receptors have been proposed as biomarkers in patients with AD, but their clinical applicability in affected children has rarely been studied. Serum concentrations of sCD14, sCD23, sCD25, and sCD30 did not significantly correlate with disease severity in children with AD and were not differentially expressed in patients of different AD phenotypes. According to this study, these soluble receptors cannot be regarded as clinically useful biomarkers for the assessment of childhood AD (71). However, another study in allergic and non-allergic children showed that serum level of sCD23 showed an age-dependent change and was significantly higher in allergic than in non-allergic infants aged 7–12 months, but not in other age groups (29). Soluble cytokine receptors can either act as inhibitors, by competitively inhibiting cytokines from binding to their membrane-bound receptors, or as enhancers, by serving as cytokine carriers. Serum levels of soluble Il-2 receptor (sIL-2R) were examined in children with allergic disease compared to age-matched controls. The results showed age-related decreases in the serum levels of sIL-2R and a significant elevation of sIL-2R was observed in sera from children with atopic eczema or history of an anaphylactic reaction to food (72). Both sCD23 and sIL-2R are studies conducted in the early 90s, and more (recent) data will be needed. In a population-based birth cohort, the relationship between Interleukin-5 receptor α-subunit (IL-5Rα) and the development of allergic phenotypes in childhood was investigated. Increased serum s-IL-5Rα level at age 5 was associated with atopic sensitization and with subsequent development of eczema by age 8 (73). An overview of the soluble immune receptors is depicted in Table S3.

Soluble adhesion molecules

The adhesion of leukocytes and thrombocytes to vascular endothelium is considered a critical step in the immunologic and inflammatory processes, leading to their migration from the vascular compartments into the airway tissues and the accumulation and expansion of their functions in the target tissue. Soluble intercellular adhesion molecule-1 (sICAM-1) was studied in children with mild or moderate asthma before and three months after inhaled steroids. Significant higher levels of sICAM-1 (but not vascular cell adhesion molecule-1 (sVCAM-1) or sP-selectin) were found before and after the treatment, paralleled to clinical improvement. Reduction in the level of sICAM-1 after the treatment may be related to the decreased inflammation in response to therapy (74). In another study in asthmatic children and adults was found that sICAM-1 (but not sE-selectin) from patients with asthma was significantly higher than healthy controls. (75). For AD in children are the results less clear. In one study, it was found that there was a close correlation between serum ICAM-1 levels and the disease severity (76), while other studies found a significant correlation between the objective SCORAD before treatment and the level of sE-selectin, but not for sICAM-1, sVCAM-1, or sP-selectin (77, 78). An overview of the soluble adhesion molecules is depicted in Table S3.

Neurotrophins

Neurotrophins are polypeptides that support growth, differentiation, and survival of neurons in developing and adult nervous systems. The prototypical neurotrophin is nerve growth factor (NGF), and this family also includes brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NTF3), and neurotrophin 4 (NTF4) (79). It was investigated whether levels of neurotrophins in serum of asthmatic children were influenced by the genotype of functional variants within genes

encoding analyzed neurotrophins and their specific receptors. Serum levels of four neurotrophins (BDNF, NGF, NTF3, and NTF4) were analyzed during exacerbation of asthma symptoms. NGF serum levels may be influenced by the genotype of NTRK1 gene individually as well as in the interaction with NGF functional genetic variant suggesting their involvement in allergic inflammation in asthma. Serum levels of the other neurotrophins did not seem to be affected by the variants in the analyzed genes (79), although serum NTF3 and NTF4 levels have found to be associated with asthma severity in children (80). BDNF gene polymorphisms have been associated with asthma (81) and AD (82). Subjects with moderate and severe asthma had higher BDNF levels than subjects with mild asthma and controls (83). BDNF levels in AD were significantly correlated with the SCORAD score (84). An overview of neurotrophins is depicted in Table S3.

Other markers for allergy

Galectins are a family of proteins defined by their binding specificity for β-galactoside sugars, which can be bound to proteins by either N-linked or O-linked glycosylation. Increasing evidence shows that galectins are involved in many fundamental biologic processes such as cellular communication, inflammation, differentiation, and apoptosis. Galectin-9 was first described as an eosinophil chemoattractant. With the progress in research, galectin-9 has come to be known as a versatile immunomodulator that is involved in various aspects of immune regulations, but the exact function still remains elusive (85). Galectin-9 attenuated asthmatic reaction in guinea pigs and suppressed passive cutaneous anaphylaxis in mice. These results indicate the mast cell stabilizing effect of galectin-9. In *in vitro* studies of mast cell degranulation was seen that galectin-9 strongly and specifically bound IgE, which is a heavily glycosylated immunoglobulin and that the interaction prevented IgE-antigen complex formation, clarifying the mode of action of the antidegranulation effect (86). Galectin-9 levels were measured in the sera of infants with AD where increased serum galectin-9 levels correlated with reduced acute allergic skin reaction (87).

Human leukocyte antigen-G (HLA-G) is a non-classical HLA class I molecule and showed an increased expression in several immunologic diseases, including asthma and allergic rhinitis (88, 89). Ciprandri et al. (89) describe that children with allergic rhinitis had significantly higher levels of sHLA-G molecules than healthy controls. In another study by Ciprandi et al. (90) was found that sHLA-G was reduced in patients with allergic rhinitis during sublingual immunotherapy and sHLA-G could therefore be a marker for response to therapy. Zheng et al. investigated sHLA-G in combination with IL-10. Plasma sHLA-G in atopic asthma was dramatically higher compared with that of the controls. IL-10 levels in the asthmatic children were significantly lower than that of the normal controls. These findings indicate that sHLA-G might be considered as a biomarker for the atopic asthmatic patients and dramatically increased sHLA-G combined with decreased IL-10 levels may have implications in the pathogenesis of atopic asthma (33).

YKL-40, also called human cartilage glycoprotein 39 or chitinase 3-like-1, is a glycoprotein belonging to the chitinase family. Serum YKL-40 has been reported to be elevated in a number of diseases characterized by inflammatory and tissue remodeling responses. It is known that YKL-40 is involved in bronchial remodeling by promoting BSM cell proliferation and migration through a PAR-2-dependent mechanism (91). YKL-40 levels were significantly higher in asthmatics compared to a control group. Although Konradsen et al. (92) found in a study within a pediatric asthma group that YKL-40 levels were increased in children with severe, therapy-resistant asthma compared to children with controlled asthma, the usefulness of YKL-40 as a marker for asthma severity, especially in children, was later contradicted by Santos et al. (93).

The costimulatory molecule OX40 and its soluble ligand, sOX40L, are key mediators of allergic airway inflammation, including eosinophilic airway inflammation, airway hyperresponsiveness, and Th2 polarization (94). Serum levels of sOX40L were investigated in asthmatic children. The median and mean serum sOX40L levels were significantly higher in asthmatic patients during acute attacks in comparison with patients in between asthma attacks and in comparison with controls. sOX40L values were higher among patients who presented with acute severe asthma exacerbations than in patients with mild or moderate asthma exacerbations. During stability, patients with severe persistent asthma had significantly higher levels when compared with patients with moderate or mild persistent asthma. Upregulation of sOX40L may play a critical role in development of childhood atopic asthma and is in favor of asthma severity (95).

Cutaneous production of antimicrobial peptides (AMPs) is a primary system for protection, and expression of some AMPs further increases in response to microbial invasion. So far, many different peptides with antimicrobial function in the skin are known but probably best studied are the defensins and cathelicidin. Cathelicidins are unique AMPs that protect the skin through two distinct pathways, by direct antimicrobial activity or initiation of a host response resulting in cytokine release, inflammation, angiogenesis, and re-epithelialization (96). Cathelicidin LL-37 dysfunction emerges as a central factor in the pathogenesis of several cutaneous diseases, including atopic dermatitis, in which cathelicidin is suppressed. Serum LL-37 concentrations did not differ between eczema patients and controls. However, serum LL-37 concentrations increased with increasing eczema severity among the patients and can therefore serve as a severity marker for eczema (97).

Osteopontin is an extracellular matrix protein with a wide range of functions on tissue fibrosis, tumor progression, as well as immune regulation, and plays a role in T-cell-mediated immunity. Osteopontin levels have been extensively investigated in adult patients with asthma, but only one study investigated the role of osteopontin in childhood asthma. Serum osteopontin levels were significantly higher in the asthma group when compared to the control group, and in the >5 years of age asthmatic group, osteopontin levels of the patients with allergic rhinitis were higher than those of the patients without allergic rhinitis (98).

Matrix metalloproteinase-9 (MMP-9) has been shown to play a role in the infiltration of inflammatory cells in various tissues. It is part of the pathogenesis of many inflammatory diseases, including asthma and allergic rhinitis/conjunctivitis. Mean serum levels of MMP-9 in the children with asthma were significantly higher than those in the healthy controls. Serum levels of MMP-9 are associated with the occurrence of childhood asthma; however, the MMP-9-1562C/T gene polymorphism has no correlation with the pathogenesis of childhood asthma (99).

Thrombomodulin is a receptor for thrombin on the vascular endothelium and plays an essential role as a protein cofactor in thrombin-catalyzed activation of protein C. Plasma thrombomodulin levels were measured in patients with AD, and levels in patients with AD were significantly higher than those of controls. A significant correlation was observed between plasma thrombomodulin levels and SCORAD or peripheral eosinophil counts. There was also a positive correlation between plasma thrombomodulin and sVCAM-1 levels. These results suggest that plasma thrombomodulin levels may reflect a severity of AD and/or endothelial cell activation induced by an allergic inflammation (100). An overview of other markers for allergy is depicted in Table S3.

Biomarkers for allergy in other biologic fluid

A few studies have already been carried out in children to identify allergy markers in less invasive biologic fluids like urine (64, 101–109) and saliva (110–112). However, for both urine and saliva, one should be aware that analysis of biomarkers may run into some issues ranging from the fact that it does not reflect the source of the biomarker, the difficulty of adequate result expression, and method and timing of collection can have an enormous impact on the results. To investigate promising allergy markers in urine or serum, larger prospective trials in patients are needed using well-defined methods and disease characteristics.

Concluding remarks

To this date, no single or specific biomarker for allergy has been identified. IgE, either total or allergen-specific, has been a classical marker for allergic sensitization, but it is known that IgE is not exclusive or specific for allergy, and also non-IgE-mediated allergies are known. As allergy is not one disease, but a collection of a number of allergic conditions, it is therefore not very plausible that one marker would fit all, and therefore, probably a more holistic approach using a combination of clinical history, clinical readouts, and diagnostic markers will be needed. Two of the most reliable and best-studied markers in AD for disease activity and treatment seem to be TARC and CTACK, which show a good correlation with the clinical readout SCORAD. Of all the other markers described in this review, although some are promising, there are either contradictory results or not enough studies were conducted to confirm the marker as a reliable biomarker. Therefore, the search for new and reliable biomarker will continue. The evolution in biomarker discovery has resulted in an 'omics' approach, in which hundreds of biomarkers in the field of genomics, transcriptomics, proteomics, and metabolomics can be simultaneously studied, and might be a promising future approach for identifying new markers (113).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Summary of immunoglobulin isotypes, subclasses, and free light chains as biomarkers for allergy.

Table S2. Summary of cytokines and chemokines as biomarkers for allergy.

Table S3. Summary of other biomarkers for allergy.